INTRODUCTION

Radiotherapy is one of the primary treatments for head and neck cancers, used as both single and combined-modality.[1] Conventional radiation fractionation is the most widely used radiotherapy regimen because it allows healthy tissues to be exposed to a limited dose of radiation, minimizing the side effects.[1] But, it is known that the surrounding normal tissues are hardly ever preserved during head and neck radiotherapy.[2]

Patients with head and neck malignant neoplasms who will be subjected to radiotherapy treatment should preferably receive dental and endodontic treatment prior to radiation sessions in order to eliminate any form of disease present on the teeth and mucosa. During or after radiotherapy, the infection foci evolves more aggressively. Occasionally, the tumor growth is so rapid that it is not recommended to delay the radiation therapy due to pre-radiotherapy dental treatment. In these cases, endodontic treatment needs have to be addressed after radiation with proper root canal sealing, which is essential in oral health maintenance and to prevent side effects.[3]

'Radiation caries' is a complex and destructive multifactorial disease and is one of the main oral complications in patients undergoing head and neck region radiotherapy.[4] The risk of developing caries is increased substantially for their lifetime and not only during or immediately after treatment because radiation causes direct alterations in the enamel and dentine ultrastructure. Radiation can cause other important indirect effects, such as decreased salivary flow and secretion as well as changes in its composition.[5] Studies have demonstrated alterations in enamel and dentine crystalline structure,[6] chemical alterations in the tissues, morphological alterations of enamel and dentine structures, decrease of dentinal microhardness, decrease of bond strength to coronal dentine, root dentine and enamel and shearing fractures.[7] Considering the changes in dentine tissue, radio-xerostomia and radiation caries in patients subjected to head and neck radiotherapy, it is reasonable to assume that these individuals are more susceptible to develop pulpal alterations with higher chances of requiring endodontic treatment.[8]

The risk of developing osteoradionecrosis will accompany the irradiated patient throughout his entire life. The treatment is extremely complex. Teeth extractions must be avoided at all costs and all efforts must be made to prevent extractions. Consequently, endodontic treatment assumes an important role as an alternative treatment in this group of patients. The tooth must be filled with a material that is least irritating with a high sealing ability.[9]

The three-dimensional obturation of the root canal system is widely accepted as one of the major factors for the success of endodontic treatment. A wide variety of materials are available for root canal obturation. However, the gutta-percha cones along with the sealer is the most accepted material of choice. Different types of sealers have been used in conjunction with gutta-percha for root canal obturation. Due to the hydrophobic nature of gutta percha the sealer tends to pull away from the gutta-percha on setting. To overcome these drawbacks, new sealer systems have been introduced to enhance the sealing ability.[10]

The root canal sealer should be capable of creating an effective bond to the core material and to the dentin of the root canal in order to prevent microleakage at the interface.

According to Erickson, the penetration of root canal sealers into dentinal tubules is essential to achieve a good bond strength. The stability of the bond formed between the root dentin and gutta-percha interface reduces the failure associated with leakage of the material.[10]

Sealers may also exert an antibacterial effect and therefore their ability to penetrate into the dentinal tubules may be especially beneficial to control or kill bacteria that may be located there. Therefore, it is important that the percentage of the sealer/dentin interface that is covered by the sealer and the degree of tubule penetration by the sealer, be as maximum as possible in all cases, whether previously infected or not.[11]

Traditionally, endodontic sealers based on ZOE were used. However, the major disadvantage with these sealers were the poor sealing efficacy and bonding ability to the core material and canal wall. Various modifications have been made in the sealer chemistry and formulation to improve the penetration and bond strength of sealers.[12]

Epoxy resin-based sealers have shown good physiochemical properties as well as excellent apical sealing. AH Plus is an epoxy-bisphenol resin-based sealer that also contains adamantine and bonds to the root canal.[13] Resin-based conventional root canal sealers have been used, offering the advantages of reduced solubility, tight apical sealing and micro retention to the root dentin. AH Plus sealer is used in conjunction with gutta-percha (GP) in various root filling techniques.

Bioceramics are inorganic, nonmetallic, biocompatible materials that have mechanical properties similar to dental hard tissues. They are chemically stable, noncorrosive and interact well with organic tissue. Newer Bioceramic sealers possess very high bond strength with dentin walls by formation of hydroxyapatite crystals.[14] BioRoot RCS sealer is composed mainly of tricalcium silicate and zirconium oxide powder that must be mixed with a liquid containing calcium chloride. In recent studies comparing epoxy resin-based and calcium silicate sealers, BioRoot RCS showed excellent biocompatibility in both the fresh and set states.[15, 16, 17]

According to Martins et al. radiation therapy performed before endodontic treatment reduces marginal adaptation of the filling material to root dentin, regardless of the sealer type used, as it damages the dentin collagen fiber network.[18]

Increase in collagen fiber degradation by collagenase enzyme is seen in irradiated dentin. This can be modified by bioceramic sealer, which has the ability to form a mineral infiltrated zone, which improves the long-term stability of the exposed collagen fibers, thus increasing the bond strength.

Therefore, considering the radiation induced changes in the collagen fiber network in the intertubular, peritubular and intratubular dentin as well as the ability of bioceramic sealer to stabilize the collagen structure, it is important to evaluate the behaviour of different types of root canal sealers before root canal filling of teeth which have undergone radiation therapy.

The ability of any one particular sealer cement to penetrate dentinal tubules consistently and effectively and to produce less gaps between sealer and dentin will be one of the many factors which will influence the choice of material considered for root canal filling. It is therefore important to compare the penetrability and gap formation of different types of cements used. It is also important to validate the results from in vitro studies with findings from clinical cases.

AIM AND OBJECTIVES

AIM

To Evaluate the Influence of Therapeutic cancer Radiation on marginal adaptation of root canal sealer to dentin in teeth filled with AH-Plus, Sealapex and BioRoot RCS sealers.

OBJECTIVES

1) Quantitative and Qualitative Analysis of sealer dentin interface for sealer penetrability by Scanning Electron Micrography in irradiated and non-irradiated samples.
2) Qualitative and Quantitative Assessment of sealer dentin interface for gap formation by Scanning Electron Micrography in irradiated and non-irradiated samples.
3) Evaluate the difference in marginal adaptation of sealer in apical, middle and cervical one thirds of the root in irradiated and non-irradiated samples by scanning electron microscopy.
4) To compare the sealing ability of different sealer materials at different levels of root by scanning electron microscopy.

REVIEW OF LITERATURE & BACKGROUND

- **Jervoe et al[6] (1970)** conducted a study on mature human premolar and molar to determine the changes in the crystalline structure of enamel and dentine after experimental and in situ radiation. The X-ray diffraction investigation demonstrates that experimental radiation with 1 M-rad, in both single doses as cumulatively, of mature human teeth as well as in situ radiation with 12.000 R induces changes in the crystalline structure of human enamel and dentine. The results of the study showed that experimental radiation of dentine requires a 100 times greater dose to obtain the same crystalline change as the in situ irradiated molar dentine.[6]

- **Hutton et al[18] (1974)** conducted an invitro study in which, the maxillary arches of two monkeys were irradiated with cobalt-60 in therapeutic doses ranging from 3,000 to 7,013 rads. Study demonstrated No histologic differences in the dental tissues due to radiation at any dose levels. Study concluded that Cobalt-60 radiation in a dose range up to 7,013 rads had no demonstrable adverse effect on the dental pulps of mature permanent teeth of monkeys.[18]

- **Carrigen et al[19] (1984)** conducted a scanning electron microscopic examination of human dentinal tubules, according to the age of the subject and specific area of the tooth from which the specimen was obtained. The results showed that the number of dentinal tubules decreased with increasing age (e.g. the mean number of tubules = 242,775 for age group 20 to 34; 149,025 for age group 80 and above), and apical location (e.g. the mean number of tubules = 265,460 for coronal dentin; 49,140 for apical root dentin). These results explain the marked sensitivity and increased bacterial penetration of coronal dentin when compared with the minimal bacterial and irritant penetration of apical dentin.[19]

- **Seto et al[20] (1985)** studied the outcome of endodontic therapy in 16 patients irradiated for head and neck cancer. Thirty-five post radiation endodontically treated teeth (54 roots) were included in the study. The follow-up period ranged from 6 months to 54 months. No osteoradionecrosis was seen in association with teeth that had been endodontically treated. Results shows that endodontic therapy is a viable method of treating diseased teeth in patients irradiated for oral neoplasms.[20]

- **Anneroth et al[21] (1985)** conducted a study on 54 teeth from 20 patients irradiated for treatment of malignant tumors of the head and neck region. The samples were examined clinically, histologically and rnicro radiographically. 18 teeth from 8 patients were used as controls. A significantly higher degree of hyposialia, radiation mucositis and atypical caries was found in the irradiated group as compared to the control material. Occurrence of narrow gaps between dentin and cementum was also higher in the former group as compared with the latter.[21]

- **Barnet et al[22] (1989)** conducted a study to determine the sealing ability of two calcium hydroxide-containing root canal sealers, CRCS and Sealapex. Leakage was demonstrated by the penetration of India ink and was evaluated using a stereomicroscope. Study concluded that Significantly less leakage occurred with both calcium hydroxide-containing sealers than with the traditional zinc oxide-eugenol sealer.[22]

