EFFECT OF SEALER AGITATION TECHNIQUE ON THE PERCENTAGE AND DEPTH OF PENETRATION OF TWO TYPES OF BIOCERAMIC SEALERS: AN IN VITRO STUDY

By

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2020-2023

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "Effect of Sealer Agitation Techniques on the Percentage and Depth of Penetration of Two Types of Bioceramic Sealers: An In Vitro Study" is a bonafide and genuine research work carried out by me under the guidance of Prof. Dr Jain Mathew, Department of Conservative Dentistry and Endodontics, St Gregorios Dental College, Chelad, Kothamangalam.

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ABSTRACT

Aim:

To compare the effect of three root canal sealer activation techniques on percentage and depth of sealer penetration of NISHIKA ROOT CANAL SEALER BG and BIOROOT RCS sealers at 2 different levels of the root canal system.

Materials and Methods

Sixty-six teeth prepared till F3 ProTaper size were divided into three equal Groups based on sealer activation technique (G1: Ultrasonics, G2: Lentulo spiral, and G3: Counter-clockwise rotary motion). Each Group was further divided into two equal Subgroups based on the type of sealer used SUBGROUP A: Nishika Root Canal Sealer Bioactive Glass (Nishika Nippon Shikha Yakuhin CO Ltd) and SUBGROUP B: BioRoot RCS sealer (Septodont) and obturated with gutta-percha. Horizontal sections at 3 and 6 mm from the apex were obtained and the percentage and depth of penetration of sealers into dentinal tubules were measured using confocal laser scanning microscopy (CLSM). Statistical analysis was performed utilizing Kruskal-Wallis and Mann-Whitney U tests with a significance level of 5%.

Results

Results: G1 showed significantly ($P < 0.001$) higher percentage and depth of sealer penetration for Subgroups A and B at both Levels 3 and 6 when compared with other Groups. Higher value of percentage and depth of penetration was seen at Level 6 than Level 3 which was statistically significant among all the tested groups. The differences among G2 and G3 was also statistically significant. Lowest values were seen for G2 for Subgroup B at Level 3 in terms of percentage depth of penetration and G3 for Subgroup A at Level 3 in terms of maximum depth of penetration.

Conclusion

Percentage and depth of sealer penetration are influenced by the type of sealer used, the technique used for sealer activation and by the root canal level. Ultrasonic method of sealer activation with Nishika Root Canal Sealer Bioactive Glass showed the best result. Highest values were seen for Level 6 when compared with Level 3.

Keywords: Confocal laser scanning microscopy; Counter-clockwise motion; lentulospiral; percentage and depth of sealer penetration; ultrasonics

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INTRODUCTION

INTRODUCTION

The complete sealing and filling of the cleaned and shaped root canal system are important steps that can affect the long-term success of root canal treatment. Due to the complexity of the root canal system, root canal sealers are necessary for filling the irregularities and for penetrating into the dentinal tubules to obtain a hermetic seal of the root canal system. They should also provide adherence between gutta-percha and the dentinal walls in order to avoid gap occurrence at the sealer-dentine interface.[1]

Grossman outlined the ideal properties of a root canal sealer, including the following: provide good adhesion between the gutta percha and the canal wall when set; establish a hermetic seal; no shrinkage upon setting; insoluble in tissue fluids; tissue tolerant; and others. The most recently introduced sealer is the calcium silicate-based sealers. At present not one of the existing sealers satisfies all the criteria.[1]

Poorly filled areas of the root canal system can be a source of bacterial growth, as seen in a report which stated that 58% of treatment failures were due to incomplete obturation. Although gutta-percha, the most commonly used core filling material can be adapted reasonably well to the canal walls, it is essential to place a root canal sealer, because of the canal irregularities and size of the dentinal tubules, thus improving the adaptation of root filling at the dentin material interface.[2]

Single cone technique is an obturation technique that uses a single fitted gutta-percha cone with sealer. It is a simple technique that is easy to master and saves time for clinicians. Neither vertical nor lateral pressure existing in condensed filling techniques is used on the root canal walls in single cone technique. Therefore, the risk of root fracture is decreased in teeth obturated by using single cone technique. In addition, no thermal damage was seen in the periodontal membrane with the single cone technique as was seen when using warm vertical compaction. However, as no condensation pressure exists during single cone obturation, the canal always contains a mass of sealer, which is much more than root canals that are obturated using cold lateral and warm vertical techniques.[1]

A sealer’s antibacterial activity is proportional to its tubular penetration. Placement and activation of a sealer into the root canal system should be predictable and should be done to completely cover the dentin walls. The various recognized modes of sealer placement include the use of endodontic files or reamers, lentulospiral, GP cones, paper points, ultrasonic files, and counter clockwise motion of rotary systems. Each technique may produce a different distribution of the sealer onto the canal walls, which could ultimately affect the sealing. At present, there is no evidence to suggest that one method is better and reliable than the other.

Ultrasonic activation (UA) of the sealer is shown to give promising results in improving obturation. It favours better penetration of the sealer into areas of anatomical complexities, more internally into the dentinal tubules and with less presence of gaps. The activation occurs through the use of specific ultrasonic tips connected to devices that produce high frequency vibrations (25-30 kHz), promoting acoustic transmission and cavitation. Sonic activation uses low-frequency vibration (1–6 kHz) through flexible tips, which when combined with short pecking movements inside the root canal, acts in a synergistic way by creating a hydrodynamic phenomenon that is responsible for the increased penetration of the sealer into the lateral canals.[3,4,5]

Ceramics, a class of biomaterial, are polycrystalline materials that display characteristic hardness, brittleness, strength, stiffness, resistance to corrosion and wear, and low density. Bioceramics are utilized to restore functionality to diseased or damaged hard tissues and are used in several different fields such as dentistry, orthopaedics, and medical sensors. Presently available bioceramics are available as three basic types: bioinert, bioactive, and bioresorbable ceramics.[6]

The first generation of bioceramics was comprised of alumina and zirconia. The main features of first-generation bioceramics were their good mechanical properties, especially their wear resistance. The second generation of bioceramics was comprised of bioactive glass (BG), hydroxyapatite, and calcium phosphate-based cement. Second generation bioceramics bonded and integrated with the living bone of the body without forming a fibrous tissue around them and without promoting either inflammation or toxicity. Unique among the second-generation bioceramics is BG which has instigated a revolution in healthcare appliances and has paved the way for modern biomaterial-driven medicine.[6]

There are two well-known commercialized root canal sealers that include BG. One is Gutta Flow Bioseal (GFB) (Coltène/Whaledent AG, Altstätten, Switzerland), which is composed of gutta-percha, polydimethylsiloxane, platinum catalyser, zirconium dioxide and BG. GFB has shown low solubility, low porosity, alkalization capacity, dentin penetrability, and cytocompatibility. At present, only limited evidence is available concerning either the mechanism of GFB hardening or its ability to seal the canal or its removal for retreatment. The second product is Nishika Canal Sealer BG (CS-BG). Presently, there exists compelling evidence concerning its physicochemical properties, biocompatibility, sealing ability and removability. CS-BG was developed from BG-based biomaterials and was originally intended for both dental pulp and bone regeneration therapies. CS-BG is a two-phased paste; Paste A consists of fatty acids, bismuth subcarbonate, and silica dioxide while Paste B consists of magnesium oxide, calcium silicate glass (a type of BG) and silica dioxide.

Ideally, root canal sealers must be biocompatible, should have low surface tension and better wettability, thus providing fluid-tight seal. The bioceramic sealer, BioRoot RCS (Septodont, Louisville, USA) is a water-based sealer composed of tricalcium silicate, zirconium oxide, and calcium chloride. When it comes in contact with the physiologic solution, these sealers release calcium and form an interfacial calcium phosphate (apatite) layer, thus developing a chemical bond with the dentinal walls.[2]

Stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy, and confocal laser scanning microscopy (CLSM) are the different microscopy techniques that are currently used to evaluate the sealer/dentin interface. However, the CLSM has the advantage of providing detailed information of the root canal walls at a relatively low magnification in comparison to the conventional SEM.[5]

Confocal laser scanning microscopy (CLSM) provides information about the sealer penetration and distribution inside the dentinal tubules of root canal walls with the use of fluorescent rhodamine dye marker mixed with the sealers.[2]

Therefore, the purpose of this study was to evaluate the percentage and depth of dentinal tubule sealer penetration of BioRoot RCS sealer when agitated with three different techniques and to compare it with Nishika Canal Sealer BG sealer at two levels - 3 mm and 6 mm from the apex using CLSM for evaluation. The null hypotheses to be tested was that the use of ultrasonic, sonic and manual dynamic techniques have no statistically significant influence on the sealer penetration in the dentinal tubules.

AIM & OBJECTIVES

AIM & OBJECTIVES

AIM

To compare the effect of different agitation techniques on the percentage and penetration depth of two Bioceramic sealers into root dentin at two different levels - 3 mm and 6 mm from the apex.

OBJECTIVES

To compare and study the effect of three different sealer agitation techniques on two types of bioceramic sealers at 3 mm and 6 mm from the apex with the help of CLSM the following:

1. The percentage depth of sealer penetration into the dentinal tubules.
2. The maximum depth of penetration into dentinal tubules.

REVIEW OF LITERATURE AND BACKGROUND

REVIEW OF LITERATURE

- **Robert R. White et al. (1984)[7]** in their in vitro study to evaluate what influence the smear layer has on tubular penetration using two plastic filling materials, pHEMA (Hydron; National Patent Development, New York, NY) and silicone (Endo-fill; Lee Pharmaceuticals, South El Monte, CA) reported that the removal of smear layer enabled certain filling materials to enter the dentinal tubules. Further studies were planned to evaluate the leakage as well as gutta-percha and sealer techniques.

- **T. OKSAN et al. (1993)[8]** in their study evaluated the effect of smear layer on the penetration of four root canal sealers into dentinal tubules. The root canals were filled with Diaket, N2 Universal, SPAD and Forfenan as recommended by the manufacturers. They concluded that the presence of smear layer prevented the penetration of root canal filling materials used in this study into dentinal tubules but after the removal of smear layer, the penetration into dentinal tubules was better with Diaket, N2 Universal and SPAD, but not with Forfenan and the chemical and physical properties of the root canal filling materials affected tubular penetration in the absence of smear layer.

- **Leonidas P. Vassiliadis et al. (1994)[9]** in their study to evaluate the depth of penetration of Grossman sealer and its appearance in the dentinal tubules in vivo, performed root canal therapies to study the differences in the sealer, if any, between endodontically treated teeth that had remained in the dental arch for different time periods. 1% NaOCl was used for irrigation using lateral condensation technique with Grossman type sealer for obturation. The teeth were fractured and prepared for and viewed by a scanning electron microscope equipped with an electron dispersive spectrometer. The study concluded that the sealer was found deepest in the middle third of the root. The smear layer did not stop the sealer from entering the dentinal tubules. The differences in the depth of penetration or in the appearance of the sealer could not be attributed to the different time periods that the teeth remained in the arch after endodontic treatment.

- **B.H SEN et al. (1996)[10]** studied the possible correlation between penetration of four root canal sealers into the dentinal tubules and microleakage of external fluids into the canal using a dye leakage method and scanning electron microscopy. The sealers used were Diaket, Endomethasone, CRCS and KetacEndo. They reported that Diaket had lower microleakage scores than the other sealers and KetacEndo demonstrated the least penetration among all the sealers.

- **Kouvas V et al. (1998)[11]** in their study examined the effect of the smear layer on the penetration depth of Sealapex, Roth 811, and CRCS root canal sealers into the dentinal tubules. They concluded that the removal of smear layer allowed penetration of all three sealers into the dentinal tubules. The presence of smear layer at the root canal walls obstructed the penetration of all sealers into the dentinal tubules.

- **Semra Qalt et al. (1999)[12]** studied the dentinal tubule penetration of root canal sealers after root canal dressing with calcium hydroxide. The sealers used were CRCS, AH26, and Ketac Endo followed by obturation with a lateral condensation technique. They concluded that sealers did not penetrate into the dentinal tubules when only NaOCI was used. EDTA followed by NaOCl irrigation resulted in complete removal of $Ca(OH)_2$ after which the root canal sealers penetrated into the dentinal tubules.

- **Matthew C. Davis et al. (2002)[13]** studied coronal distribution and colour changes of four commonly used sealers placed in the pulp chamber after 2 years. Sealers evaluated were AH 26, Kerr Pulp Canal Sealer, Roth 801, and Sealapex. They concluded that there was no measurable penetration of sealer into dentin for all groups and no dentin discoloration occurred. The sealers displayed marked discoloration after 2 years and remained confined primarily to the pulp chamber.

- **S. SEVIMAY et al. (2003)[14]** studied dentinal penetration and adaptation of three endodontic sealers using scanning electron microscope (SEM). The canals were obturated with AH 26, CRCS, RSA sealers and gutta-percha using lateral condensation technique. They concluded that AH 26 was the best sealer for penetrating into the dentinal tubules and adapted well to the dentinal walls when compared with the CRCS and RSA.

- **Andreas B. Kokkas et al. (2004)**[15] studied the effect of smear layer on the penetration depth of three different root canal sealers into the dentinal tubules by examining 64 recently extracted single-rooted teeth. Examination in scanning electron microscope revealed that the smear layer obstructed all the sealers from penetrating the dentinal tubules. In contrast, smear layer removal allowed the penetration of all sealers to occur to a varying depth. They concluded that that smear layer plays an important role in sealer penetration into the dentinal tubules, as well as in potential clinical implications.