- **Grotz et al[23] (1997)** conducted a systematic study, comparing teeth with radiation caries, clinically caries free teeth, with tooth specimens after an experimental enoral (in situ) irradiation and after in vitro irradiation. 60 Co was the irradiation source. Sound teeth were used as a standard Results showed that Tooth samples from radiotherapy patients (cancer therapeutic doses, long interval before extraction; group 1) showed three characteristic changes: 1, rarefaction of the branching (ramification) of odontoblastic processes near the junction. 2, dentine tubules end in front of the interface to the hard tissue and 3, in dentine the interface is characterized by a zone (about 10 microns wide) of low intensity of the remitted light. Study concluded that the obliteration of the dentine tubules, preceded by a degeneration of the odontoblastic processes, is obviously the result of a direct radiogenic cell damage with hampered vascularization and metabolism particularly in the area of the terminations of the odontoblastic processes. The deficit in metabolism combined with a latent damage of the parenchyma (hypo-remitting zone) is the evidence for the functional symptoms (subsurface caries).[23]

- **Lee et al[10] (2002)**, in vitro study, four classes of endodontic sealers (Kerr, a ZOE-based sealer; Sealapex, a calcium hydroxide-based sealer; AH 26, an epoxy resin-based system; and Ketac-Endo, a glass-ionomer based sealer) were compared for their ability to bond to dentin or gutta-percha. Results showed that AH 26 gave the significantly highest bonds to gutta-percha.[10]

- **Saleh et al[11] (2003)** conducted a study in which the microscopic details of the debonded interfaces between endodontic sealers and dentin or gutta-percha were assessed Using Grossman's sealer, Apexit, Ketac-Endo, AH Plus, RoekoSeal Automix, or RoekoSeal Automix with an experimental primer. Grossman's sealer, RoekoSeal Automix with an experimental primer, AH Plus / EDTA sealers penetrated into the dentinal tubules when the dentin surface had been pretreated with acids. The study concluded that penetration of the endodontic sealers into the dentinal tubules when the smear layer was removed was not associated with higher bond strength.[11]

- **Weis et al[24] (2004)** compared the average sealer cement film thickness and the extent and pattern of sealer penetration into dentinal tubules in association with four obturation techniques (SimpliFill, continuous wave, Thermafil and 0.04 matched taper (master cones) lateral compaction obturation groups) in curved root canals. Average sealer cement thickness (measured at 10 points around the canal wall), depth of dentinal tubule penetration and frequency of voids were determined at the 1, 3 and 5 mm levels. Results shows that Thermafil demonstrated superior GP adaptation at all levels. Followed by lateral compaction, continuous wave and SimpliFill. SimpliFill also demonstrated the highest frequency of voids. Sealer cement penetrated dentinal tubules as far as the outer one-third of dentine, with greater penetration observed buccally or lingually. Penetration was not significantly affected by obturation technique, but on average was deeper and more frequent at the 3 and 5 mm levels than at the 1 mm level. From the results they concluded that sealer thickness was strongly dependent on obturation technique. Consistent, extensive sealer penetration into dentinal tubules was seen and was unrelated to the obturation technique.[24]

- **Ordinola et al[25] (2009)** conducted a study to compare 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. For the study thirty root canals filled with the lateral compaction technique using GuttaFlow (n = 10), Sealapex (n = 10), or Sealer 26 (n = 10) were analyzed using confocal microscopy. The teeth were sectioned at 3 and 5 mm from the apex. Results showed that Sealapex showed the deepest sealer penetration at both levels evaluated. The study concluded that although Sealapex displayed deeper penetration into the dentinal tubules there was no difference in the percentage of adaptation to the root canal walls among the 3 sealers evaluated.[25]

- **Zhang et al[26] (2009)** investigated the apical sealing ability of a newly introduced bioceramic root canal sealer. Sixty-eight extracted human anterior single-root teeth were used. The coronal part of each tooth was removed and the root canals were prepared with protaper files. The specimens were divided into 3 groups of 20 teeth each. Group A specimens were filled with iRoot SP using the continuous wave condensation technique; Group B specimens were obturated with iRoot SP using a single cone technique; Group C specimens were filled with AH plus by means of the continuous wave condensation technique. Evaluation of the apical leakage was performed with a fluid filtration method at 24 hours and 1, 4, and 8 weeks. Scanning electron microscopy (SEM) was used to qualitatively assess what mechanisms might be responsible for leakage of the different group. There was no significant difference in fluid leakage among the groups, as well as no time effect on leakage ($P > .05$). SEM revealed both gap free regions and gap-containing regions in canals filled with both materials. From the results the study concluded that iRoot SP was equivalent to AH Plus sealer in apical sealing ability.[26]

- **Rosales et al[27] (2009)** conducted a study to analyze the dental needs in 357 patients who received radiotherapy in the head and neck region. Study showed that dental examination before radiotherapy was not performed in 148 patients (41.5%) and was done in 209 patients (58.5%). From the total of examined patients, 94 (45%) did not require dental procedures at the moment of examination, while 115 (55%) presented some sort of dental need. Following the patients after the radiotherapy, it was observed that the group of patients that was evaluated before radiation presented less need of restorations, root canal filling and dental extractions than those who were not evaluated. The results of this study confirm that the evaluation of oral conditions prior to radiotherapy is essential to minimize the dental needs, emphasizing the importance of the dentist in the multidisciplinary team that treats cancer patients.[27]

- **Soares et al[28] (2010)** conducted a study to evaluate if the tubule and prism orientation, location, and irradiation have an effect on the ultimate tensile strength of dental structures. 20 human third molars subjected to 60 Gy of gamma irradiation, in daily increments of 2 Gy. The specimens were evaluated by microtensile testing. Results showed that irradiation treatment significantly decreased the UTS of coronal and radicular dentin and of enamel, regardless of tubule or prism orientation. With or without irradiation, enamel was significantly stronger when tested parallel to its prismatic orientation. Coronal and radicular dentin of non-irradiated specimens presented significantly higher UTS when tested perpendicularly to tubule orientation. Study also showed that when the teeth were irradiated, the influence of tubule orientation disappeared, demonstrating that irradiation is more harmful to organic components.[28]

- **Steier et al[29] (2010)** conducted a study to compare the interface dentin-sealer of two sealers (RealSeal and AH Plus) using two magnifications under Scanning Electron Microscope (SEM). Results shows that RealSeal produced less gaps than AH Plus. It could be assumed that 150x is good enough to show defects in the interface dentin-sealer.[29]

- **Naves et al[30] (2012)** evaluated the effect of gamma radiation on the microtensile bond strength of resin-based composite restoration to human enamel and dentin performed either before or after radiotherapy. Radiation therapy was defined by application of 60-Gy dose fractionally with daily exposures of 2 Gy, 5 days a week, over 6 weeks. Restorations were carried out using Adper Single Bond adhesive system and Filtek Z250 resin composite. It was observed that Bond strength to enamel was significantly higher than to dentin irrespective radiation therapy. Radiotherapy applied before restoration significantly reduced the bond strength to both substrates. A predominance of adhesive failures was detected for control groups and groups restored before radiotherapy. Cohesive failures in dentin and enamel increased when the specimens were restored after irradiation. From the results it was concluded that the gamma radiation had a significant detrimental effect on bond strength to human enamel and dentin when the adhesive restorative procedure was carried out after radiotherapy.[30]

- **Chandra et al[31] (2012)** had done a study to evaluate the depth of penetration of 4 different endodontic resin sealers into the radicular dentinal tubules with the aid of confocal microscopy. Eighty single-rooted teeth were instrumented and divided into 4 groups composed of 20 teeth each. The samples were obturated with AH Plus, RealSeal, EndoRez, and RoekoSeal resin sealers, respectively. The core material in all the groups was Resilon. The teeth were sectioned at the coronal, middle, and apical thirds and viewed under confocal microscope to determine the depth of penetration of the sealer into the dentinal tubules. The results showed that the maximum penetration was exhibited by RealSeal resin sealer, followed by AH Plus, RoekoSeal, and EndoRez. The coronal third showed the maximum penetration, followed by middle third and least at the apical third.[31]

- **Goncalves et al[5] (2014)** in a study evaluated, in vitro, the mechanical and micro-morphological properties of enamel and dentin of permanent teeth after ionizing radiation. Enamel and dentin microhardness were evaluated at three depths (superficial, middle and deep) prior to (control) and after every 10Gy radiation dose up to a cumulative dose of 60Gy by means of longitudinal microhardness. Enamel and dentin morphology were assessed by scanning electron microscopy (SEM). Study concluded that, Dentin microhardness decreased after the radiation doses compared with the control, with the greatest reduction of microhardness in the middle area. More evident interprismatic portion, presence of fissures and obliterated dentinal tubules, and progressive fragmentation of the collagen fibers was more evident with the increase of the radiation doses. This study shows that irradiation affects microhardness and micro-morphology of enamel and dentin of permanent teeth. The effects of gamma irradiation on dental substrate might contribute to increased risk of radiation tooth decay associated with salivary changes, and micro biota shift .[5]

- **Sequira Mallara et al[32] (2014)** conducted a study to evaluate the effects of therapeutic radiation on deciduous teeth. For the study, the enamel and dentin microhardness were evaluated at 3 depths, both before and after each 10 Gy of irradiation and up to a dose of 60 Gy. The morphology was evaluated by scanning electron microscopy (SEM). Results shows that there was a significant difference in the microhardness of non irradiated and irradiated dentin that was irradiated with doses of 10 Gy, 20 Gy, 30 Gy, and 40 Gy. There was no difference was noted between nonirradiated dentin and dentin irradiated with 60 Gy. From the results they concluded that the enamel microhardness increased at a dose of 60 Gy, whereas the value of the dentin microhardness did not change. Increased radiation dose resulted in a progressive disruption of enamel and dentin morphology.[32]

- **Pawwar et al[33] (2014)** an *in vitro* study evaluated and compared the microleakage of three sealers; Endosequence bioceramic (BC) sealer, AH Plus and Epiphany. Microleakage was evaluated using dye penetration method which was done under stereomicroscope (30X magnification).The dye penetration in Group B was more than in Group A and C in both vertical and horizontal directions, suggesting that newly introduced BC sealer and Epiphany sealer sealed the root canal better compared to AH Plus Sealer. It was concluded that newer root canal sealers seal the root canal better but cannot totally eliminate leakage.[33]

- **Reed et al[34] (2015)** conducted a study to understand radiotherapy-induced dental lesions characterized by enamel loss or delamination near the dentine–enamel junction (DEJ), this study evaluated enamel and dentine Nano-mechanical properties and chemical composition before and after simulated oral cancer radiotherapy. The study concluded that simulated radiotherapy produced an increase in the stiffness of enamel and dentine near the DEJ. Increased stiffness is speculated to be the result of the radiation-induced decrease in the protein content, with the percent reduction much greater in the enamel sites. Such changes in mechanical properties and chemical composition could potentially contribute to DEJ biomechanical failure leading to enamel delamination that occurs post-radiotherapy[34]

- **Lo guidance et al[35] (2015)** conducted a study is to analyse the root canal dentin going from coronal to apical zone to find the ratio between the intertubular dentin area and the surface occupied by dentin tubules varies. A SEM analysis of the data obtained in different canal portions showed that, in the coronal zone, dentinal tubules had a greater diameter (4.32 µm) than the middle zone (3.74 µm) and the apical zone (1.73 µm). The average number of dentinal tubules (in an area of 1 mm^2) was similar in coronal zone and apical zone, while in the middle zone they were lower in number. However, intertubular dentin area was bigger going from apical to coronal portion. The differences between the analysed areas must be considered for the choice of the adhesive system.[35]

- A study conducted by **Afaf et al[36] (2015)** evaluated and compared the sealer thickness and interfacial adaptation of bioceramic sealers (Sankin Apatite III, MTA Fillapex®, EndoSequence® BC) to root dentin against AH Plus sealer.

Percentage of gap-containing region to canal circumference was calculated using a confocal laser microscope. Sealer thickness was significantly higher at apical and middle levels than at coronal level. EndoSequence BC had the significantly highest thickness compared with MTA Fillapex and AH Plus. The coronal level had significantly less interfacial gaps compared with apical and middle levels. Bioceramic sealers showed more gaps compared with AH Plus, with no significant differences among them.[36]

- **Martini et al[37] (2016)** studied the effect of cancer radiation on marginal adaptation of root canal sealer to dentin. For the study Thirty-two maxillary canines were selected and were assigned to 2 groups. One group was not irradiated, and the other was subjected to a cumulative radiation dose of 60 Gy and each group was divided into 2 subgroups (n = 8) according to the sealer – AH Plus or MTA Fillapex – using the single-cone filling technique. SEM revealed more gap-containing regions and fewer tags at the sealer/dentin interface in irradiated specimens, with more tag formation and fewer gaps with AH Plus sealer. From the results they concluded that Radiation was associated with a decrease in the marginal adaptation to intraradicular dentin and formation of more gaps and fewer tags at the sealer/dentine interface.[37]

- **Khader et al[38] (2016)** compared the penetration depth of three root canal sealers most commonly available., AH Plus, TubliSeal, and Apexit with different compositions using SEM. Results showed that there was no statistically significant difference among the means of measured depth of penetration of AH Plus and Apexit Plus sealer. It was concluded that Zinc oxide eugenol-based sealer (Tubli-Seal™) shows less depth of penetration as compared to the calcium hydroxide-based sealer (Apexit® Plus) and resin-based sealer (AH Plus®).[38]

- **Viapina et al[39] (2016)** conducted a study to investigate the ability of BioRoot RCS, a tricalcium silicate-based root canal sealer and AH Plus to effectively fill the root canals. BioRoot RCS exhibited significantly more percentage of voids than AH Plus. There was no difference in fluid flow and microsphere penetration. BioRoot RCS exhibited a different pattern of sealer penetration and interaction with the dentine walls compared to AH Plus.[39]

- **Mohammadian et al[40] (2017)** in a study aimed to evaluate the dentine-sealer interface in three different sealers using scanning electron microscopy (SEM) with BC Sealer, AH-Plus and Dorifill. It was observed that that BC Sealer and AH-Plus had less gaps than Dorifill in coronal area. In addition, BC Sealer had better dentine interface in middle and coronal area compared to AH-Plus, and both performed better than Dorifill. Reverse relationship was observed between the mean gap width and dentine-sealer interface quality.[40]

- **Mamootil et al[41] (2017)** compared the depth and consistency of penetration of three different root canal sealer cements into dentinal tubules in extracted teeth and to measure the penetration of an epoxy resin-based sealer cement *in vivo*. The study concluded that depth and consistency of dentinal tubule penetration of sealer cements appears to be influenced by the chemical and physical characteristics of the materials. Resin-based sealers displayed deeper and more consistent penetration. Penetration depths observed for the epoxy resin-based sealer *in vivo* were consistent with that found in the experimental model.[41]

- **Siboni et al[42] (2017)** evaluate the chemical and physical properties of a tricalcium silicate root canal sealer containing povidone and polycarboxylate (BioRoot RCS), a calcium silicate MTA-based sealer containing a salicylate resin (MTA Fillapex), a traditional eugenol-containing sealer (Pulp Canal Sealer) and an epoxy resin-based root canal sealer (AH Plus). The study concluded that BioRoot RCS had bioactivity with calcium release, strong alkalizing activity and apatite-forming ability, and adequate radiopacity.[42]

- **Remy et al[43] (2017)** compared the marginal adaptation and sealing ability [mineral trioxide aggregate (MTA)-Fillapex, AH Plus, Endofill sealers] of root canal sealers. Among the three maximum marginal adaptations were seen with AH Plus sealer which is followed by Endofill sealer and MTA-Fillapex sealer. Between the coronal and apical marginal adaptation, significant statistical difference was seen in AH Plus sealer. Study proves that AH Plus sealer has a better marginal adaptation when compared with other sealers used. For sealing space between dentin wall and main cone in root canal treatment, sealers play an important role. The other advantages of sealers are that they are used to fill voids and irregularities in root channel, secondary, lateral channels, and space between applied gutta-percha cones and also act as tripper during filling.[43]

- **Rodrigues et al[44] (2018)** conducted a study to evaluate the biomechanical properties of dentin and the micro tensile bond strength performed before or after radiotherapy (RT). SEM image showed a disorganized dentin structure. This study concluded that RT alters the absorption bands and SEM images showed a disorganization of the dentin structure. RT causes changes that contribute to increased risk of tooth decay. Restorative treatments can be performed using adhesive procedures, but it is preferable to be performed before of the irradiation protocol, to guarantee better adhesive properties to restoration.[44]

- **Paiola et al[45] (2018)** had done a study to evaluate the influence of radiation therapy on root canal sealer push-out bond strength (BS) to dentin and the sealer/dentin interface after different final irrigation solutions (NaOCl, EDTA, and chitosan. Lower BS was obtained after irradiation regardless of the final irrigation solution used. The NaOCl group had the lowest BS in the irradiated and non-irradiated groups, whereas the EDTA and chitosan groups demonstrated a higher BS. The highest values were observed in the coronal third (3.17±1.38) when compared to the middle (2.74±1.36) and apical ones (2.09±0.97). There were more cohesive failures and more gaps in irradiated specimens, regardless of the final solution. The study showed that radiation was associated with a decrease in BS, regardless of the final solution used, whereas chitosan increased BS in teeth subjected to radiation therapy.[45]

- **Arikatla et al[46] (2018)** conducted a study to evaluate the interfacial adaptation and penetration depth of BioRoot RCS and MTA Plus sealers to root dentin

AH Plus sealer has shown significantly higher depth of penetration and minimum gaps than bioceramic sealers MTA Plus sealer exhibited significantly more interfacial gaps and less penetration depth than BioRoot RCS. At all root regions, AH plus sealer exhibited minimum gaps and more tubular penetration whereas MTA Plus sealer exhibited more gaps and less penetration.[46]

- **Marangoni et al[47] (2019)** in their study assessed whether radiotherapy causes changes in the mineral composition, hardness, and morphology of enamel and dentin of primary teeth. Thirty specimens of primary teeth were subjected to radiotherapy. At baseline and after 1,080, 2,160, and 3,060 cGy, the specimens were subjected to microhardness, FT-Raman spectroscopy, and scanning electron microscopy (SEM) analysis. The results showed that the microhardness of the enamel surface decreased after 2,160 cGy when compared to baseline. For dentin, the surface hardness decreased after 1,080 cGy and 2,160 cGy when compared to baseline. They concluded that after radiotherapy there is reduction in surface hardness, changed mineral and organic composition, and promoted morphological changes on the enamel and dentin of primary teeth.[47]

- Another study was done by **Kim et al[48] (2019)** to compare the penetration ability of calcium silicate root canal sealers and conventional resin-based sealer using confocal laser scanning microscopy. The maximum sealer penetration depth was low in the apical area and high in the coronal area in the AH Plus and Endoseal MTA groups. In the BioRoot RCS group, maximum sealer penetration was observed in the middle third. In conclusion, there were significant differences in sealer penetration pattern and distance according to the root level and sealer type.[48]

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RELEVANCE

Therapeutic cancer radiation is widely used in the treatment of head and neck carcinomas. Studies have proved that changes occur in the structure of dentin after radiation such as obliteration of dentinal tubules and collapse of collagen fibers which can adversely affect the physical and mechanical properties of dentin. This reduces the marginal adaptation of obturating materials and dentin, which in turn affects the outcome of the endodontic treatment.