- **M. V. Weis et al. (2004)**[16] compared the average sealer cement film thickness and the extent and pattern of sealer penetration into dentinal tubules in association with four obturation techniques in curved root canals. The obturation techniques used were SimpliFill, continuous wave, Thermafil and 0.04 matched taper (master cones) lateral compaction obturation. They concluded that the Sealer thickness was strongly dependent on obturation technique. Thermafil resulted in the best outcome. Extensive sealer penetration into dentinal tubules was seen and was unrelated to the obturation technique.

- **De Deus GA et al. (2004)**[17] compared the depth of sealer penetration into the dentinal tubules with three root-filling techniques (lateral condensation, warm vertical compaction of gutta-percha and Thermafil system) using a methodology combining light microscopy and digital image processing. They concluded that the samples that were root-filled with thermoplasticised gutta-percha technique lead to deeper penetration of the root canal sealer into the dentinal tubules.

- **Patel DV et al. (2007)**[18] compared the penetration depth into dentinal tubules of RealSeal with that of a well-established endodontic sealer (Tubliseal) by means of confocal microscopy. Confocal microscopy was used to assess the penetration depths of the sealers at three sites for each specimen (coronal, middle and apical). They concluded that the penetration depth of RealSeal into the root dentinal tubules was significantly greater than that of Tubliseal.

- **K. Mamootil et al (2007)**[19] compared the depth and consistency of penetration of three different root canal sealer cements into dentinal tubules in extracted teeth and measured the penetration of epoxy resin-based sealer cement in vivo. Root canals were prepared and obturated using three different sealer cements based on epoxy resin (AH26), zinc oxide eugenol (Pulp Canal Sealer EWT) and methacrylate resin (EndoREZ). They concluded that the depth and consistency of dentinal tubule penetration of sealer cements appeared to be influenced by the chemical and physical characteristics of the materials. Resin-based sealers displayed deeper and more consistent penetration.

- **Ronald Ordinola-Zapata et al (2007)**[20] compared the percentage and depth of sealer penetration into dentinal tubules during obturation using Sealer 26, GuttaFlow, or Sealapex in root canals filled with the lateral compaction technique. The specimens were analyzed using confocal microscopy. They concluded that Sealapex displayed deeper penetration into the dentinal tubules.

- **Young-Mi Moon et al (2010)**[21] evaluated the effect of different final irrigation regimens on the sealer penetration into dentinal tubules of curved root canals. The specimens were obturated with gutta-percha and AH Plus sealer labelled with fluorescent dye. Transverse sections at 2 mm (apical) and 5 mm (coronal) from the root apex were examined by using confocal laser scanning microscopy. The total percentage and maximum depth of sealer penetration was measured. They concluded that the final flushing with 3.5% NaOCl after the use of 17% EDTA had no additional effect on sealer penetration. Regardless of final flush regimen, the use of EDTA before canal obturation resulted in significantly better sealer penetration at both levels.

- **Eric Balguerie et al (2011)**[22] studied the penetration depth and the adaptation to the root canal walls of five different sealers used in combination with softened gutta-percha cones in vitro in the apical, middle, and coronal third of the root canal. The roots were cross sectioned and prepared for scanning electron microscopic evaluation. They concluded that the tubular penetration and adaptation varies with the different physical and chemical properties of the sealers used. AH Plus showed the most optimal tubular penetration and adaptation to the root canal wall among the sealers tested.

- **Bird DC et al. (2012)**[23] evaluated the ability of Capasio and MTA to penetrate human dentinal tubules when used as a root-end filling material and to examine the interaction of Capasio and MTA with synthetic tissue fluid (STF) and endodontically prepared root canal walls in extracted human teeth. STF was chosen to simulate the in vivo conditions in which root-end filling materials were used. They concluded that when used as a root-end filling material, Capasio was more likely to penetrate dentinal tubules and equally likely to promote apatite deposition when compared with MTA.

- **Young-Mi Moon et al. (2012)**[24] investigated the efficacy of laser-activated irrigation (LAI) of 1320-nm Nd:YAG laser on sealer penetration into dentinal tubules in the presence of 5.25% NaOCl or 17% EDTA. Transverse sections at 2 and 5 mm from root apex were examined with confocal laser scanning microscopy, and the percentage of sealer penetration into dentinal tubules was measured. They concluded that the 1320-nm Nd:YAG laser activation with either NaOCl or EDTA was much better than NaOCl irrigation alone.

- **Saurabh S. Chandra et al. (2012)**[25] evaluated the depth of penetration of 4 different endodontic resin sealers into the radicular dentinal tubules with the aid of confocal microscopy. The samples were obturated with AH Plus, RealSeal, EndoRez, and RoekoSeal resin sealers, respectively. The core material used in all the groups was Resilon. They concluded that RealSeal resin sealer exhibited the maximum penetration.

- **Jordan A. Bolles et al. (2013)**[26] compared the effect of conventional syringe irrigation and 2 different sonic irrigation systems, the Vibringe and EndoActivator, on sealer penetration into dentinal tubules of single-rooted extracted teeth by using confocal laser scanning microscopy. They concluded that the use of sonic activation with either the EndoActivator or Vibringe did not significantly improve sealer penetration when compared with conventional irrigation.

- **Armita Rouhani et al. (2013)**[27] compared the depth of dentinal tubule sealer penetration in the apical thirds of severely curved root canals obturated with Resilon/Epiphany or gutta-percha/AH Plus using scanning electron microscopy. Sealer penetration was evaluated in 2 mm sections of the apical thirds of roots using scanning electron microscopy. They concluded that average penetration into dentinal tubules in the apical thirds of severely curved roots did not differ significantly between Epiphany and AH Plus.

- **Astrit Kuci et al. (2014)**[28] tested the dentinal tubule penetration of AH26 (Dentsply DeTrey, Konstanz, Germany) and MTA Fillapex (Angelus, Londrina, PR, Brazil) in instrumented root canals obturated by using cold lateral compaction or warm vertical compaction techniques in either the presence or absence of the smear layer. Sealer penetration in the dentinal tubules was measured by using confocal laser scanning microscopy. They concluded that greater sealer penetration could be achieved with either the MTA Fillapex–cold lateral compaction combination or with the AH26–warm vertical compaction combination. Smear layer removal was critical for the penetration of MTA Fillapex.

- **Aysun Kara Tuncer et al. (2014)**[29] compared the effect of the EndoVac irrigation system (SybronEndo, Orange, CA) and conventional endodontic needle irrigation on sealer penetration into dentinal tubules. All teeth were instrumented using the ProFile rotary system (Dentsply Maillefer, Ballaigues, Switzerland) and obturated with gutta-percha and AH Plus sealer (Dentsply DeTrey, Konstanz, Germany) labelled with fluorescent dye. Transverse sections at 1, 3 and 5 mm from the root apex were examined using confocal laser scanning microscopy. The total percentage and maximum depth of sealer penetration were then measured. They concluded that the EndoVac irrigation system significantly improved the sealer penetration at the 1to 3 mm level over that of conventional endodontic needle irrigation.

- **Aysun Kara Tuncer et al. (2015)**[30] evaluated the effect of final irrigation with QMix 2 in1 (Dentsply Tulsa Dental, Tulsa, OK), on sealer penetration into dentinal tubules using confocal laser scanning microscopy. The teeth were instrumented with sodium hypochlorite (NaOCl) irrigation and then divided into 3 groups according to the final irrigation regimen used. All teeth obturated with gutta-percha and rhodamine B– labelled AH26 sealer (Dentsply DeTrey, Konstanz, Germany). After setting, the roots were sectioned horizontally at 3, 5 and 8 mm from the root apex. Sealer penetration into the dentinal tubules was examined by confocal laser scanning microscopy. They concluded that the use of EDTA + CHX or QMix during final irrigation significantly improved sealer penetration when compared with the control group in the middle and coronal sections of the roots. However, no effect was observed in the apical sections.

- **Vineeta Nikhil et al. (2015)**[31] compared the effect of three root canal sealer activation techniques on the percentage and depth of sealer penetration of MTA Fillapex and AH Plus sealers. Sixty teeth prepared till F5 ProTaper size were divided into three equal groups on the basis of sealer activation technique (G1: Ultrasonics, G2: Lentulo spiral, and G3: Counter-clockwise rotary motion). Each group was further divided into two equal subgroups on the basis of the type of sealer used: AH Plus (Denstply, Konstanz, Germany) or MTA Fillapex (Angelus, Londrina, PR, Brazil) and obturated with gutta-percha. Horizontal sections at 3 and 6 mm from the apex were obtained and the percentage and depth of penetration of sealers into dentinal tubules were measured using confocal laser scanning microscopy (CLSM). They concluded that the percentage and depth of sealer penetration were influenced by the type of sealer used, sealer activation technique and by the root canal level. Ultrasonic method of sealer activation and MTA Fillapex showed the best results.

- **Greer E. McMichael et al. (2016)**[32] studied the dentinal tubule penetration of EndoSequence BC Sealer (Brasseler USA, Savannah, GA), QuickSet2 (Avalon Biomed, Bradenton, FL), NeoMTA Plus (Avalon Biomed), and MTA Fillapex (Angelus, Londrina, Brazil) sealers using continuous wave (CW) and single-cone (SC) obturation techniques. Teeth were sectioned at 1 mm and 5 mm from the apex and examined under a confocal laser microscope. The percentage of sealer penetration and the maximum sealer penetration were measured. The CW and SC techniques produced similar tubule penetration at both the 1mm and the 5 mm level when compared with the tricalcium silicate sealers BC Sealer, QuickSet2 and NeoMTA Plus.

- **Alexander Pompermayer Jardine et al. (2016)**[33] compared the effect of QMix, BioPure MTAD, 17 % EDTA and saline on the penetrability of a resin-based sealer into dentinal tubules using a confocal laser scanning microscope (CLSM). They concluded that 17% EDTA and QMix promoted sealer penetration superior to that achieved by BioPure MTAD and saline.

- **Luigi Generali et al. (2017)**[34] compared the effect of conventional endodontic needle irrigation with other irrigant delivery and agitation systems on sealer penetration into dentinal tubules. The different cleansing system used were conventional endodontic needle irrigation, EndoActivator, Irrisafe, Self-Adjusting File, and EndoVac. After instrumentation, all teeth were filled by Thermafil obturators and rhodamine B dye labelled TopSeal sealer. Teeth were transversally sectioned at 2, 5 and 7mm levels from the apex and observed under confocal laser scanning microscope. Maximum, mean, and percentage of sealer penetration inside tubules around the root canal were measured. They concluded that sealer penetration into dentinal tubules is not affected by the irrigant delivery and agitation systems used in the study.

- **Ji Wook Jeong et al. (2017)**[35] investigated the depths of penetration of a calcium silicate–based sealer in dentinal tubules by using 3 different obturation methods. The obturation methods were C Point single cone (CPSC), gutta-percha single cone (GPSC), gutta-percha vertical condensation (GPVC). The roots of the teeth in each group were axially cross-sectioned, and the surfaces were examined under confocal laser scanning microscopy. They concluded that the pressure derived from hygroscopic expansion of C Point or warm vertical condensation did not enhance penetration depths of the calcium silicate–based sealer. Sealer penetration into the dentinal tubules occurred independent of the obturation technique.

- **Roula El Hachem et al. (2018)**[36] compared the effects of a conventional endodontic needle with an agitation system on a novel tricalcium silicate-based sealer (NTS) in terms of dentinal tubule penetration and interfacial adaptation to a root canal. Two different final cleansing systems were used, Conventional endodontic needle and Endo Activator. After instrumentation, all the teeth were filled with gutta-percha using single cone technique in conjunction with the novel tricalcium silicate-based sealer. Teeth were horizontally sectioned at 1 and 5 mm from the apex and were observed under a confocal laser scanning microscope (CLSM). They concluded that irrigant activation did not improve the novel tricalcium silicate-based sealer penetration into the dentinal tubules. However, the interfacial adaptation of the sealer was improved with Endo Activator.

- **Yemi Kim et al. (2019)**[37] compared the penetration ability of calcium silicate root canal sealers and conventional resin-based sealer using confocal laser scanning microscopy (CLSM). The specimens were randomly divided into three experimental groups. They concluded that the maximum sealer penetration was higher at the apical third in the AH Plus group compared with BioRoot RCS and Endoseal MTA, and similar sealer penetration was observed at the middle and coronal third when comparing between AH Plus and BioRoot RCS.

- **Zeliha Uğur Aydın et al. (2019)**[38] compared the effect of chitosan nanoparticle, QMix, and 17% EDTA on the penetrability of a calcium silicate-based sealer into dentinal tubules using a confocal laser scanning microscope (CLSM). Tooth in each group were filled with a TotalFill BC sealer with single gutta-percha cone and 0.1% rhodamine B. The specimens were horizontally sectioned at 3 and 5 mm from the apex, and the slices were analysed in CLSM (4×). They concluded that QMix and EDTA promoted sealer penetration superior to that achieved by chitosan nanoparticle.

- **Ayca Yilmaz et al. (2020)**[39] compared the efficacy of various techniques used for final irrigation on sealer penetration in the apical one-third of curved root canals. The experimental groups were based on the final irrigation protocols.