Major advances in materials have taken place. A number of sealers with improved properties are available in the market such as bioceramic sealer, epoxy resin-based sealer and calcium hydroxide sealer.

The ability of any one particular sealer cement to penetrate dentinal tubules consistently and effectively can nullify the gap between the sealer and dentin. This is one of the many factors which will influence the choice of material considered for root canal therapy in patients who have undergone radiotherapy in the head and neck region.

This study is aimed to evaluate the influence of radiotherapy on the ability of 3 root canal sealers to penetrate the dentinal tubules and reduce the gap between the sealer and dentin before and after radiotherapy at three levels of the root canal system.

MATERIALS AND METHODS

Research Approach

Qualitative and Quantitative analysis

Study design

In vitro study

Study Setting

Study was conducted at

- St. Gregorios Medical Mission Hospital, Parumala,
- St. Gregorios Dental College, Chelad, Kothamangalam,
- Amritha Institute of Medical Science, Ernakulam.

SAMPLE AND SAMPLE SIZE

- Sample size is calculated using statistical package G*power (3.1.5).
- The minimum sample size is obtained n = 54
- 54 samples divided into 2 Groups
- Each Group is further divided into 3 subgroups with 9 samples per subgroup.

The materials and methodology used for this study are described under the following headings.

1. Selection of specimens
2. Armamentarium
3. Materials used for the study
4. Specimen preparation
    - Radiation protocol
    - Root canal preparation
    - Mounting and Sectioning
    - Chemical preparation
5. SEM analysis
6. Statistical analysis

SELECTION OF SPECIMENS

Human second premolars extracted for orthodontic purposes were collected from the Department of Oral and Maxillofacial Surgery, St. Gregorios Dental College, Kerala and St. Mary's Dental Clinic, Kerala.

100 mandibular second premolars are selected

The teeth are radiographed at two angulations

Inclusion criteria

- Single-rooted premolars
- Non carious teeth
- Teeth with complete root formation

Exclusion criteria

- Immature teeth, more than one canal.
- Canal with moderate or accentuated curvature.
- Calcifications in the pulp chamber.
- Internal resorptions.
- Previous endodontic treatment and metallic dental restorations, which could produce secondary radiation.

54 samples were selected and divided into two groups.

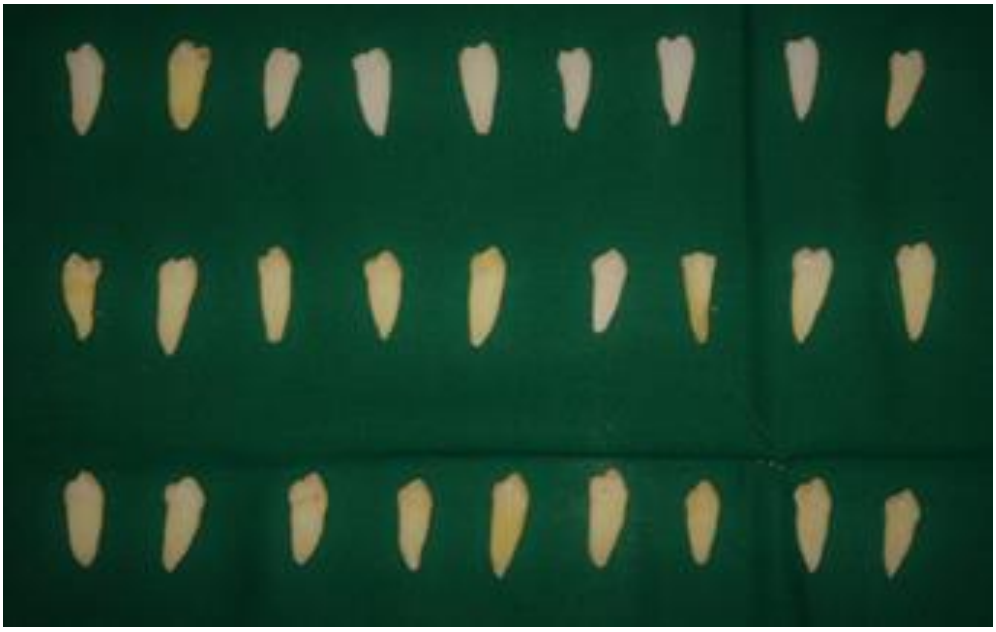


Figure 1: Irradiated samples (Group1)

Figure 2: Non Irradiated Samples (Group 2)

The teeth are stored in labelled plastic vials containing artificial saliva, renewed daily. The teeth are randomly assigned to 2 Groups as irradiated and non irradiated which are again divided in to 3 Subgroups based on the sealers.

IRRADIATED - 27 samples

NON IRRADIATED - 27 samples

- <u>GROUP 1- IRRADIATED</u>

  Subgroup 1a - AH Plus Irradiated (n=9)

  Subgroup 1b - Sealapex Irradiated (n=9)

  Subgroup 1c - BioRoot RCS Irradiated (n=9)

- <u>GROUP 2 – NON IRRADIATED</u>

  Subgroup 2a - AH Plus Non Irradiated (n=9)

  Subgroup 2b - Sealapex Non Irradiated (n=9)

  Subgroup 2c - BioRoot RCS Non irradiated (n=9)

ARMAMENTARIUM

Linear accelerator (RS 2000, RAD Source Technologies, Inc., Suwanee, GA, USA), Aerotor hand piece, X-Smart;9 Dentsply Maillefer), size 10 k file, Protaper gold(Dentsply Tulsa Dental, Tulsa, OK, USA),5.25% NaOCl, 17%EDTA, Normal Saline, paper point, side vented irrigation needle, lentulospiral, size F2 GP cones, Sealers(BioRoot RCS, Sealapex, AH Plus)

Hand pluggers, Self-cure acrylic, moulds, Precision saw, Scanning Electron (Isomet 1000; Buehler, Lake Forest, IL, USA). Scanning Electron Microscope (EvoMa10, Carl Zeiss, Munich, Germany). (Figure 3)

Figure 3: Armamentarium

MATERIALS USED FOR THE STUDY

1. BioRoot RCS – Bioceramic based root canal sealer manufactured by Septodont (Saint Maur des Fosses, France)

2. AH Plus – Epoxy resin-based root canal sealer marketed by Dentsply (Dentsply DeTrey GmbH, Konstanz, Germany

3. Sealapex – Calcium hydroxide-based root canal sealer marketed by Kerr Sybron, USA

Figure 4: Sealers Used in the study

SPECIMEN PREPARATION

1. IRRADIATION PROTOCOL

27 samples were irradiated with 60 Gy cumulative radiation for 5 consecutive days per week for 6 weeks over a period of 30 days.

Samples were stored in artificial saliva in between the radiation renewed daily.

27 TEETH (Group 1- irradiated) (Figure1)

PLACED IN PLASTIC VIALS (containing artificial saliva)

RADIATION DOSE – A cumulative radiation dose of 60 Gy fractioned in 30 fractions (2 Gy per fraction) were delivered using linear accelerator (Figure 5 and 6)

RADIATION TIME INTERVEL - 5 consecutive days per week, over 6 weeks

Figure 5: Linear Accelerator

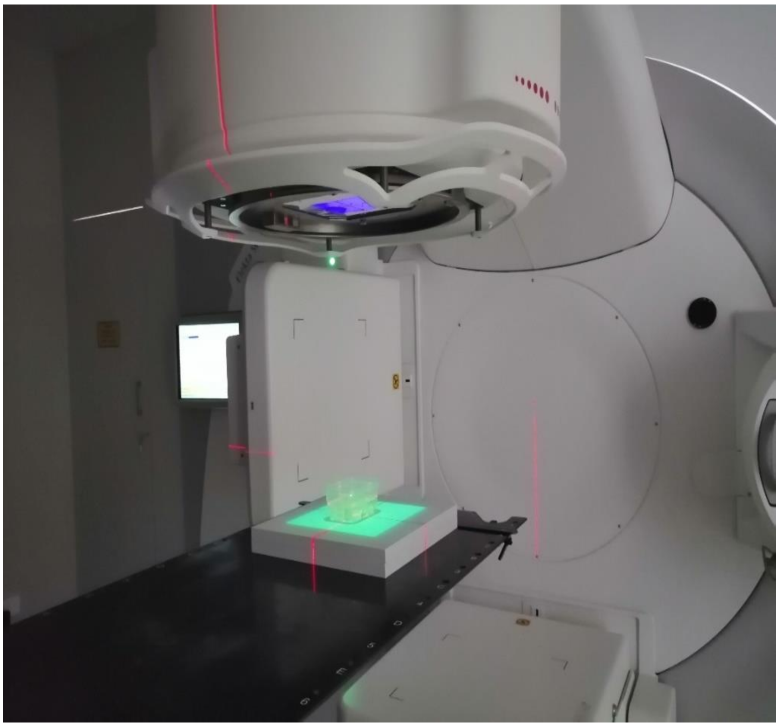


Figure 6: Sample positioning in linear accelerator

2. ROOT CANAL PREPARATION

Conventional access cavities were made for both group 1 and group 2, (Endo access bur, Dentsply) followed by irrigation with 5 mL 5.25% NaOCl. Working length was established 0.5 mm short of the apical foramen. Protaper Gold instruments were activated in pecking motion driven with the Xmart (Dentsply). The instruments were moved in the apical direction using an in-and-out pecking motion of about 3 mm in amplitude with a light apical pressure, being cleaned after three pecking motions. Canals were irrigated with 5.25% NaOCl between each preparation step. Canals were enlarged up to file size F3. At the end of preparation, the canals were flushed with 17% EDTA followed by 5.25% NaOCl irrigation and final rinse with saline. In each group, the canals were divided into 3 subgroups with 9 samples each filled with AH Plus, filled with Sealapex, filled with BioRoot RCS using a lentulospiral. Single-cone technique was done with GP size F3.