All teeth were obturated with gutta-percha and AH Plus sealer labelled with fluorescent dye. Transverse sections at 2 mm and 4 mm distance from the root apex were examined with the aid of confocal laser scanning microscopy. Total percentage (%) and maximum depth (µm) of sealer penetration was measured. They concluded that Passive Ultrasonic Irrigation, Sonic Irrigation and Manual Dynamic Activation did not significantly improve sealer penetration in the apical portion of curved root canals when compared to conventional needle irrigation.

- **Tushar Kanti Majumdar et al. (2021)[40]** assessed and evaluated the sealer penetration depth and interfacial adaptation of AH Plus, Apexit Plus, and GuttaFlow Bioseal sealer to root dentin**.** 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. Longitudinal sections were obtained for the rest half of the samples to gauge the difference of sealer adaptation using the scanning electron microscope. They concluded that at all root regions, 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.

- **Tufan Ozasir et al. (2021)[41]** evaluated the effects of different final irrigation regimens on the dentin tubule penetration of three different root canal sealers using confocal laser scanning microscopy (CLSM). Specimens were divided into five groups according to the solution used in the final rinse protocol. Specimens were sectioned and observed by CLSM to evaluate the percentage and maximum depth of sealer penetration at the apical, middle and coronal levels. They concluded that AH Plus showed the greatest tubule penetration while Tech BioSealer Endo showed the least. Resin-based sealers displayed deeper and more consistent penetration. CHX irrigation positively influenced sealer tubule penetration.

- **Dilara Koruk et al. (2022)[42]** compared the efficacy of 1-hydroxyethylidene-1, 1-diphosphonic acid (HEDP) and ethylenediaminetetraacetic acid (EDTA) by using various final irrigation techniques on penetration of sealer. One hundred mandibular premolars were selected. Final irrigation was performed with HEDP or EDTA by conventional syringe irrigation (CI), EndoActivator (EA), passive ultrasonic irrigation (PUI), photon-induced photoacoustic streaming (PIPS) and shock wave-enhanced emission photoacoustic streaming (SWEEPS) methods. After obturation of root canals with the EndoSequence BC Sealer, samples were evaluated using a confocal laser scanning microscope (CLSM), which enabled measurement of the maximum depth, percentage and penetration area. They concluded that HEDP and EDTA showed similar effects on the amount of penetrated sealer into the dentinal tubules and the PUI, PIPS and SWEEPS methods provided enhanced EndoSequence BC Sealer penetration compared with the Conventional syringe irrigation and EndoActivator methods.

- **Riccardo Tonini et al. (2022)[43]** investigated the effects of sonicated Thermafil (Dentsply, Tulsa Dental Specialties, Johnson City, TN, USA) on sealer penetration into the dentinal tubules. Thirty teeth with single round shaped root canals were used to compare Sonicated Thermafil with sonication, System B (EIE Analytical Technology, Orange, CE, USA), and Thermafil without sonication. A confocal laser scanning microscope (CLSM) was used to determine the depth, area and percentage of sealer penetration into the dentinal tubules. They concluded that Sonic activation can improve the carrier-based obturation technique due to deeper sealer penetration and thus aid in better retention of materials.

RELEVANCE

RELEVANCE

The goal of root canal obturation is to obtain a three-dimensional seal of the root canal system. Accomplishment of an ideal root canal treatment is attributed to various essential factors such as proper instrumentation, biomechanical preparation, obturation and post endodontic restoration. The access to areas such as isthmuses, ramifications, deltas, accessory and lateral canals is difficult and residual bacteria are most often located there, due to the communication of accessory canals with the periodontal membrane which is a potential pathway for bacteria.

Therefore, the penetration of sealers into these areas might play a role in the eradication of bacteria from the dentinal tubules. The analysis of the dentin/sealer interface can be done using Confocal Laser Scanning Microscope (CLSM). In comparison to conventional scanning electron microscope, CLSM provides the advantage of detailed information about the presence and distribution of sealers within the dentinal tubules at a relatively low magnification using fluorescent Rhodamine-marked sealers.

The aim of this study is to evaluate the percentage depth of penetration and maximum depth of penetration of two bioceramic sealers into dentinal tubules using three different sealer agitation techniques at two levels of the root canal, namely 3 mm and 6 mm from the apex, using CLSM.

MATERIALS AND METHODS

MATERIALS AND METHODS

REASEARCH APPROACH

Quantitative and Qualitative analysis

STUDY DESIGN

In Vitro Study

STUDY SETTING

Study will be conducted at:

1. St Gregorios Dental College, Chelad, Kothamangalam
2. Amrita Centre for Nanosciences and Molecular Medicine, Ponnekara P O, Kochi, Kerala

SAMPLE AND SAMPLE SIZE

- Sample size is calculated using statistical package G* Power (0.8459)
- The minimum sample size obtained, n=66
- 66 samples divided into 3 main Groups and each Group was divided into 2 Subgroups.

INCLUSION CRITERIA

- Mandibular second premolar
- Non carious tooth with complete root formation

EXCLUSION CRITERIA

- Immature teeth with open apex and other structural anomalies.
- Canal with moderately accentuated curvature.
- Calcifications in the pulp chamber
- Internal resorption
- Previously endodontically treated tooth
- Presence of fracture lines in the root

SAMPLING PROCEDURE

Freshly extracted human permanent mandibular second premolars with mature apex were collected. Teeth with single canals, free of cracks or caries or resorption or calcification, without previous endodontic treatment and with less than 10˚root curvature were selected. They were cleaned with ultrasonic scaler and pumice. They were then disinfected with 5.25% NaOCl and stored in 0.1% thymol solution. Specimens were subsequently assigned to 3 Groups and each Group was divided into 2 Subgroups (n = 11). Teeth were stratified to have similar averages of tooth dimensions in each Group so that the influence of size and shape variations on the results were minimized.

Figure 1: Tooth samples used in the study

STUDY GROUPS

The groups are as follows:

• Group 1A: Ultrasonics activation – Nishika Root Canal Sealer Bioactive Glass

• Group 1B: Ultrasonics activation – BioRoot RCS

• Group 2A: Lentulospiral activation - Nishika Root Canal Sealer Bioactive Glass

• Group 2B: Lentulospiral activation - BioRoot RCS

• Group 3A: Counter-clockwise rotary motion activation - Nishika Root Canal Sealer Bioactive Glass

• Group 3B: Counter-clockwise rotary motion activation - BioRoot RCS

Each Subgroup was evaluated at 2 levels under the CLSM at 3 mm and 6 mm from the apex.

ARMAMENTARIUM

Endodontic preparation:

Endodontic access: FG 1 Coarse 21 mm diamond Endo access bur.

Canal negotiation: #15 K-file 25 mm (Dentsply).

Canal instrumentation: Protaper Universal – SX, S1, F1, F2, F3.

Irrigants: 5.25% Sodium Hypochlorite, 17% EDTA solution.

Sealer activators: Protaper Rotary file F1, Ultrasonic Endodontic tips (ACTEON), Lentulospiral size 30.

Obturation: Gutta percha - single-cone size 30, 0.04 taper.

Sealers: Nishika Root Canal Sealer Bioactive Glass, BioRoot RCS

Figure 2: Armamentarium used

Figure 3: Armamentarium used

Figure 4: Ultrasonic activator

ROOT CANAL SEALERS

Nishika Root Canal Sealer Bioactive Glass (Nishika Nippon Shikha Yakuhin CO Ltd) and BioRoot RCS sealer (Septodont)

Figure 5: Sealers used in the study

METHODOLOGY

Shaping and cleaning of the root canal system:

For standardization of the root length at 10 mm, the crowns were resected with a water-cooled high-speed saw (ISOMET 5000) with constant water cooling.

Figure 6: ISOMET 5000 – Linear Precision Saw

The working length were determined with a # 15 K-file (Dentsply Maillefer) which was placed in the canal until it was just seen at the apical foramen. 0.5 mm was then subtracted from this length.

The canal was instrumented using Protaper Universal root canal files (Dentsply Maillefer) in a sequential manner from S1 to F3. The canals were irrigated in between with 2 ml of 5.25% NaOCl.

To eliminate smear layer, 2 ml of 17% ethylene diamine tetra acetic acid (EDTA) (pH 7.7) was used for 3 minutes, followed by a final rinse of 2 ml of distilled water. A 1ml tuberculin syringe was used to dispense 0.05 ml sealer within each canal. Each canal was then dried with absorbent paper points.

The roots were then randomly divided into 3 Groups based on the sealer activation technique:

G1: Ultrasonic (Woodpecker DTe-D5 Ultrasonic scaler, China)

G2: Lentulospiral (Dentsply, Maillefer)

G3: Counter-clockwise rotary motion (X smart, Dentsply, Maillefer, Ballaigues, Switzerland).

Each Group was further divided into two Subgroups based on the type of sealer used:

Subgroup A - Nishika Root Canal Sealer Bioactive Glass

Subgroup B - BioRoot RCS sealer.

Each sample in the Subgroup was then sectioned at two levels.

3 mm from apex- LEVEL 3 (L3)

6 mm from apex- LEVEL 6 (L6)

So, the Groups were then depicted as

Group 1A L3: Ultrasonics activation – Nishika Root Canal Sealer Bioactive Glass at Level 3

Group 1A L6: Ultrasonics activation – Nishika Root Canal Sealer Bioactive Glass at Level 6

Group 1B L3: Ultrasonics activation – BioRoot RCS at Level 3

Group 1B L6: Ultrasonics activation – BioRoot RCS at Level 6

Group 2A L3: Lentulospiral activation - Nishika Root Canal Sealer Bioactive Glass at Level 3

Group 2A L6: Lentulospiral activation - Nishika Root Canal Sealer Bioactive Glass at Level 6

Group 2B L3: Lentulospiral activation- BioRoot RCS at Level 3

Group 2B L6: Lentulospiral activation- BioRoot RCS at Level 6

Group 3A L3: Counter-clockwise rotary motion activation - Nishika Root Canal Sealer Bioactive Glass at Level 3

Group 3A L6: Counter-clockwise rotary motion activation- Nishika Root Canal Sealer Bioactive Glass at Level 6

Group 3B L3: Counter-clockwise rotary motion activation- BioRoot RCS at Level 3

Group 3B L6: Counter-clockwise rotary motion activation- BioRoot RCS at Level 6

The specimens were then kept in an incubator at 37˚C and 100% humidity for 2 days.

CONFOCAL LASER SCANNING MICROSCOPY (CLSM)

To calculate the percentage of sealer penetration around the root canal, each image was first imported into the Image J software and the circumference of the root canal was measured using its ruler tool.

Nishika Root Canal sealer Bioactive Glass and BioRoot RCS sealer was mixed according to the manufacturer's instructions and analysed under the CLSM.

Each sealer was mixed with Rhodamine B dye (Mayor Diagnostics, Mumbai, India) to an approximate concentration of 0.1% (by weight).

Figure 7: Rhodamine B Dye

Each root was sectioned at 90˚ to the long axis with a diamond disc. 1 mm sections were obtained at distances of 3 mm and 6 mm from the apex (Isomet 1000).

Figure 8: 1 mm serial sections

The Coronal surface of each section was polished with sand paper (Politriz, Arotec, Cotia, SP, Brazil). The dentin segments were examined with a CLSM (Olympus Fluoview FV 1000). The respective absorption and emission wave lengths for the Rhodamine B dye are 540 nm and 590 nm.

Dentin samples were analyzed using the 10X lens. Then areas around the canal walls, where the sealer penetrated into the dentinal tubules were outlined and measured using the same method. Subsequently, the percentage of root canal sealer penetration in that section was established.

To determine the maximum depth of penetration of the sealer, the point of deepest penetration was measured from the canal wall to the maximum depth of penetration into the dentinal tubule.

Figure 9: Confocal Laser Scanning Microscope

STATISTICS

STATISTICS

This study deals with testing whether there is any significant difference in the mean value of percentage depth of penetration and maximum depth of penetration among the sealers, NISHIKA ROOT CANAL SEALER BG and BIOROOT RCS. Kruskal-Wallis test is used for the analysis. In all the analysis, the significance level is taken to be 0.05 (i.e., if the p-value is less than 0.05, reject the null hypothesis or it can be concluded that the null hypothesis is statistically significant).

DESCRIPTIVE STATISTICS

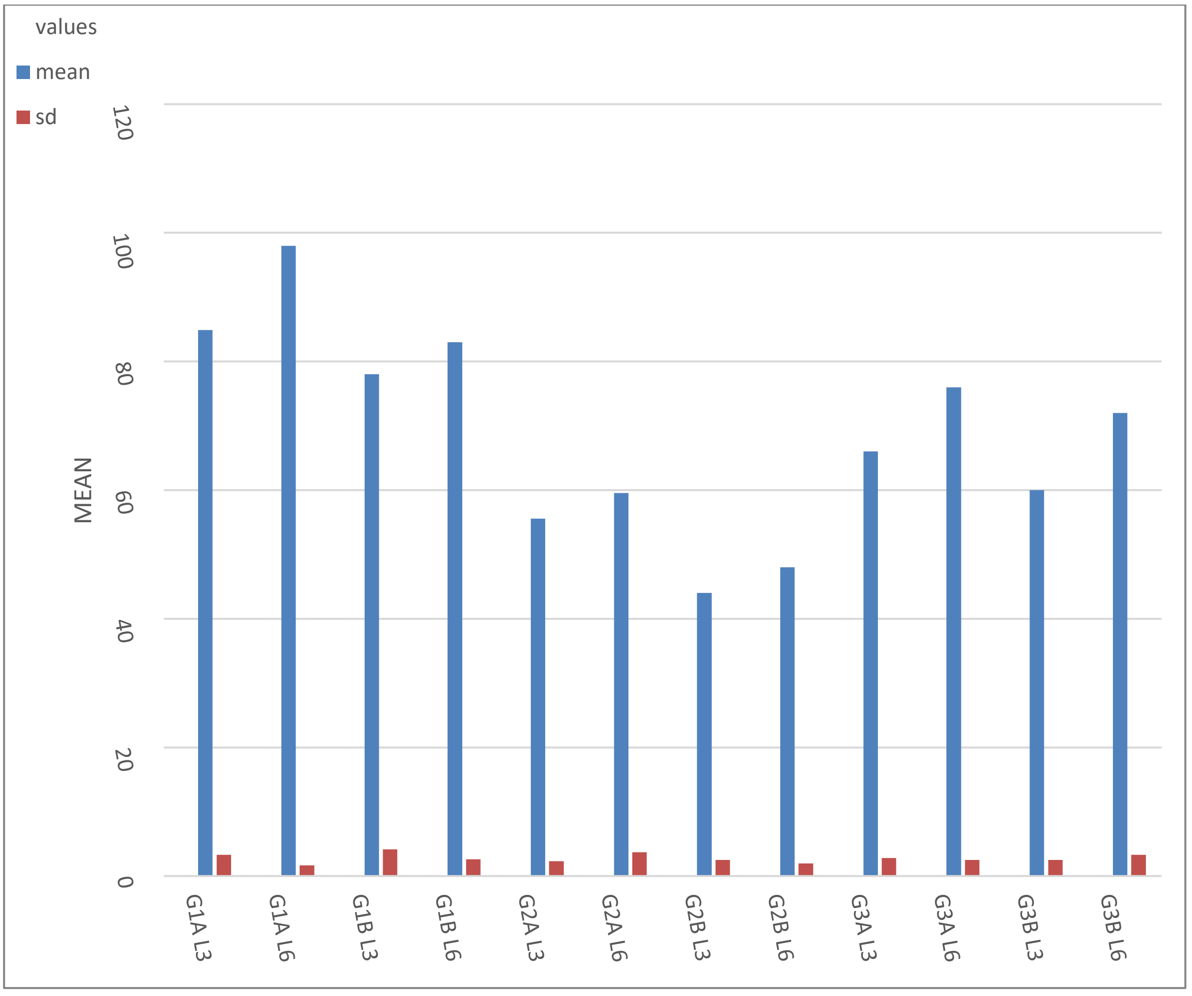
DESCRIPTIVE STATISTICS		**PERCENTAGE DEPTH OF PENETRATION**		**MAXIMUM DEPTH OF PENETRATION**	
GROUPS	N	MEAN	SD	MEAN	SD
G1A L3	11	84.91	3.300	1833.4345	123.98466
G1A L6	11	98.00	1.673	2029.0109	153.40969
G1B L3	11	78.00	4.171	766.0182	38.97784
G1B L6	11	83.00	2.608	1229.7964	59.18017
G2A L3	11	55.55	2.296	1266.3355	78.51102
G2A L6	11	59.55	3.725	1633.1809	83.03085
G2B L3	11	44.00	2.490	878.7991	74.38520
G2B L6	11	48.00	1.949	1308.9373	135.91073
G3A L3	11	66.00	2.828	605.2364	63.69333
G3A L6	11	76.00	2.490	947.4855	52.92390
G3B L3	11	60.00	2.490	1122.5064	77.34014
G3B L6	11	72.00	3.300	1325.3464	62.81722

TABLE 1: DESCRIPTIVE STATISTICS

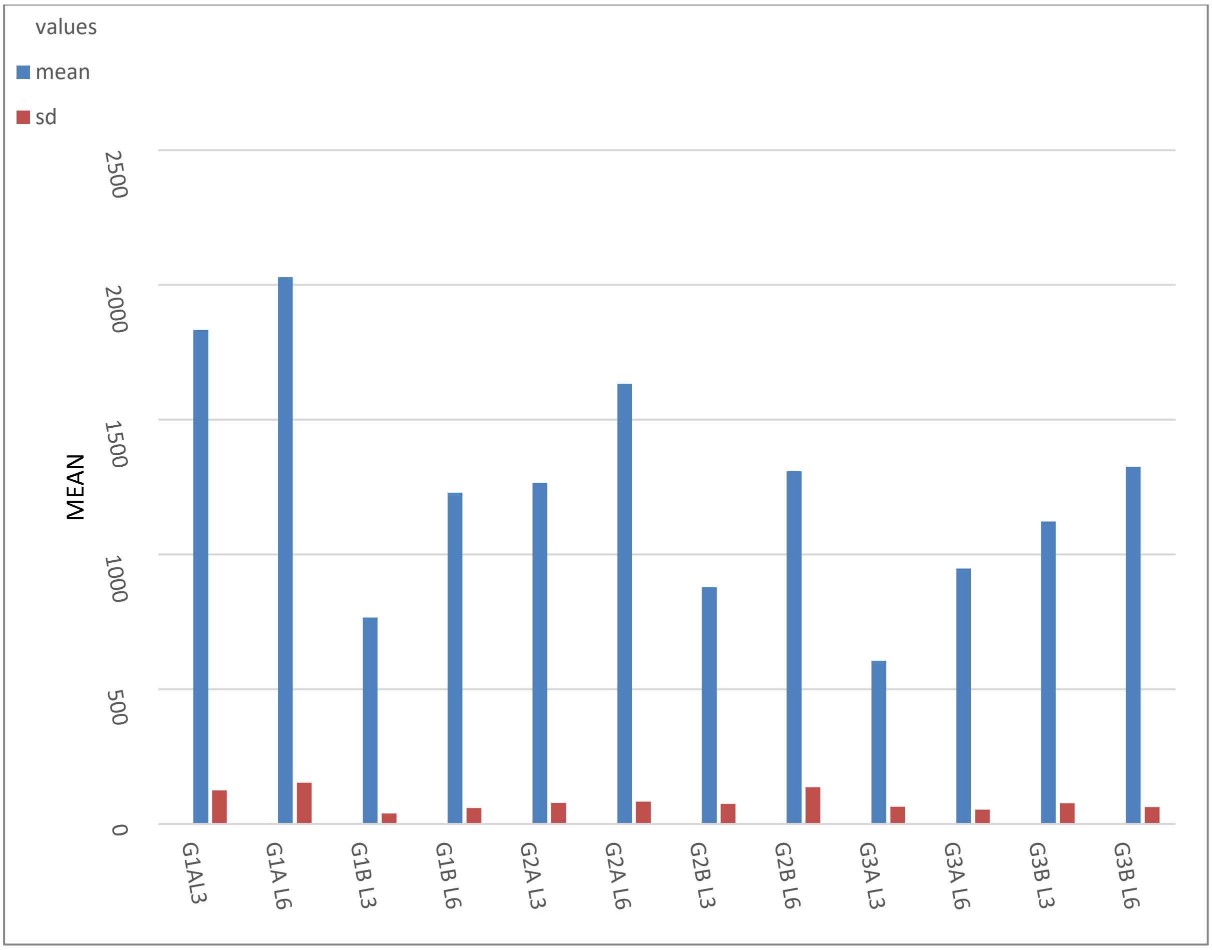
INFERENCE

From the table we can infer that G1A L6 showed the highest percentage of sealer penetration and maximum depth of sealer penetration amongst all the Groups and Subgroups, while Group 2B L3 showed the least percentage of sealer penetration and G3A L3 showed the least maximum depth of sealer penetration among all the Groups and Subgroups.

There is a statistically significant difference in the percentage of sealer penetration with $\chi^2(11) = 126.233$, and $\chi^2(11) = 124.079$ for maximum depth of sealer penetration with a p value of 0.000 between the different Groups and Subgroups as obtained by Kruskal Wallis test.



GRAPH 1: GRAPH FOR PERCENTAGE DEPTH OF PENETRATION



GRAPH 2: GRAPH OF MAXIMUM DEPTH OF PENETRATION

WILCOXON TEST FOR TESTING SIGNIFICANCE OF LEVEL 3 AND LEVEL 6 PERCENTAGE OF PENETRATION AND DEPTH OF PENETRATION

COMPARISON	PERCENTAGE DEPTH OF PENETRATION p value	DEPTH OF PENETRATION p value
G1A L 3 Vs G1A L6	0.003	0.008
G1B L 3 Vs G1B L6	0.028	0.003
G2A L3 Vs G2A L6	0.007	0.003
G2B L3 Vs G2B L6	0.011	0.003
G3A L3 Vs G3A L6	0.003	0.003
G3B L3 Vs G3B L6	0.003	0.003

TABLE 2: WILCOXON TEST FOR TESTING SIGNIFICANCE

INFERENCE

Since the overall p values was less than 0.05, all comparisons among the different Groups and Subgroups were significantly different at 5% level of significance by applying Wilcoxon signed ranks test.

MANN WHITNEY TEST FOR TESTING THE SIGNIFICANCE OF DIFFERENCE OF 3 AND 6 LEVELS OF DIFFERENT GROUP COMBINATIONS.

Intergroup comparison of depth and percentage of sealer penetration at Level 3 and Level 6:

G1A vs G1B	**G1A vs G3A**	**G1B vs G3A**	**G2A vs G3B**
G1A vs G2A	**G1A vs G3B**	**G1B vs G3B**	**G2B vs G3A**
G1A vs G2B	**G1B vs G2A**	**G2A vs G2B**	**G2B vs G3B**
G1A vs G3B	**G1B vs G2B**	**G2A vs G3A**	**G3A vs G3B**

TABLE 3: MANN WHITNEY TEST

The above-mentioned comparisons at Levels 3 and 6, corresponding to the depth and percentage of sealer penetration have p value less than 0.000. These comparisons are highly significant.

RESULTS

RESULTS

- Group 1A L6 showed the highest values for maximum depth of penetration (2029±153.40969) and percentage depth of penetration (98±1.673) among all the tested Groups and Subgroups in the study.
- Group 2B L3 (44±2.490) showed the least value for percentage depth of penetration and Group 3A L3 (605.23±63.693) for maximum depth of penetration among all the tested Groups and Subgroups.
- Among all the Groups/ Subgroups tested for sealer penetration and maximum depth of penetration, statistically significant increase in sealer penetration and maximum depth of penetration was observed at Level 6 (6 mm from apex) than Level 3 (3 mm from apex). This is also evident from the analysis by Wilcoxon signed rank test.
- In the Level 3 Group/ Subgroup, for percentage depth of penetration the highest to lowest is as follows: G1A > G1B > G3A > G3B > G2A > G2B.
- In the Level 3 Group/ Subgroup, for maximum depth of penetration the highest to lowest is as follows: G1A > G2A > G3B > G2B > G1B > G3A.
- In the Level 6 Group/ Subgroup, for percentage depth of penetration the highest to lowest is as follows: G1A > G1B > G3A > G3B > G2A > G2B.
- In the Level 6 Group/ Subgroup, for maximum depth of penetration the highest to lowest is as follows: G1A > G2A > G3B > G2B > G1B > G3A
- From Table 2, we see that the overall p values is less than 0.05. All comparisons among the different Groups and Subgroups were significantly different at 5% level of significance by applying Wilcoxon signed ranks test.
- From Table 3, we see that the p value is less than 0.000. These comparisons are highly significant.
- In Tables 4, 5 and 6, we observe that the Maximum depth and percentage of penetration was highest for Ultrasonic agitation irrespective of the sealer used when compared with all the Groups and Subgroups. Between the 2 sealers placed with Ultrasonic agitation technique, Nishika Root Canal Sealer Bioactive Glass showed maximum depth and percentage of penetration when compared with BioRoot RCS.