3. MOUNTING AND SECTIONING OF SAMPLES

Tooth is then mounted on to acrylic stumps (Figure: 7) and 1mm thin serial sections of the tooth is made using water cooled low speed saw. (Figure: 8 and 9) (Buehler Isomet 1000)

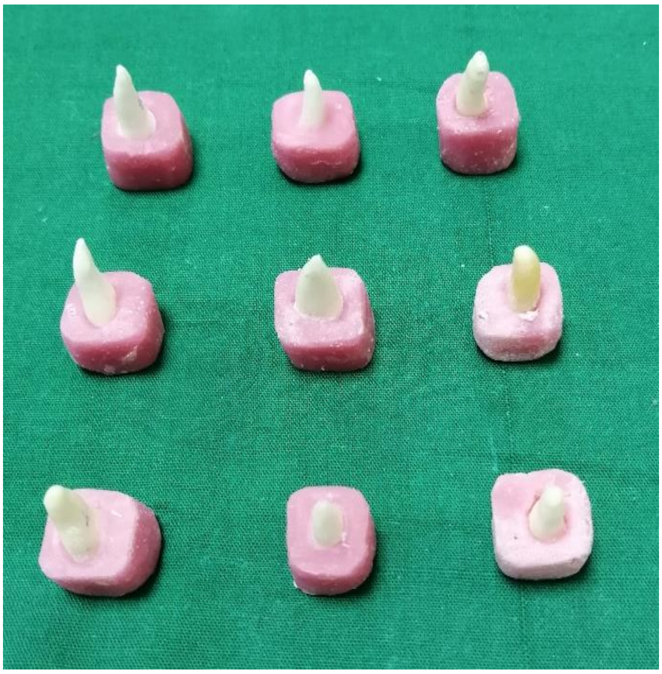


Figure 7: Samples mounted on acrylic stumps for sectioning

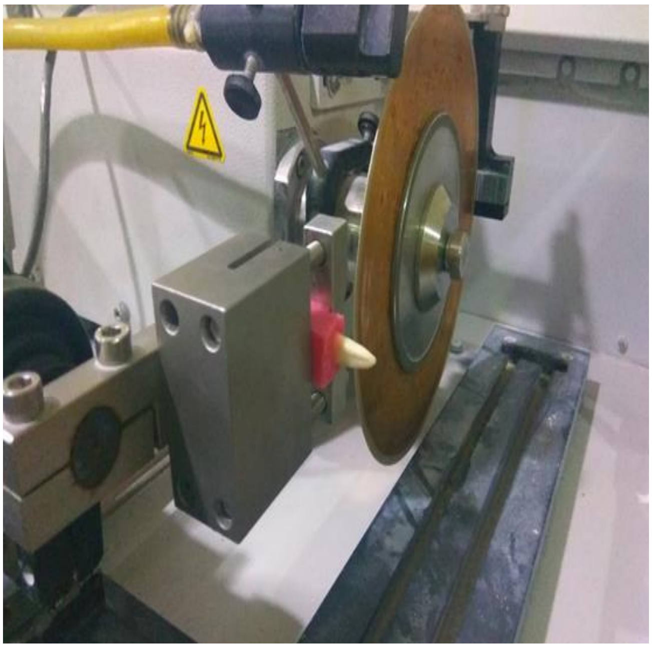


Figure 8: Sectioning of tooth

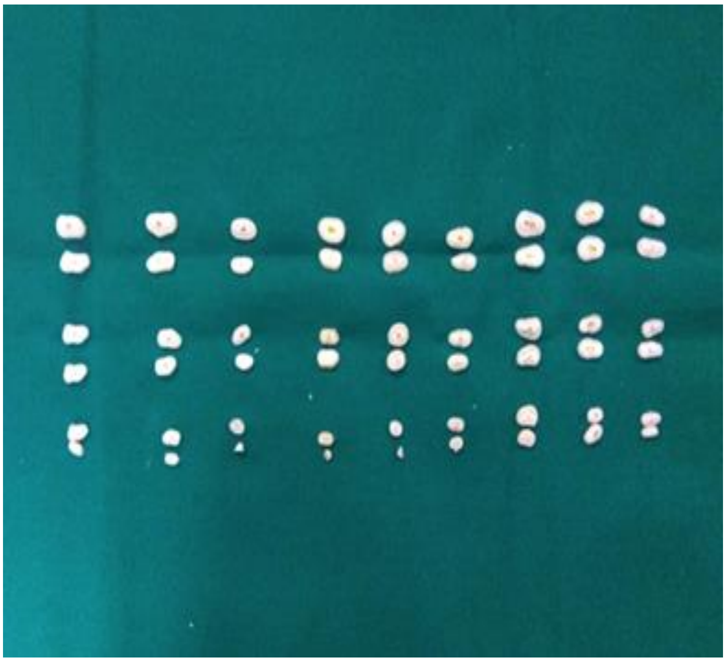


Figure 9: 1mm serial sections

4. CHEMICAL PREPARATION

For assessment of gaps, the slices were dehydrated in 99% isopropyl alcohol.

For assessment of sealer penetrability, the tooth sections were subjected to demineralization with hydrochloric acid 6 mol / L followed by deproteinization in 2.5% NaOCl.

Figure 10: Reagents for chemical preparations

5. SCANNING ELECTRON MICROSCOPIC ANALYSIS

All specimens were mounted on a disc provided and sputter coated with gold–palladium. After this they were viewed in a scanning electron microscope

1) Assessment of sealer penetrability

- The maximum depth of sealer penetration = Distance from sealer /gutta-percha interface to the highest depth of sealer penetration to root dentin

2) Assessment of gaps.

- Gap formation = Distance from Sealer to root dentin interface

Figure 11: Mounting of samples on disc for Sputter coating and SEM study

Figure 12: Sputter coating

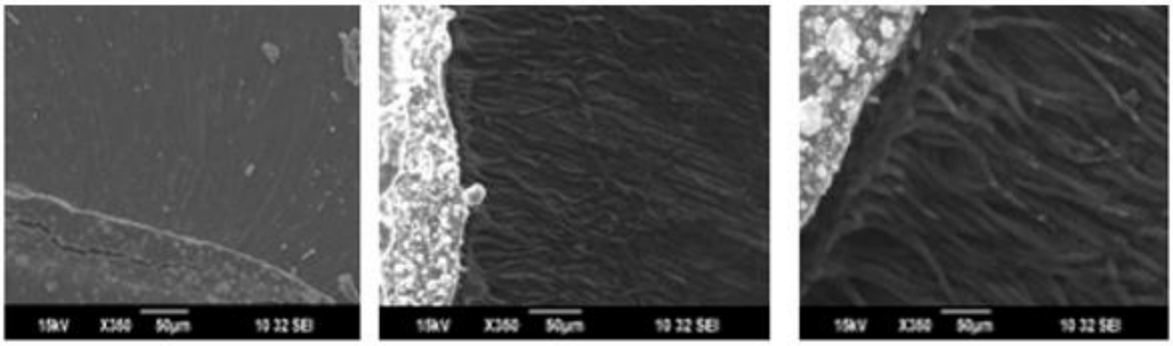
Figure 13: Scanning Electron Microscopic Analysis