PERCENTAGE DEPTH OF PENETRATION INTO DENTINAL TUBULES (in %)

S NO	G1A L3	G1A L6	G1B L3	G1B L6	G2A L3	G2A L6	G2B L3	G2B L6	G3A L3	G3A L6	G3B L3	G3B L6
1.	83%	**100%**	81%	**81%**	54%	**63%**	45%	**49%**	67%	**77%**	61%	**73%**
2.	87%	**96%**	75%	**85%**	56%	**55%**	43%	**47%**	65%	**78%**	63%	**69%**
3.	88%	**97%**	82%	**80%**	53%	**57%**	41%	**50%**	63%	**77%**	62%	**70%**
4.	82%	**99%**	74%	**86%**	57%	**61%**	47%	**46%**	69%	**75%**	64%	**71%**
5.	81%	**100%**	79%	**87%**	59%	**66%**	45%	**45%**	64%	**80%**	59%	**74%**
6.	89%	**100%**	77%	**79%**	58%	**58%**	43%	**51%**	68%	**76%**	60%	**71%**
7.	90%	**96%**	83%	**81%**	52%	**54%**	42%	**47%**	67%	**73%**	59%	**75%**
8.	80%	**96%**	73%	**82%**	53%	**64%**	46%	**49%**	65%	**79%**	61%	**74%**
9.	84%	**97%**	72%	**84%**	57%	**60%**	44%	**46%**	61%	**72%**	57%	**72%**
10	86%	**99%**	84%	**85%**	55%	**58%**	40%	**50%**	71%	**74%**	58%	**70%**
11	85%	**98%**	78%	**83%**	57%	**59%**	48%	**48%**	66%	**75%**	56%	**73%**

TABLE 4: PERCENTAGE DEPTH OF PENETRATION INTO DENTINAL TUBULES (in %)

MAXIMUM DEPTH OF PENETRATION INTO DENTINAL TUBULES AT L3 (in µm)

S NO	G1A L3	G1B L3	G2A L3	G2B L3	G3A L3	G3B L3
1.	**1786.72 µm**	892.27µm	**767.36 µm**	510.11 µm	**1296.43 µm**	1249.32 µm
2.	**1895.62 µm**	886.72 µm	**745.82 µm**	613.24 µm	**1298.88 µm**	979.72 µm
3.	**1785.75 µm**	875.86 µm	**721.96 µm**	632.18 µm	**1262.65 µm**	1175.95 µm
4.	**1632.46 µm**	926.52 µm	**807.14 µm**	576.86 µm	**1147.83 µm**	1159.07 µm
5.	**1954.84 µm**	988.68 µm	**810.18 µm**	532.42 µm	**1320.51 µm**	1161.12 µm
6.	**1963.72 µm**	716.72 µm	**796.73 µm**	609.17 µm	**1211.83 µm**	1065.94 µm
7.	**1972.84 µm**	820.52 µm	**758.98 µm**	633.89 µm	**1306.74 µm**	1187.06 µm
8.	**1656.63 µm**	881.83 µm	**727.63 µm**	658.72 µm	**1292.61 µm**	1067.74 µm
9.	**1788.72 µm**	829.96 µm	**787.59 µm**	664.96 µm	**1107.81 µm**	1143.98 µm
10.	**1961.62 µm**	971.85 µm	**697.36 µm**	705.86 µm	**1361.54 µm**	1117.69 µm
11.	**1768.86 µm**	988.83 µm	**805.45 µm**	520.19 µm	**1322.86 µm**	1039.98 µm

TABLE 5: MAXIMUM DEPTH OF PENETRATION INTO DENTINAL TUBULES AT LEVEL 3 (in µm)

MAXIMUM DEPTH OF PENETRATION INTO DENTINAL TUBULES AT L6 (in µm)

S NO	G1A L6	G1B L6	G2A L6	G2B L6	G3A L6	G3B L6
1.	**1986.52 µm**	1282.86 µm	**1320.62 µm**	976.42 µm	**1656.72 µm**	1402.38 µm
2.	**2135.61 µm**	1386.51 µm	**1298.54 µm**	929.18 µm	**1727.64 µm**	1207.22 µm
3.	**1863.48 µm**	1275.18 µm	**1186.32 µm**	1020.1 µm	**1737.72 µm**	1389.26 µm
4.	**1993.72 µm**	1416.32 µm	**1170.52 µm**	863.98 µm	**1526.82 µm**	1274.34 µm
5.	**2211.98 µm**	1413.29 µm	**1256.41 µm**	872.63 µm	**1575.96 µm**	1348.72 µm
6.	**2168.58 µm**	1186.02 µm	**1263.72 µm**	997.42 µm	**1624.96 µm**	1304.87 µm
7.	**1972.63 µm**	1461.79 µm	**1188.69 µm**	962.41 µm	**1633.42 µm**	1389.56 µm
8.	**1958.94 µm**	1392.91 µm	**1300.18 µm**	988.72 µm	**1699.75 µm**	1279.04 µm
9.	**2103.62 µm**	1371.86 µm	**1205.23 µm**	885.13 µm	**1720.51 µm**	1375.71 µm
10.	**2204.86 µm**	1352.96 µm	**1175.42 µm**	953.41 µm	**1551.21 µm**	1270.75 µm
11.	**1719.18 µm**	1241.64 µm	**1162.11 µm**	972.94 µm	**1510.28 µm**	1336.96 µm

TABLE 6: MAXIMUM DEPTH OF PENETRATION INTO DENTINAL TUBULES AT LEVEL 6 (in µm)

OBSERVATIONS

OBSERVATIONS:

PERCENTAGE DEPTH AND MAXIMUM DEPTH OF PENETRATION G1A L3 vs G1B L3

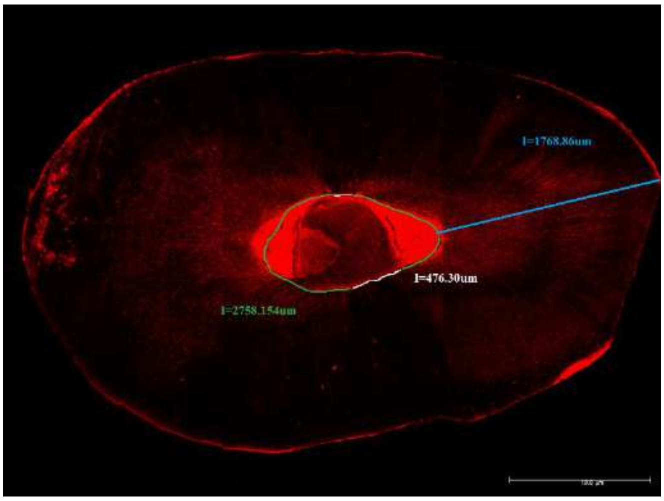


Figure 10: Percentage depth and maximum depth of penetration G1A L3

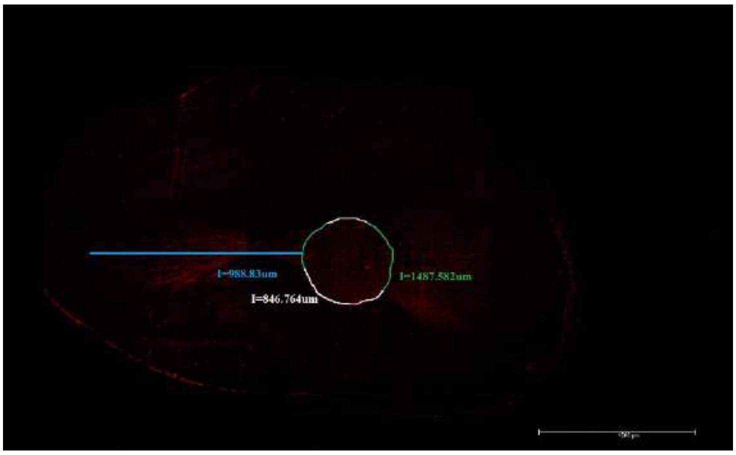


Figure 11: Percentage depth and maximum depth of penetration G1B L3

PERCENTAGE DEPTH AND MAXIMUM DEPTH OF PENETRATION G2A L3 vs G2B L3

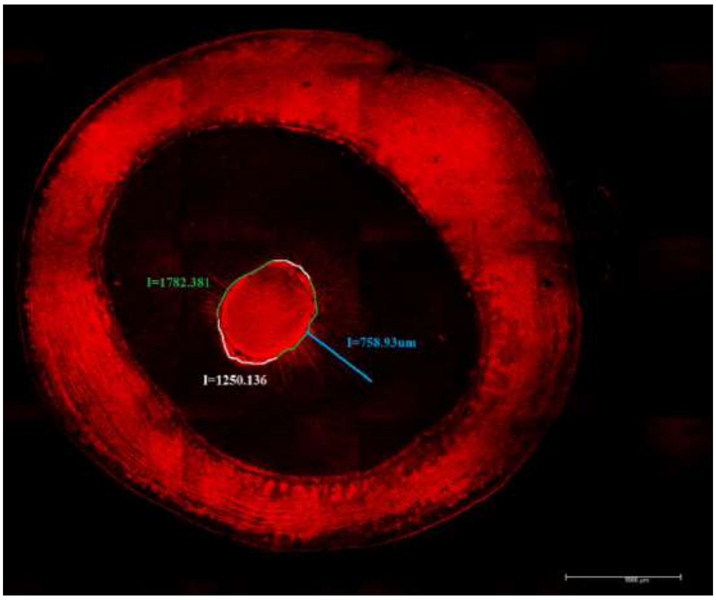


Figure 12: Percentage depth and maximum depth of penetration G2A L3

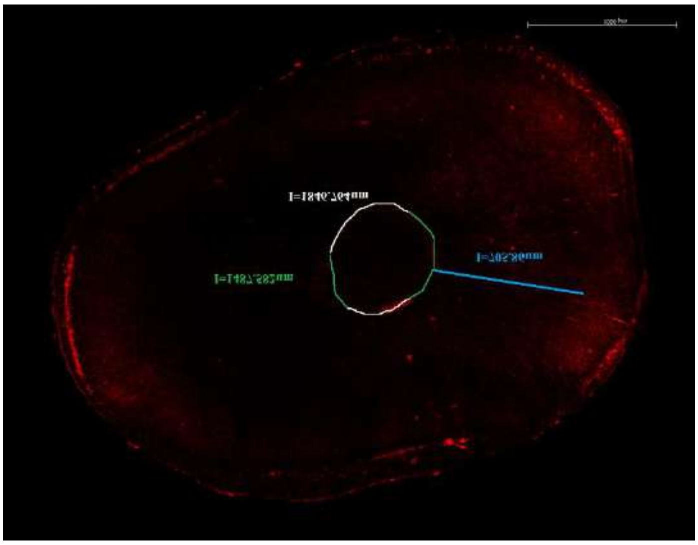


Figure 13: Percentage depth and maximum depth of penetration G2B L3

PERCENTAGE DEPTH AND MAXIMUM DEPTH OF PENETRATION G3AL3 vs G3BL3

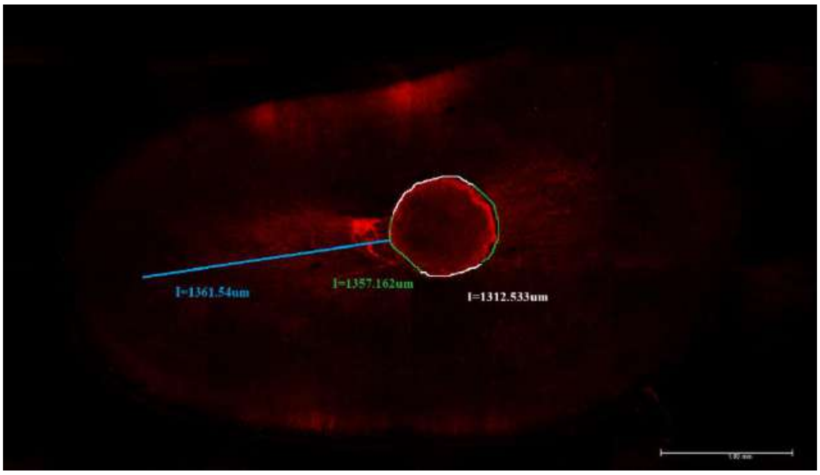


Figure 14: Percentage depth and maximum depth of penetration G3A L3

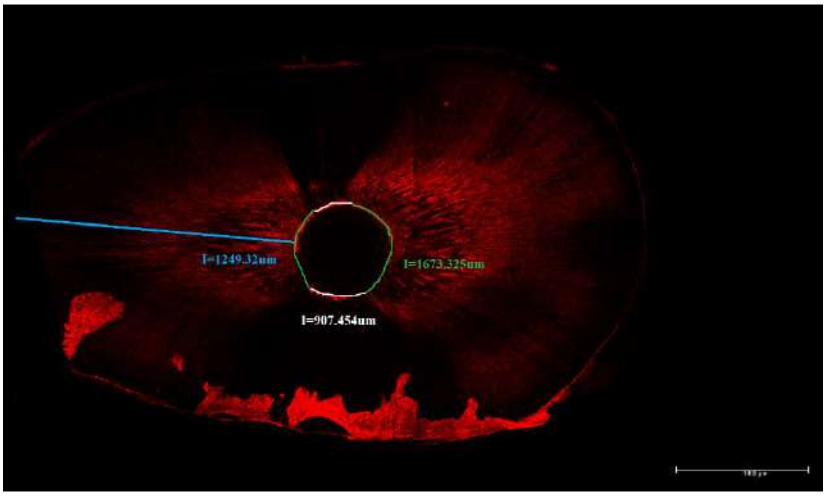


Figure 15: Percentage depth and maximum depth of penetration G3B L3

PERCENTAGE DEPTH AND MAXIMUM DEPTH OF PENETRATION G1AL6 vs G1BL6

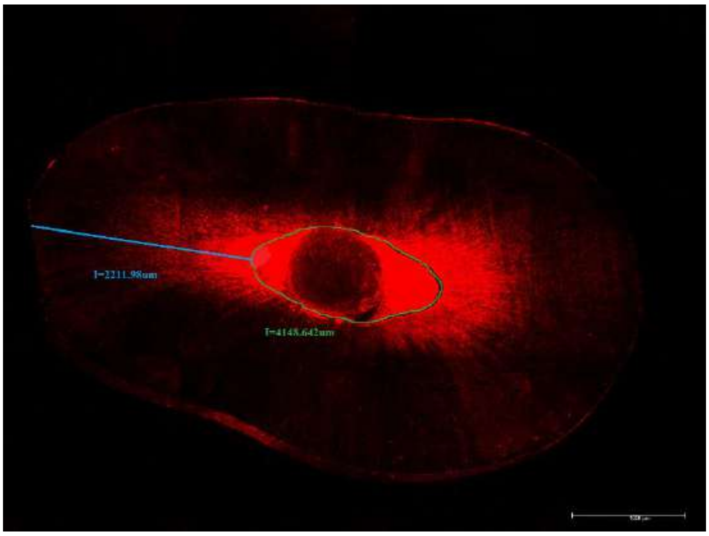


Figure 16: Percentage depth and maximum depth of penetration G1A L6

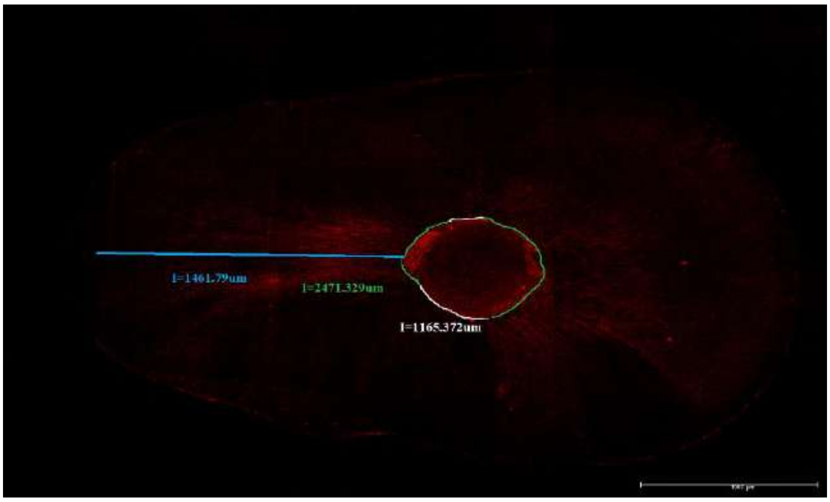


Figure 17: Percentage depth and maximum depth of penetration G1B L6

PERCENTAGE DEPTH AND MAXIMUM DEPTH OF PENETRATION G2AL6 vs G2BL6

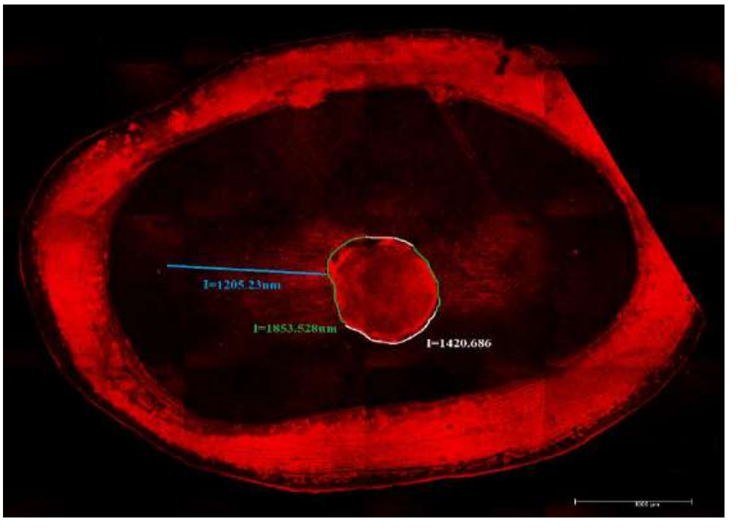


Figure 18: Percentage depth and maximum depth of penetration G2A L6

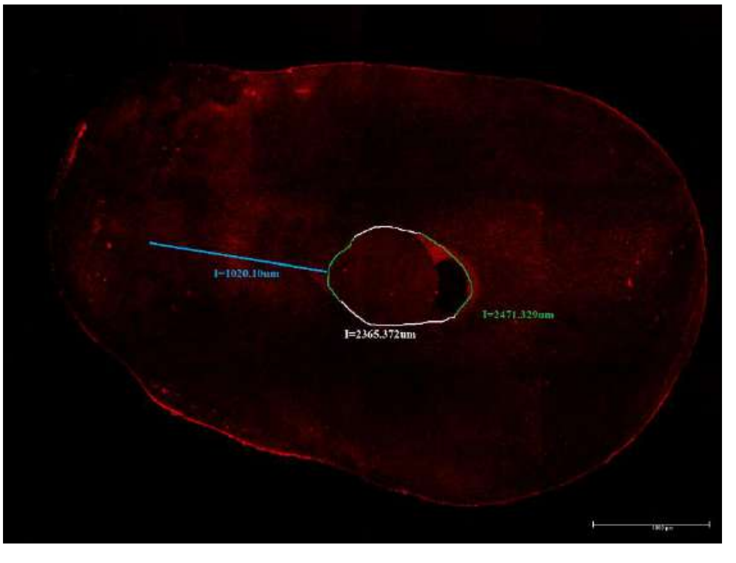


Figure 19: Percentage depth and maximum depth of penetration G2B L6

PERCENTAGE DEPTH AND MAXIMUM DEPTH OF PENETRATION G3AL6 vs G3BL6

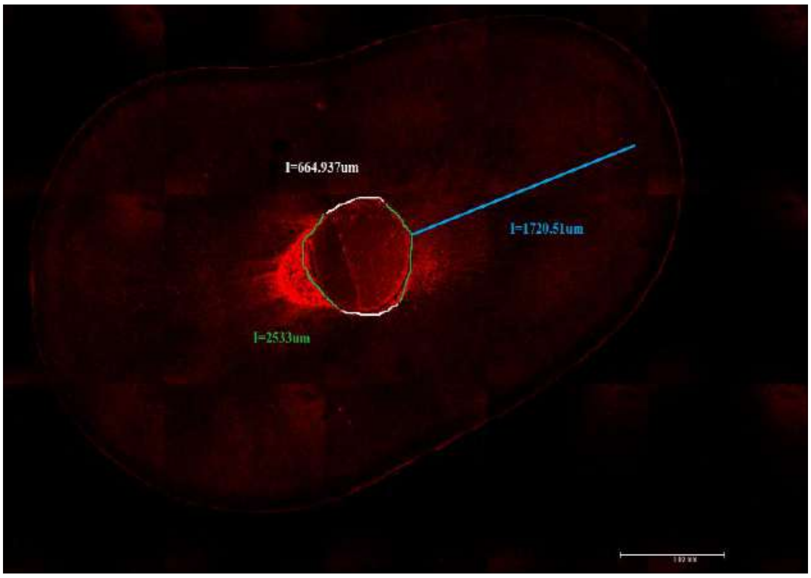


Figure 20: Percentage depth and maximum depth of penetration G3A L6

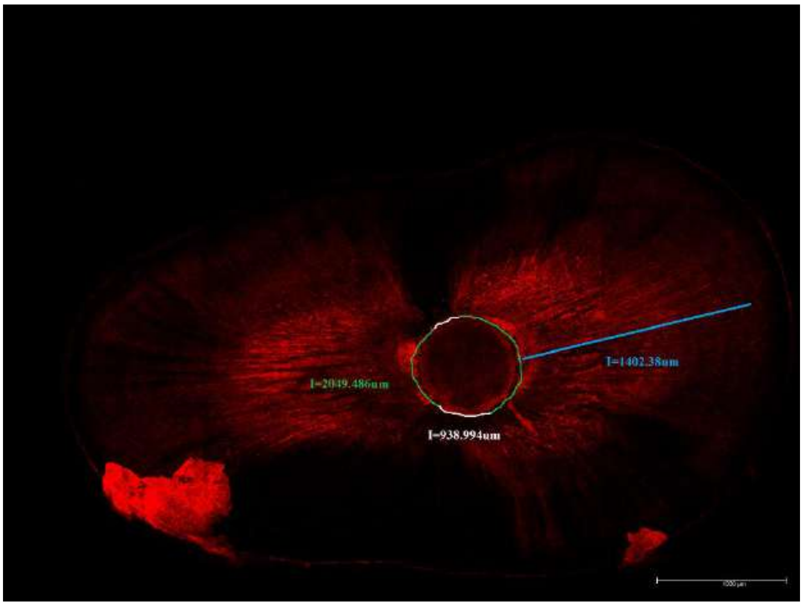


Figure 21: Percentage depth and maximum depth of penetration G3B L6

DISCUSSION

DISCUSSION

The major objective of root canal filling is to prevent microleakage between the oral cavity, the root canal system and the periradicular tissues, thus providing a hindrance against any re-infection.

Sealers are used to achieve an impervious seal between the core material and root canal walls. Sealer penetration into the dentinal tubules increases the interface between the obturating material and the dentin. The expansion in the interface improves the sealing ability of the obturation. The removal of the smear layer from the root canal walls is regarded as an essential step of root canal treatment.[44,45]

Sealers are necessary to seal the space between the dentinal wall and the obturating core interface. In addition, they often can penetrate areas such as lateral canals and dentinal tubules. This property is highly important because the penetration of sealer cements into dentinal tubules increases their surface contact with the root canal dentin thus improving the sealing ability. Sealer plugs inside the dentinal tubules allows a mechanical interlocking, improving the retention of the filling material. The penetration of sealers into the dentinal tubules may be biologically advantageous because laboratory studies have shown that endodontic sealers can exert antibacterial effects against bacteria in infected dentinal tubules.[46] Bacterial penetration into the dentinal tubules may reach 100-1,000 µm and it can be increased by the absence of smear layer.[47] Many species seen in the infection of the root canal have the propensity to penetrate deeply into the dentinal tubules, such as facultative and anaerobic species.[48-50] These microorganisms penetrate till the dentinal- cementum junction.[51] Sealer cements may entomb any residual bacteria in the tubules rendering them harmless. The sealer serves as a reasonable blocking agent that may prevent bacterial repopulation or inactivate them in the tubules. Further, it has been proposed that penetration of the sealer into the dentinal tubules may have a root strengthening effect due to filling of the voids.

Thus, the capability of a sealer to penetrate the dentinal tubules effectively may be one of the factors influencing the choice for selection of a sealer and sealer placement techniques during obturation. The apical 3 to 6 mm region of a root canal is a critical area for the placement of a sealer. It is important for successful obturation because it is in this area that accessory canals are most often found. Since accessory canals communicate with the periodontal membrane, they can form a periodontic-endodontic pathway for potential bacterial penetration to and from the periodontium.[52,53] Sealer placement technique performed using a combination of gutta-percha and a root canal sealer might not be adequate to provide an optimal seal.[54] Sealers which can penetrate into the dentinal tubules exert bactericidal effect by having a closer contact with the residual bacteria within the tubules. In addition, sealer plugs inside the dentinal tubules provide a mechanical interlocking, thereby improving the retention of the filling material and reducing the microleakage along the root canal walls. Thus, both percentage and depth of sealer penetration might influence the outcome or success rate of endodontic therapy.[31]

The smear layer is the organic and inorganic debris that is created after cavity preparation or root canal instrumentation and coats the dentin as well as clogs the orifice of the dentinal tubules. Theoretically, this layer is assumed to stop the penetration of disinfectants and root canal sealers into the dentinal tubules; therefore, its removal, by using agents such as EDTA, should be preferred for better adaptation of sealers.[28] The sealer penetration depth in the dentinal tubules depends on many factors like smear layer removal, dentinal permeability (the number and the diameter of tubules), root canal dimension, and the physical and chemical properties of the sealer. The flow is one of the main chemical or physical factors to influence the tubular penetration and is defined as the capability of a sealer to penetrate in irregularities, lateral canals, or dentinal tubules of the root canal system. The flow is determined by the consistency, particle size, shear rate, temperature, time, internal diameter of the root canal, and the rate of insertion.[22]

Dentinal tubules are smaller at the apex and larger toward the crown, and the tubule diameter at the pulpal wall is variable, ranging between 2 to 3.2 µm. To attain tubule penetration, the particle size of the material must be smaller than the tubule diameter; the larger the tubule, the deeper a particle can penetrate.[32] It is paramount that the percentage of the sealer/dentin inter-face that is covered by the sealer and the degree of tubule penetration by the sealer be as great as possible in all cases, whether previously infected or not.[20]

In this study comparison of the effect of different agitation techniques in percentage and depth of sealer penetration of two bioceramic sealers into root dentin was assessed at 2 different levels. The sealers used in this study were Nishika Root Canal Sealer Bioactive Glass (Nishika Nippon Shikha Yakuhin CO Ltd) and BioRoot RCS sealer (Septodont).

Nishika Root Canal Sealer Bioactive Glass was developed from BG-based biomaterials and originally intended for both dental pulp and bone regeneration therapies. CS-BG is a two-phased paste; Paste A consists of fatty acids, bismuth sub carbonate and silica dioxide, whereas Paste B consists of magnesium oxide, calcium silicate glass (a type of BG) and silica dioxide. By pushing the plunger of a double syringe, the two-phase paste can be dispensed at a 1:1 ratio. The dispensed paste can be mixed easily and quickly. The final pH of this material is optimal for the formation of HAP on the BG surface. In vitro and in vivo studies show that CS-BG has excellent biocompatibility with periapical tissue.

When a root canal was filled with CS-BG by the single-cone technique, the leakage was less than that observed for the CS-GB material applied by the lateral condensation technique. This showed the excellent Material sealing ability of CS-BG, especially when applied by the single cone method.[6]

BioRoot ™ RCS is an endodontic sealer based on tricalcium silicate material benefiting from both Active Biosilicate Technology and Biodentine™. The first provides medical grade level of purity and unlike “Portland cement” based materials, it ensures the purity of the calcium silicate content with the absence of any aluminate and calcium sulfate. BioRoot ™ RCS is a mineral based root canal sealer using tricalcium silicate setting system. The powder part additionally contains zirconium oxide as biocompatible radiopacifier and a hydrophilic biocompatible polymer for adhesion enhancing. The liquid part contains mainly water, calcium chloride as a setting modifier and a water reducing agent. BioRoot ™ RCS is bioactive by stimulating bone physiological process and mineralization of the dentinal structure. Thus, it creates a favourable environment for periapical healing and bioactive properties including biocompatibility, hydroxyapatite formation, mineralization of dentinal structure, alkaline pH and sealing properties.[55]

Sealer agitation or placement into the root canal system should be done in a manner which is predictable and completely covers the dentin walls. Accepted means of sealer placement include the use of endodontic files or reamers, lentulospirals, gutta-percha cones, paper points, and recently ultrasonic files.