<u>OBSERVATIONS</u>

GROUP 1 - IRRADIATED

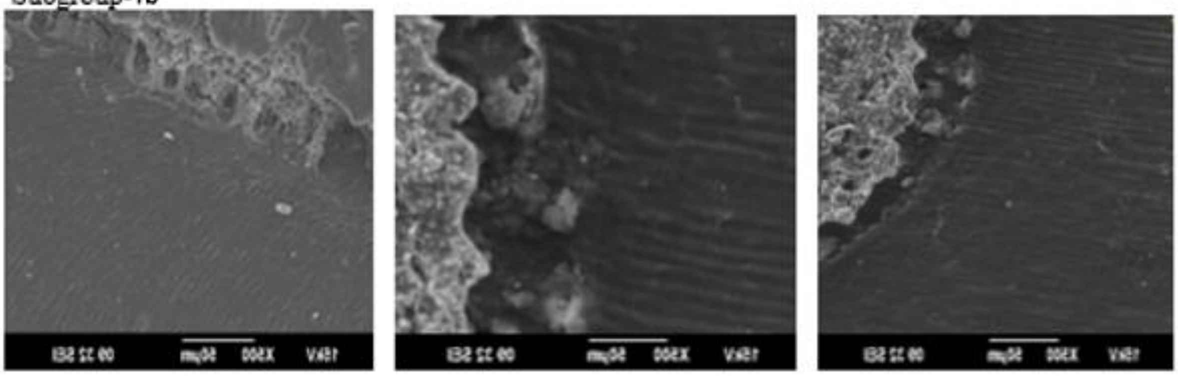
SEALER PENETRABILITY

Subgroup -1a



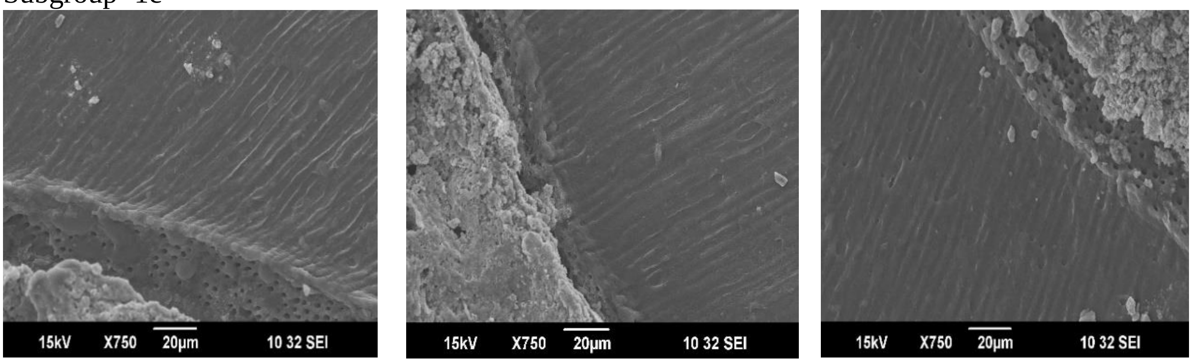
APICAL MIDDLE CERVICAL

Subgroup-1b



APICAL MIDDLE CERVICAL

Subgroup -1c

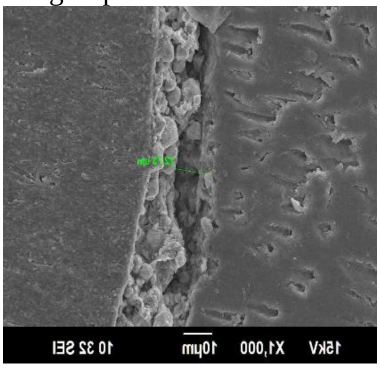


APICAL MIDDLE CERVICAL

GROUP 1 - IRRADIATED

GAP FORMATION

Subgroup -1a



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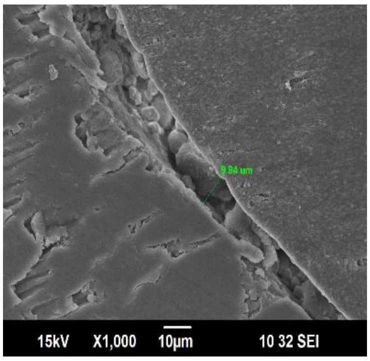

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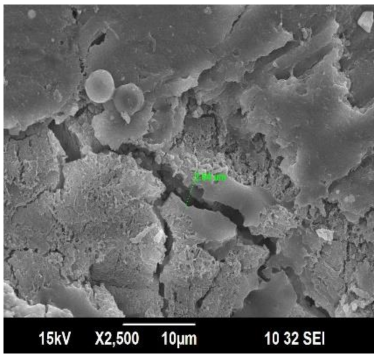
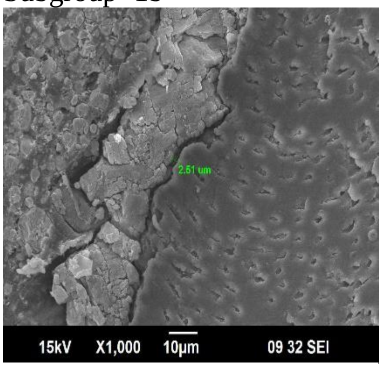


CERVICAL

Subgroup -1b



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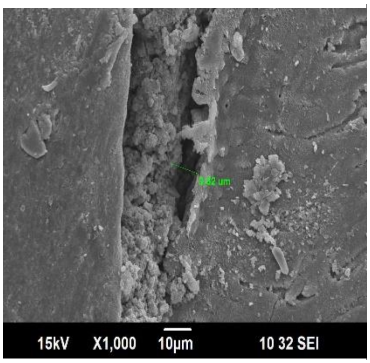

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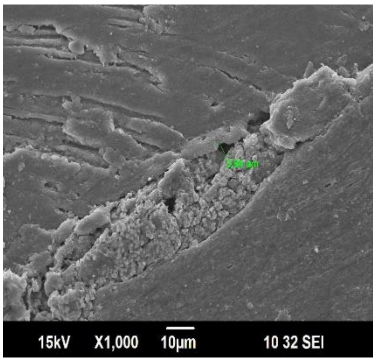
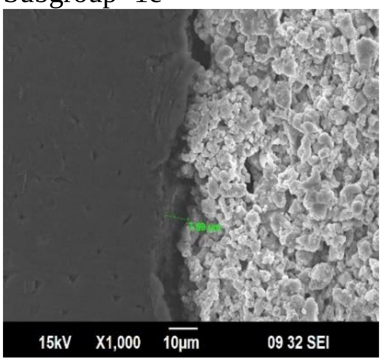


CERVICAL

Subgroup -1c



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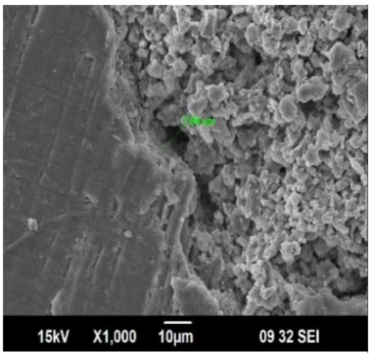

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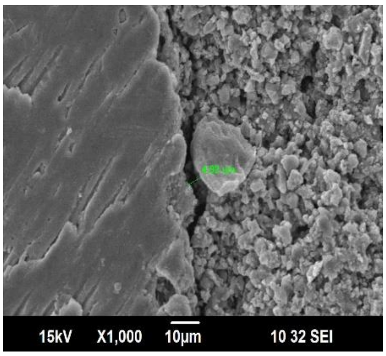
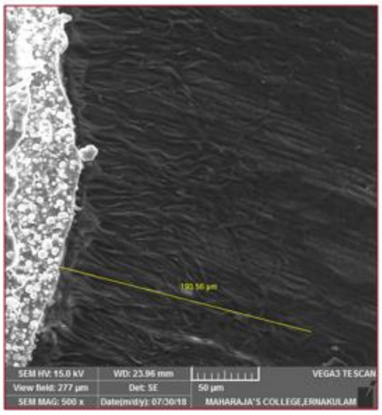


CERVICAL

GROUP 2 - (NON IRRADIATED)

SEALER PENETRABILITY

Subgroup – 2a



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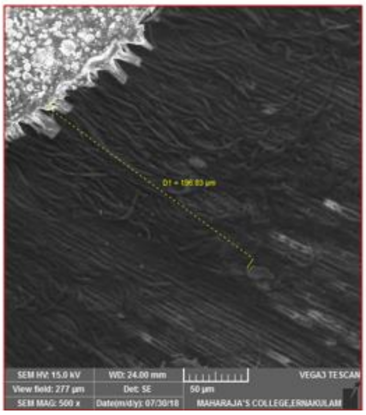

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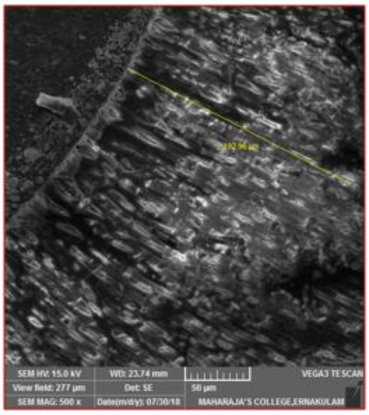
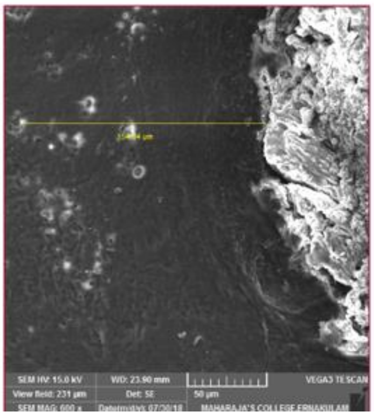


CERVICAL

Subgroup - 2b



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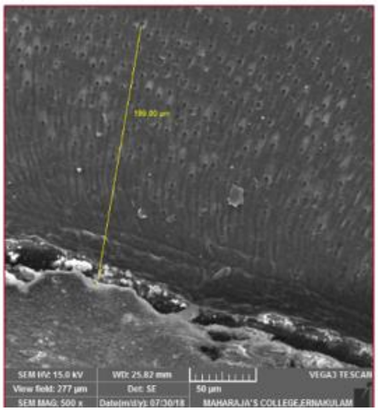