In the ultrasonic system properties of irrigant activation, cavitation and acoustic streaming are apparently responsible for the enhanced cleaning of the root canal system. The same actions may be the reason for more thorough placement of a root canal sealer.[31]

Studies performed using Lateral Condensation technique, showed that different sealer placement or agitation techniques did not interfere with the quality of the filling. However, one of the main disadvantages of lateral compaction technique has been the lack of a 3-dimensional root canal sealing, especially in oval canals or irregular canals.[55] Lateral compaction shows contradictory results regarding the ability of sealers to penetrate dentinal tubules[14,17,19].

In Single Cone method, Sealer placement technique plays a important role to produce a three-dimensional root canal filling without any voids. Hence in the present study we have used Single cone obturation method with three commonly used sealer placement techniques.

Scanning electron microscopy (SEM), light microscopy, and confocal laser scanning microscopy (CLSM) have been used for the analysis of sealer penetration. In the present study, CLSM was used because this technique has several advantages over SEM. CLSM does not require any special specimen processing and observations can be made under near normal conditions. The preparation of samples for CLSM also tends to produce fewer artifacts than does sample preparation for SEM.[56] CLSM permits image acquisition from several optical sections, even from thick specimens, which are further reconstructed to achieve the final image. However, SEM permits the visualization of only one plane. Rhodamine B dye was used to promote the fluorescence of the sealer because CLSM works with high contrast points to identify the sealers within the dentinal tubules.[33] Another advantage when using CLSM in segments is that the sealer can be visualised at various depths, including the ability to control the depth of field, reduction of background information away from the focal plane.[57] Picoh et al. have reported that when using CLSM, artefacts can be practically excluded.[58]

As very few studies have been conducted on the effect of sealer activation and sealing ability of root canal sealers, three activation techniques (ultrasonics, lentulo spiral, and rotary counter-clockwise motion) were chosen to be studied in this study along with analysing the sealer penetration. The amount of sealer, extent of activating instrument and time for activation were standardized to minimize the errors.

Not many studies have been done regarding the sealer penetrability of Nishika Root

Canal Sealer Bioactive Glass. Hence in this study, Nishika Root Canal Sealer Bioactive Glass was chosen as one of the sealers to be studied and compared it with BioRoot RCS sealer in terms of dentinal tubule penetration at Level 3 (3 mm from apex) and Level 6 (6 mm from apex) following various sealer activation/placement methods.

In this study, Nishika Root Canal Sealer Bioactive Glass when placed with Ultrasonic agitation technique at 6 mm from apex (Group 1A L6) was seen to have the highest value for maximum depth of penetration (2029±153.40969) and percentage depth of penetration (98±1.673) among all the tested groups. This is evident in Figure 16, Graph 1 and Graph 2. This might be due to the smaller sealer particles which may result in a thinner film thickness and enhance dentinal tubules penetration. The film thickness of Nishika Root Canal Sealer Bioactive Glass is 27.9µm.[6] Bioceramic-based sealers were claimed to exhibit smaller particle size, greater fluidity, and hydrophilicity which allow them to form more sealer tags when in contact with the dentinal walls, resulting in greater sealer penetration and adaptation. Bioceramic-based sealers have also been discovered to show high hydraulic conductivity which can form tag-like structure 'mineral infiltration zone' and obstruct dentinal tubules, allowing for greater bond strength and tubular penetration.[64]

Nishika Root Canal Sealer Bioactive Glass showed the maximum percentage of depth of sealer penetration and maximum depth of penetration when compared to BioRoot RCS sealer at both level 3 and level 6 in almost all the studied groups. This was evident from Graph 1, Graph 2 and Table 1. This was attributed to the lesser particle size, greater fluidity and hydrophilicity of Nishika Root Canal Sealer Bioactive Glass compared to BioRoot RCS. This was appreciated in all the comparisons done in Figures 10 and 11, Figure 12 and 13, Figure 14 and 15, Figure 16 and 17, Figure 18 and 19 and Figure 20 and 21.

Irrespective of technique of activation and type of sealer, the depth and percentage of penetration of sealer was significantly better at the 6 mm level than 3 mm level. There was significant difference among the observations of the two levels as evident in Table 2. We can appreciate this finding in Figures 10 and 16, Figures 12 and 18, Figures 14 and 20 for Nishika Root Canal Sealer Bioactive Glass and Figures 11 and 17, Figure 13 and 19 and Figure 15 and 21 for BioRoot RCS. It is also evident from the graphs 1 and 2. These findings are similar to other studies[25,59,60] and could be because the number and diameter of dentinal tubules decreases on descending apically in the root canal and superior removal of smear layer. In addition, the apical dentin is irregular in direction and density; even some areas are devoid of dentinal tubule.

Ultrasonics for activation of sealers led to significantly more ($P < 0.001$) percentage and depth of both the sealers. This is evident in Figure 10 and 11 and Figure 16 and 17. This is also evident in Graphs 1 and 2. This is in accordance to study by Guimaraes BM et al.[59] The explanation for this is that the oscillating movement of ultrasonic files transmits the acoustic microstreaming energy and cause a greater depth of dentinal sealer penetration and coverage of root canal walls in the same manner as it enhances the penetration of irrigants in an area of anatomic complexities and the dentinal tubules. Significantly improved percentage of sealer penetration and depth of sealer penetration was observed in ultrasonic group, substantiating the findings of previous studies[61,62,63]. All these studies concluded that the use of ultrasonic method of sealer placement results in better sealer placement than other compared techniques. The ultrasonic and sonic energy apparently propels the relatively viscous sealer along the length of file to an appropriate depth [61] while lentulo spiral centrifugally pushes the sealer.

Studies have shown that the rotary lentulo spiral group will produce a better adaptation of the sealer onto the canal walls with even thickness which in turn leads to a better seal, but the results of this study did not correlate with their findings. Group 2B L3(44 ± 2.490) showed the least value for percentage depth of penetration among all the tested groups. This was evident in Graph 1. This may be attributed to several factors. First, increased amount of sealer was introduced into the canal as compared with other techniques, and as the sealer shrank during setting, more gaps and voids were formed that contributed to the highest value of microleakage. Second, the use of rotary lentulo spiral during sealer placement may force some air bubbles into the material that will lead to void formation and microleakage. This was evident in the Figures 12 and 13 and Figures 18 and 19.

Group 3A L3(605.23 ± 63.693) showed least value for maximum depth of penetration among all the tested groups. In counter-clockwise rotation with a file, the sealer tends to pool toward the tip of file but is not forced toward the walls. This might be the reason for decreased percentage depth of penetration and maximum depth of penetration while sealers are activated through counter clockwise rotary motion when comparing with the ultrasonic groups. This was appreciated in the figures 14 and 15 and figure 20 and 21.

In Tables 4, 5 and 6, we observe that the Maximum depth and percentage of penetration was highest for Ultrasonic agitation irrespective of the sealer used when compared with all the Groups and Subgroups. Between the 2 sealers placed with Ultrasonic agitation technique, Nishika Root Canal Sealer Bioactive Glass showed maximum depth and percentage of penetration when compared with BioRoot RCS.

Under the parameters of present study, it can be concluded that Nishika Root Canal Sealer Bioactive Glass sealer exhibited better percentage and depth of penetration in the radicular dentinal tubules irrespective of the level in the root canal than BioRoot RCS sealer. Ultrasonic agitation Technique significantly increased the percentage and depth of penetration of sealers in Root canal when compared with the other 2 methods.

CONCLUSION

CONCLUSION

Within the limitations of the present study, it can be concluded that:

- Nishika Root Canal Sealer Bioactive Glass sealer exhibited better percentage depth of penetration and maximum depth of penetration in the radicular dentinal tubules than BioRoot RCS sealer irrespective of the level in the root canal.
- Ultrasonic agitation technique of placement of sealers significantly increased the percentage and depth of penetration of sealers into the root canals when compared with lentulospiral and Counter clockwise rotary motion sealer agitation techniques.

SUMMARY

SUMMARY

The main objective of a root canal filling is to seal the root canal system to prevent reinfection. Normally, a root canal filling is associated with a hard core, like gutta-percha, and a sealer to better adapt the root canal filling material and complete the seal of the root canal filling in the most effectual manner. Therefore, the sealer root canal wall interface is crucial for the sealing of the root canal system. The sealer can fill the irregularities of the root canal wall and the dentinal tubules, which cannot be filled by gutta-percha. Sealer penetration into the tubules could affect the seal of the root filling because an increase of the contact surface between filling material and dentin is related to an improvement of the sealability. Also, sealer penetration can promote an antimicrobial effect in the tubules, which increases when in closer contact with the microbes.

This study is aimed to compare the effect of three root canal sealer activation techniques on percentage and depth of sealer penetration of NISHIKA ROOT CANAL SEALER BG and BIO ROOT RCS sealers at two levels of the root canal system.

Sixty-six teeth prepared till F3 ProTaper size were divided into three equal groups on the basis of sealer activation technique (G1: Ultrasonics, G2: Lentulo spiral, and G3: Counter-clockwise rotary motion). Each group was further divided into two equal subgroups on the basis of type of sealer used: Nishika Root Canal Sealer Bioactive Glass (Nishika Nippon Shikha Yakuhin CO Ltd) and BioRoot RCS sealer (Septodont) and obturated with gutta-percha. Horizontal sections at 3 and 6 mm from the apex were obtained and the percentage and depth of penetration of sealers into dentinal tubules were measured using confocal laser scanning microscopy (CLSM). Statistical analysis was performed utilizing Kruskal-Wallis and Mann-Whitney U tests with a significance level of 5%.

In this study, it was found that Nishika Root Canal Sealer Bioactive Glass sealer exhibited maximum percentage and depth of penetration into the radicular dentinal tubules than BioRoot RCS sealer irrespective of the level within the root canal.

As per this study, it was seen that Ultrasonic agitation technique of placement of sealers significantly increased the percentage and depth of penetration of sealers into the root canals when compared with lentulospiral and Counter clockwise rotary motion sealer agitation techniques.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Wang Y, Liu S, Dong Y. In vitro study of dentinal tubule penetration and filling quality of bioceramic sealer. PLoS One. 2018 Feb 1;13(2): e0192248.
2. Arikatla SK, Chalasani U, Mandava J, Yelisela RK. Interfacial adaptation and penetration depth of bioceramic endodontic sealers. Journal of conservative dentistry: JCD. 2018 Jul;21(4):373.
3. De Bem IA, de Oliveira RA, Weissheimer T, Bier CA, Só MV, da Rosa RA. Effect of ultrasonic activation of endodontic sealers on intratubular penetration and bond strength to root dentin. Journal of Endodontics. 2020 Sep 1;46(9):1302-8.
4. Pérez-Alfayate R, Algar-Pinilla J, Mercade M, Foschi F. Sonic activation improves bioceramic sealer's penetration into the tubular dentin of curved root canals: A confocal laser scanning microscopy investigation. Applied Sciences. 2021 Apr 26;11(9):3902.
5. Hegde V, Jain A, Mhadgut RP. Evaluation of percentage and depth of smartpaste bio versus AH plus sealer penetration using three different activation techniques: A confocal laser scanning microscopic study. Endodontology. 2017 Jul 1;29(2):130.
6. Washio A, Morotomi T, Yoshii S, Kitamura C. Bioactive glass-based endodontic sealer as a promising root canal filling material without semisolid core materials. Materials. 2019 Nov 29;12(23):3967.
7. White RR, Goldman M, Lin PS. The influence of the smeared layer upon dentinal tubule penetration by plastic filling materials. Journal of Endodontics. 1984 Dec 1;10(12):558-62.
8. Okşan T, Aktener BO, Şen BH, Tezel H. The penetration of root canal sealers into dentinai tubules. A scanning electron microscopic study. International Endodontic Journal. 1993 Sep;26(5):301-5.
9. Vassiliadis LP, Sklavounos SA, Stavrianos CK. Depth of penetration and appearance of Grossman sealer in the dentinal tubules: an in vivo study. Journal of endodontics. 1994 Aug 1;20(8):373-6

10. Şen BH, Pişkin B, Baran N. The effect of tubular penetration of root canal sealers on dye microleakage. International endodontic journal. 1996 Jan;29(1):23-8.
11. Kouvas V, Liolios E, Vassiliadis I, Parissis-Messimeris S, Boutsioukis A. Influence of smear layer on depth of penetration of three endodontic sealers: an SEM study. Dental Traumatology. 1998 Aug;14(4):191-5.
12. Çalt S, Serper A. Dentinal tubule penetration of root canal sealers after root canal dressing with calcium hydroxide. Journal of endodontics. 1999 Jun 1;25(6):431-3.
13. Davis MC, Walton RE, Rivera EM. Sealer distribution in coronal dentin. Journal of endodontics. 2002 Jun 1;28(6):464-6.
14. Sevimay S, Dalat D. Evaluation of penetration and adaptation of three different sealers: a SEM study. Journal of oral rehabilitation. 2003 Sep;30(9):951-5.
15. Kokkas AB, Boutsioukis AC, Vassiliadis LP, Stavrianos CK. The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. Journal of endodontics. 2004 Feb 1;30(2):100-2.
16. Weis MV, Parashos P, Messer HH. Effect of obturation technique on sealer cement thickness and dentinal tubule penetration. International Endodontic Journal. 2004 Oct;37(10):653-63.
17. De Deus GA, Gurgel-Filho ED, Maniglia-Ferreira C, Coulinho-Filho T. The influence of filling technique on depth of tubule penetration by root canal sealer: a study using light microscopy and digital image processing. Australian Endodontic Journal. 2004 Apr;30(1):23-8.
18. Patel DV, Sherriff M, Ford TP, Watson TF, Mannocci F. The penetration of RealSeal primer and Tubliseal into root canal dentinal tubules: a confocal microscopic study. International endodontic journal. 2007 Jan;40(1):67-71.
19. Mamootil K, Messer HH. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. International endodontic journal. 2007 Nov;40(11):873-81.

20. Ordinola-Zapata R, Bramante CM, Graeff MS, del Carpio Perochena A, Vivan RR, Camargo EJ, Garcia RB, Bernardineli N, Gutmann JL, de Moraes IG. Depth and percentage of penetration of endodontic sealers into dentinal tubules after root canal obturation using a lateral compaction technique: a confocal laser scanning microscopy study. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2009 Sep 1;108(3):450-7.
21. Moon YM, Shon WJ, Baek SH, Bae KS, Kum KY, Lee W. Effect of final irrigation regimen on sealer penetration in curved root canals. Journal of endodontics. 2010 Apr 1;36(4):732-6.
22. Balguerie E, van der Sluis L, Vallaeys K, Gurgel-Georgelin M, Diemer F. Sealer penetration and adaptation in the dentinal tubules: a scanning electron microscopic study. Journal of endodontics. 2011 Nov 1;37(11):1576-9.
23. Bird DC, Komabayashi T, Guo L, Opperman LA, Spears R. In vitro evaluation of dentinal tubule penetration and biomineralization ability of a new root-end filling material. Journal of Endodontics. 2012 Aug 1;38(8):1093-6.
24. Moon YM, Kim HC, Bae KS, Baek SH, Shon WJ, Lee W. Effect of laser-activated irrigation of 1320-nanometer Nd: YAG laser on sealer penetration in curved root canals. Journal of endodontics. 2012 Apr 1;38(4):531-5.
25. Chandra SS, Shankar P, Indira R. Depth of penetration of four resin sealers into radicular dentinal tubules: a confocal microscopic study. Journal of endodontics. 2012 Oct 1;38(10):1412-6.
26. Bolles JA, He J, Svoboda KK, Schneiderman E, Glickman GN. Comparison of Vibringe, EndoActivator, and needle irrigation on sealer penetration in extracted human teeth. Journal of endodontics. 2013 May 1;39(5):708-11.
27. Rouhani A, Ghoddusi J, Naghavi N, Al-Lawati G. Scanning electron microscopic evaluation of dentinal tubule penetration of Epiphany in severely curved root canals. European Journal of Dentistry. 2013 Oct;7(04):423-8.

28. Kuçi A, Alaçam T, Yavaş Ö, Ergul-Ulger Z, Kayaoglu G. Sealer penetration into dentinal tubules in the presence or absence of smear layer: a confocal laser scanning microscopic study. Journal of endodontics. 2014 Oct 1;40(10):1627-31.
29. Tuncer AK, Ünal B. Comparison of sealer penetration using the EndoVac irrigation system and conventional needle root canal irrigation. Journal of endodontics. 2014 May 1;40(5):613-7.
30. Tuncer AK. Effect of QMix 2in1 on sealer penetration into the dentinal tubules. Journal of endodontics. 2015 Feb 1;41(2):257-60.
31. Nikhil V, Bansal P, Sawani S. Effect of technique of sealer agitation on percentage and depth of MTA Fillapex sealer penetration: A comparative in-vitro study. Journal of conservative dentistry: JCD. 2015 Mar;18(2):119.
32. McMichael GE, Primus CM, Opperman LA. Dentinal tubule penetration of tricalcium silicate sealers. Journal of endodontics. 2016 Apr 1;42(4):632-6.
33. Jardine AP, Rosa RA, Santini MF, Wagner M, Só MV, Kuga MC, Pereira JR, Kopper PM. The effect of final irrigation on the penetrability of an epoxy resin-based sealer into dentinal tubules: a confocal microscopy study. Clinical oral investigations. 2016 Jan;20(1):117-23.
34. Generali L, Cavani F, Serena V, Pettenati C, Righi E, Bertoldi C. Effect of different irrigation systems on sealer penetration into dentinal tubules. Journal of endodontics. 2017 Apr 1;43(4):652-6.
35. Jeong JW, DeGraft-Johnson A, Dorn SO, Di Fiore PM. Dentinal tubule penetration of a calcium silicate–based root canal sealer with different obturation methods. Journal of endodontics. 2017 Apr 1;43(4):633-7.
36. El Hachem R, Le Brun G, Le Jeune B, Pellen F, Khalil I, Abboud M. Influence of the EndoActivator irrigation system on dentinal tubule penetration of a novel tricalcium silicate-based sealer. Dentistry Journal. 2018 Sep 3;6(3):45.
37. Kim Y, Kim BS, Kim YM, Lee D, Kim SY. The penetration ability of calcium silicate root canal sealers into dentinal tubules compared to

conventional resin-based sealer: a confocal laser scanning microscopy study. Materials. 2019 Feb 11;12(3):531

38. Aydın ZU, Özyürek T, Keskin B, Baran T. Effect of chitosan nanoparticle, QMix, and EDTA on TotalFill BC sealers' dentinal tubule penetration: a confocal laser scanning microscopy study. Odontology. 2019 Jan;107(1):64-71.
39. Yilmaz A, Yalcin TY, Helvacioglu-Yigit D. Effectiveness of various final irrigation techniques on sealer penetration in curved roots: a confocal laser scanning microscopy study. BioMed research international. 2020 Apr 14;2020.
40. Majumdar TK, Mukherjee S, Mazumdar P. Microscopic evaluation of sealer penetration and interfacial adaptation of three different endodontic sealers: An in vitro study. Journal of Conservative Dentistry: JCD. 2021 Sep;24(5):435.
41. Ozasir T, Eren B, Gulsahi K, Ungor M. The effect of different final irrigation regimens on the dentinal tubule penetration of three different root canal sealers: a confocal laser scanning microscopy study in vitro. Scanning. 2021 Oct 14;2021.
42. Koruk D, Basmacı F, Kırmızı D, Aksoy U. The impact of laser-activated and conventional irrigation techniques on sealer penetration into dentinal tubules. Photobiomodulation, Photomedicine, and Laser Surgery. 2022 Aug 1;40(8):565-72.
43. Tonini R, Salvadori M, Bartoli M, Francinelli J, Bertoletti P, Garo ML, Salgarello S. Carrier-Based Obturation: Effect of Sonication Technique on Sealer Penetration in Dentinal Tubules: A Confocal Laser Scanning Microscope Study. Applied Sciences. 2022 Sep 4;12(17):8877.
44. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implication of the smear layer in endodontics: A review. Oral Surg Oral Med Oral Pathol Oral Radio Endod 2002; 94:658-66.
45. Jhamb S, Nikhil V, Singh V. An in vitro study to determine the sealing ability of sealers with and without smear layer removal. J Conserv Dent 2009; 12:150-3

46. Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. Survival of Enterococcus faecalis in infected dentinal tubules after root canal filling with different root canal sealers in vitro. Int Endod J 2004; 37:193-8.
47. Haapasalo M, Ørstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987; 66:1375-9.
48. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev Oral Biol Med 2002; 13:171-83.
49. Love RM. Enterococcus faecalis—a mechanism for its role in endodontic failure. Int Endod J 2001; 34:399-405.
50. Love RM. Regional variation in root dentinal tubule infection by Streptococcus gordonii. J Endod 1996; 22:290-3.
51. Peters LB, Wesselink PR, Buijs JF, Van Winkelhoff AJ. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod 2001; 27:76-81.
52. Cohen S, Burns RC. Pathways of the Pulp. 5th ed. St Louis: Mosby-Year Book, Inc; 1991. p. 215-6.
53. Solomon C, Chalfin H, Kellert M, Weseley P. The endodontic-periodontal lesion: A rational approach to treatment. J Am Dent Assoc 1995; 126:473-9.
54. Hoen MM, LaBounty GL, Keller DL. Ultrasonic Endodontic sealer placement. J Endod 1988; 14:169-74.
55. Wu MK, Kast'akova A, Wesselink PR. Quality of cold and warm gutta-percha fillings in oval canals in mandibular premolars. Int Endod J 2001; 34:485-91.
56. Tuncer AK, Tuncer S. Effect of different final irrigation solutions on dentinal tubule penetration depth and percentage of root canal sealer. Journal of endodontics. 2012 Jun 1;38(6):860-3.
57. El Hachem R, Khalil I, Le Brun G, Pellen F, Le Jeune B, Daou M, El Osta N, Naaman A, Abboud M. Dentinal tubule penetration of AH Plus, BC Sealer and a novel tricalcium silicate sealer: a confocal laser scanning microscopy study. Clinical Oral Investigations. 2019 Apr;23(4):1871-6.

58. Picoh T, Stotz S, Buff E, Duschner H, Staehle HJ. Influence of different etching times on hybrid layer formation and tensile bond strength. Am J Dent 1998; 11:202–6.

59. Guimaraes BM, Amoroso-Silva PA, Alcalde MP, Marciano MA, de Andrade FB, Duarte MA. Influence of ultrasonic activation of 4 root canal sealers on the filling quality. J Endod 2014; 40:964-8.

60. Nikhil V, Singh R. Confocal laser scanning microscopic investigation of ultrasonic, sonic, and rotary sealer placement techniques. J Conserv Dent 2013; 16:294-9.

61. Hoen MM, LaBounty GL, Keller DL. Ultrasonic Endodontic sealer placement. J Endod 1988; 14:169-74.

62. West LA, LaBounty GL, Keller DL. Obturation quality utilizing ultrasonic cleaning and sealer placement followed by lateral condensation with gutta-percha. J Endod 1989; 15:507- 11.

63. Aguirre AM, el-Deeb ME, Aguirre R. The effect of ultrasonics on sealer distribution and sealing of root canals. J Endod 1997; 23:759-64.

64. Lin GS, Chan DZ, Leong JZ, Kan Z, Xuan WM, Tee V. Dentinal tubule penetration of bioceramic-based versus epoxy resin-based root canal sealers: a systematic review and meta-analysis. Giornale Italiano di Endodonzia. 2022 Jul 1;36(2).

ANNEXURE

ST. GREGORIOS DENTAL COLLEGE

UNDER THE MANAGEMENT OF MJSCE TRUST, PUTHENCRUZ

CHELAD , KOTHAMANGALAM, ERNAKULAM DIST, KERALA - 686681

SGDC/152/2021/4010

17/02/2021

ETHICAL CLEARANCE CERTIFICATE

To,

Dr. Jimmy George K
St. Gregorios Dental College
Chelad, Kothamangalam

Dear Dr. Jimmy George K

Subject: Ethics Committee Clearance-reg

Protocol: Effect of Sealer Agitation Techniques on The Percentage And Depth of Penetration of Two Types of Bioceramic Sealers: An In Vitro Study.

At the Institutional Ethics Committee (IEC) held on 15th of January 2021, this study was examined and discussed. After consideration, the committee has decided to approve and grant clearance for the aforementioned study.

The members who attended the meeting at which the protocol was discussed were:

1) Dr .C.K.K Nair - Former BARC Scientist
2) Dr.Cinu Thomas A - Scientist, Vice Principal, Caritas College of Pharmacy.
3) Dr. Lissy Jose – Former member of Women's welfare Association.
4) Adv. Jose Aranjani – Advocate.
5) Dr. Sauganth Paul – Reader, Department of Biochemistry, St. Gregorios Dental College.
6) Dr. Eapen Cherian – Secretary, Professor, St. Gregorios Dental College
7) Dr. Jain Mathew – Principal and Head of the Department, Department of Conservative Dentistry and Endodontics.
8) Dr. George Francis – Head of the Department, Department of Prosthodontics and Crown and Bridge.
9) Dr. Binoy Kurian – Head of the Department, Department of Orthodontics and Dentofacial Orthopaedics.

Dr. C.K.K Nair
Chairman Institutional Ethics Committee
St.Gregorios Dental College, Chelad.

Dr. Eapen Cherian
Secretary

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LIST OF ABBREVIATIONS
(In alphabetical order)

S NO	Abbreviations	Descriptions
1.	BG	BioActive Glass
2.	CHX	Chlorhexidine
3.	CLSM	Confocal Laser Scanning Microscope
4.	CS-BG	Nishika canal sealer bioactive glass
5.	EA	Endo activator
6.	EDTA	Ethylene Diamine Tetra Acetic Acid
7.	GFB	Gutta Flow Bioseal
8.	GP	Gutta percha
9.	RCS	Root Canal Sealer
10.	SEM	Scanning Electron Microscope
11.	UA	Ultrasonic Activation