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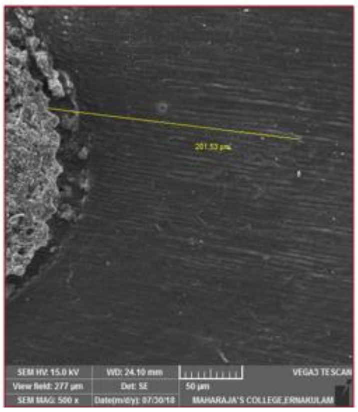

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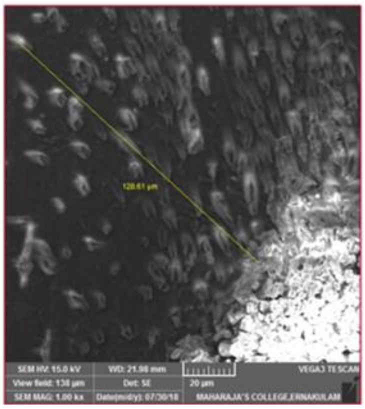
Subgroup - 2c



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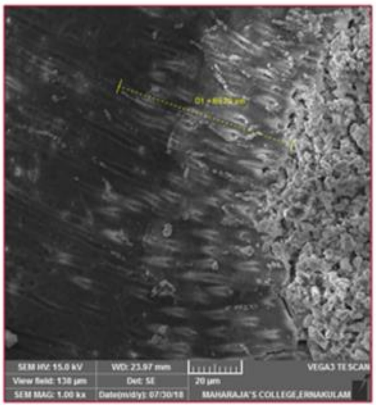

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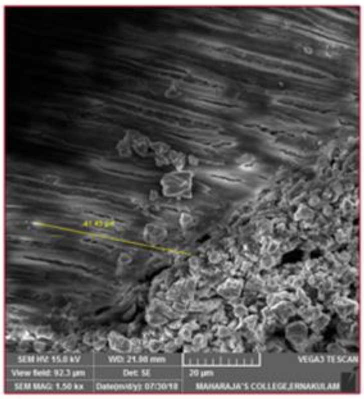
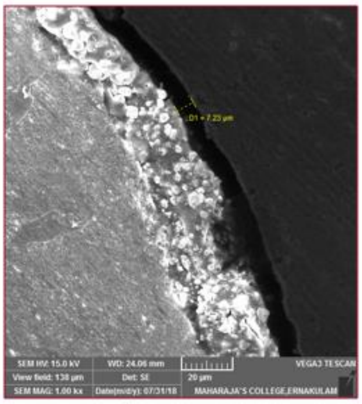


CERVICAL

GROUP 2 - (NON IRRADIATED)

GAP FORMATION

Subgroup - 2a



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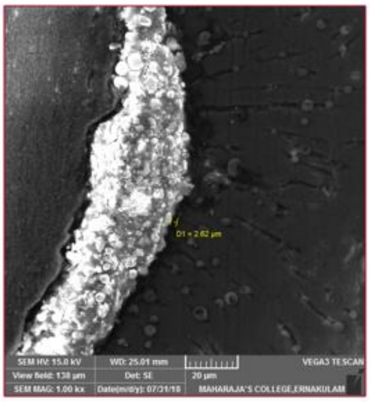

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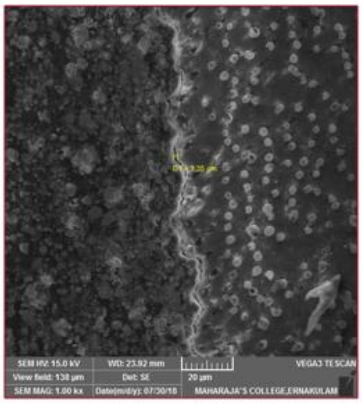
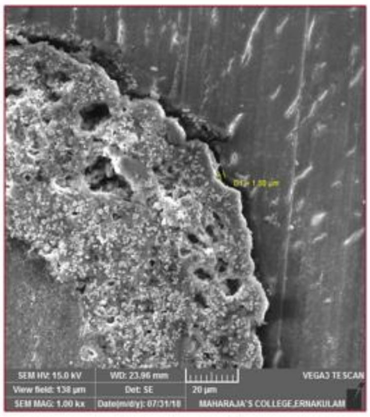


CERVICAL

Subgroup - 2b



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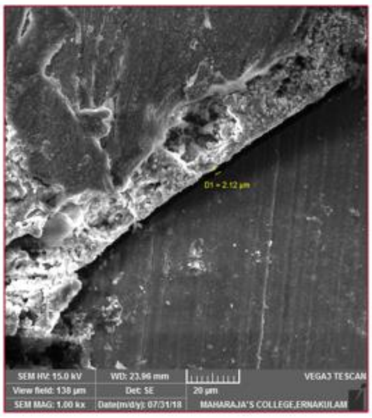

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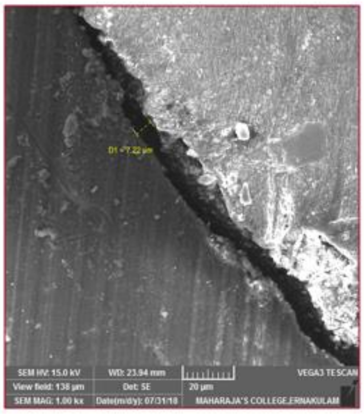
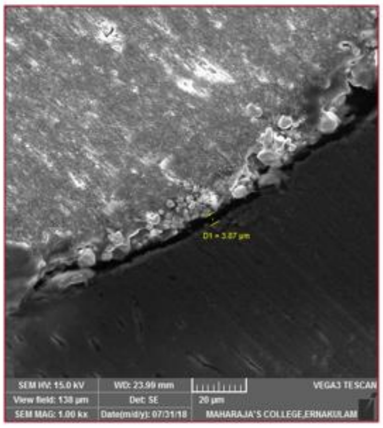


CERVICAL

Subgroup - 2c



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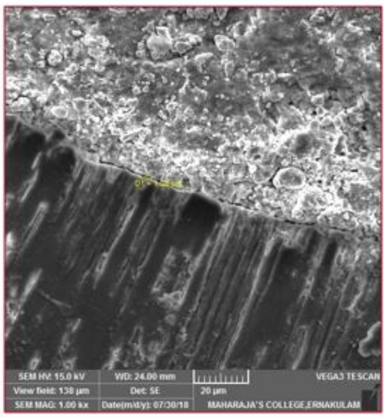

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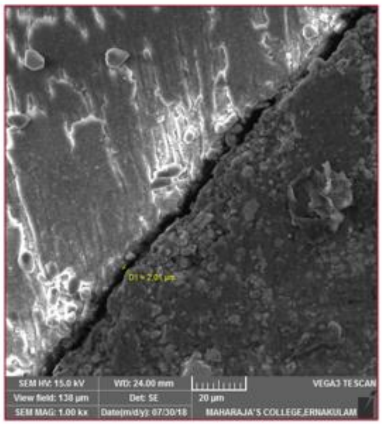


CERVICAL

STATISTICAL ANALYSIS

This study deals with testing whether there is any significant difference in mean value of sealer penetrability and gap formation among three different materials – AH plus, BioRoot RCS and Sealapex at apical, middle and cervical sections for Irradiated and Non-Irradiated tooth. One-way ANOVA is used for the analysis. Error bars are also drawn. In all the analysis 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) and the tests are two-tailed. Statistical Analysis was carried out using statistical package, SPSS (version 22.0.0.0).

Group 1: Irradiated

Difference of sealer penetrability among materials

One-way ANOVA is used to test the null hypothesis that there is no significant difference in mean value of sealer penetrability among the materials. The descriptive statistics is given below. For Apical, the sealer penetrability is highest for BioRoot RCS whereas for middle and cervical the sealer penetrability is highest for AH Plus.

		N	Mean	Std. Deviation	Std. Error
Apical	AH Plus	9	26.140	8.195	2.732
	BioRoot RCS	9	31.792	20.175	6.725
	Sealapex	9	0.679	0.275	0.092
	Total	27	19.537	18.333	3.528
Middle	AH Plus	9	61.983	11.873	3.958
	BioRoot RCS	9	18.910	14.978	4.993
	Sealapex	9	2.579	3.740	1.247
	Total	27	27.824	27.726	5.336
Cervical	AH Plus	9	70.891	11.510	3.837
	BioRoot RCS	9	43.321	12.081	4.027
	Sealapex	9	8.672	6.056	2.019
	Total	27	40.962	27.746	5.340

Table 1: Descriptive statistics

The results of the one-way ANOVA are given below.

		Sum of Squares	df	Mean Square	F	Sig.
Apical	Between Sub Groups	4944.766	2	2472.383	15.639	.000
	Within Sub Groups	3794.178	24	158.091		
	Total	8738.944	26			
Middle	Between Sub Groups	16952.716	2	8476.358	67.045	.000
	Within Sub Groups	3034.263	24	126.428		
	Total	19986.979	26			
Cervical	Between Sub Groups	17495.522	2	8747.761	83.287	.000
	Within Sub Groups	2520.767	24	105.032		
	Total	20016.289	26			

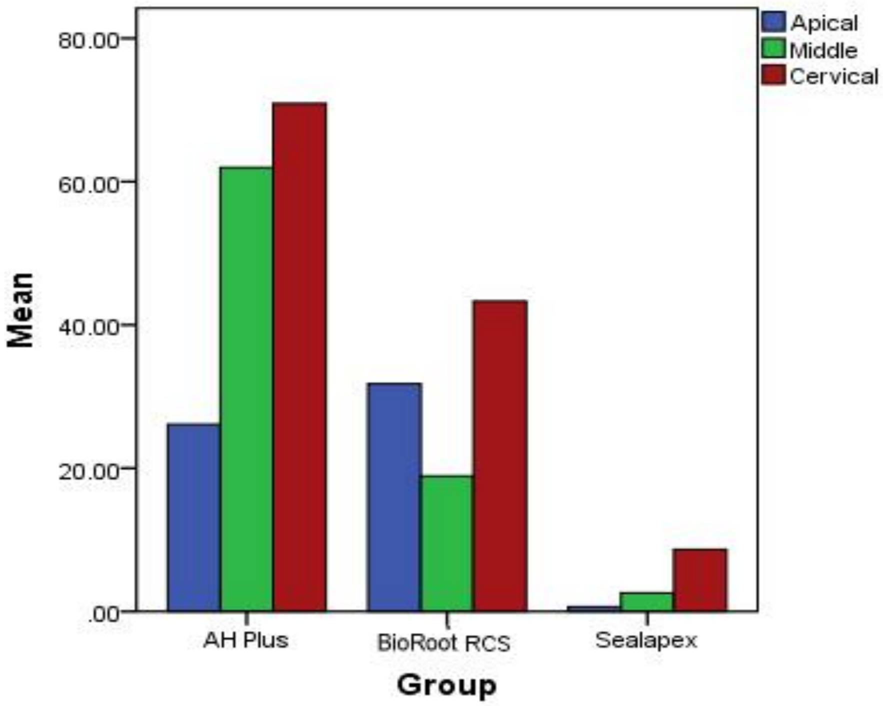
Table 2: One-way ANOVA

From the above table it can be observed that there is significant difference in mean value of sealer penetrability among the materials.

As there is significant difference, pairwise tests are conducted.

	(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.
Apical	AH Plus	BioRoot RCS	-5.652	5.927	0.612
		Sealapex	25.461	5.927	0.001
	BioRoot RCS	AH Plus	5.652	5.927	0.612
		Sealapex	31.113	5.927	0.000
	Sealapex	AH Plus	-25.461	5.927	0.001
		BioRoot RCS	-31.113	5.927	0.000
Middle	AH Plus	BioRoot RCS	43.073	5.300	0.000
		Sealapex	59.404	5.300	0.000
	BioRoot RCS	AH Plus	-43.073	5.300	0.000
		Sealapex	16.331	5.300	0.014
	Sealapex	AH Plus	-59.404	5.300	0.000
		BioRoot RCS	-16.331	5.300	0.014
Cervical	AH Plus	BioRoot RCS	27.570	4.831	0.000
		Sealapex	62.219	4.831	0.000
	BioRoot RCS	AH Plus	-27.570	4.831	0.000
		Sealapex	34.649	4.831	0.000
	Sealapex	AH Plus	-62.219	4.831	0.000
		BioRoot RCS	-34.649	4.831	0.000

Table 3: Post-hoc tests



Difference of gap formation among materials

One-way ANOVA is used to test the null hypothesis that there is no significant difference in mean value of gap formation among the materials. The descriptive statistics is given below.

		N	Mean	Std. Deviation	Std. Error
Apical	AH Plus	9	11.642	0.551	0.184
	BioRoot RCS	9	7.872	0.522	0.174
	Sealapex	9	14.297	0.678	0.226
	Total	27	11.270	2.745	0.528
Middle	AH Plus	9	9.156	0.649	0.216
	BioRoot RCS	9	7.246	0.437	0.146
	Sealapex	9	12.841	0.379	0.126
	Total	27	9.747	2.415	0.465
Cervical	AH Plus	9	8.309	1.335	0.445
	BioRoot RCS	9	7.318	0.433	0.144
	Sealapex	9	13.616	0.370	0.123
	Total	27	9.747	2.930	0.564

Table 4: Descriptive statistics

The results of the one-way ANOVA are given below.

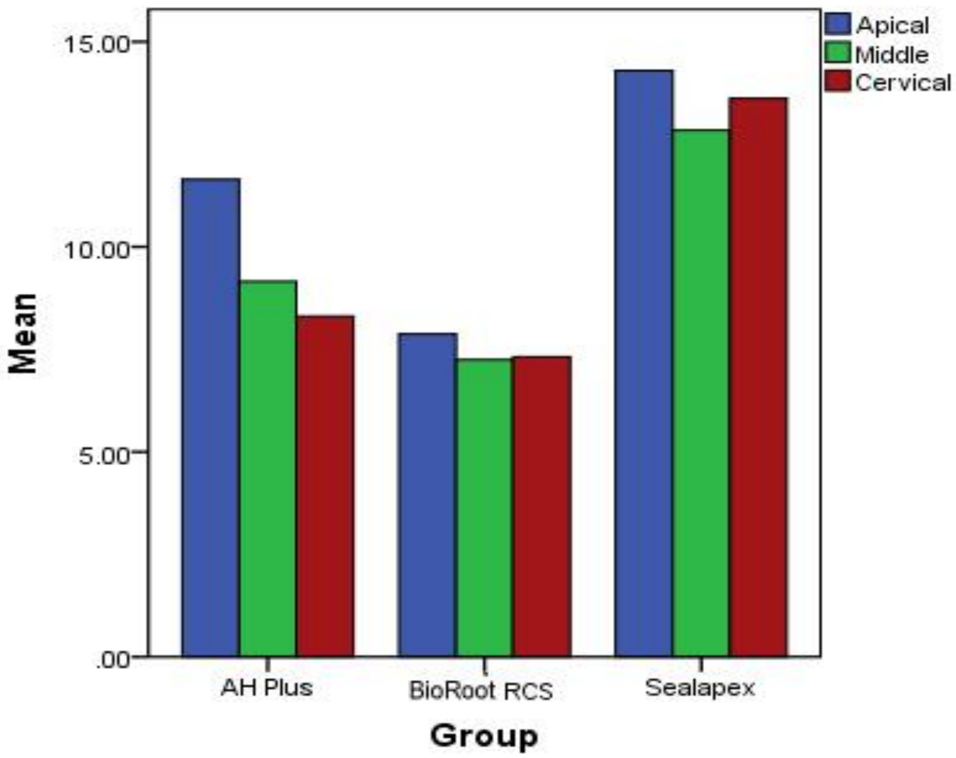
		Sum of Squares	df	Mean Square	F	Sig.
Apical	Between Groups	187.597	2	93.799	271.627	.000
	Within Groups	8.288	24	0.345		
	Total	195.665	26			
Middle	Between Groups	145.625	2	72.812	288.789	.000
	Within Groups	6.051	24	0.252		
	Total	151.676	26			
Cervical	Between Groups	206.415	2	103.208	146.940	.000
	Within Groups	16.857	24	0.702		
	Total	223.272	26			

Table 5: One-way ANOVA

From the above table it can be observed that there is significant difference in mean value of gap formation among the materials.

	(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.
Apical	AH Plus	BioRoot RCS	3.770	0.277	0.000
		Sealapex	-2.654	0.277	0.000
	BioRoot RCS	AH Plus	-3.770	0.277	0.000
		Sealapex	-6.424	0.277	0.000
	Sealapex	AH Plus	2.654	0.277	0.000
		BioRoot RCS	6.424	0.277	0.000
Middle	AH Plus	BioRoot RCS	1.910	0.237	0.000
		Sealapex	-3.686	0.237	0.000
	BioRoot RCS	AH Plus	-1.910	0.237	0.000
		Sealapex	-5.596	0.237	0.000
	Sealapex	AH Plus	3.686	0.237	0.000
		BioRoot RCS	5.596	0.237	0.000
Cervical	AH Plus	BioRoot RCS	0.991	0.395	0.049
		Sealapex	-5.307	0.395	0.000
	BioRoot RCS	AH Plus	-0.991	0.395	0.049
		Sealapex	-6.298	0.395	0.000
	Sealapex	AH Plus	5.307	0.395	0.000
		BioRoot RCS	6.298	0.395	0.000

Table 6: Post-hoc tests



GROUP 2 - NON IRRADIATED

Difference of sealer penetrability among materials

One-way ANOVA is used to test the null hypothesis that there is no significant difference in mean value of sealer penetrability among the materials. The descriptive statistics is given below.

		N	Mean	Std. Deviation	Std. Error
Apical	AH Plus	9	106.588	25.545	8.515
	BioRoot RCS	9	131.110	34.865	11.622
	Sealapex	9	57.649	39.381	13.127
	Total	27	98.449	44.949	8.650
Middle	AH Plus	9	175.376	22.621	7.540
	BioRoot RCS	9	95.723	36.584	12.195
	Sealapex	9	104.374	44.490	14.830
	Total	27	125.158	50.006	9.624
Cervical	AH Plus	9	220.269	26.539	8.846
	BioRoot RCS	9	52.344	13.078	4.359
	Sealapex	9	145.002	47.502	15.834
	Total	27	139.205	76.561	14.734

Table 7: Descriptive statistics

The results of the one-way ANOVA are given below.

		Sum of Squares	df	Mean Square	F	Sig.
Apical	Between Groups	25178.667	2	12589.334	11.047	.000
	Within Groups	27351.954	24	1139.665		
	Total	52530.621	26			
Middle	Between Groups	34381.428	2	17190.714	13.467	.000
	Within Groups	30635.184	24	1276.466		
	Total	65016.612	26			
Cervical	Between Groups	127347.462	2	63673.731	60.995	.000
	Within Groups	25054.173	24	1043.924		
	Total	152401.635	26			

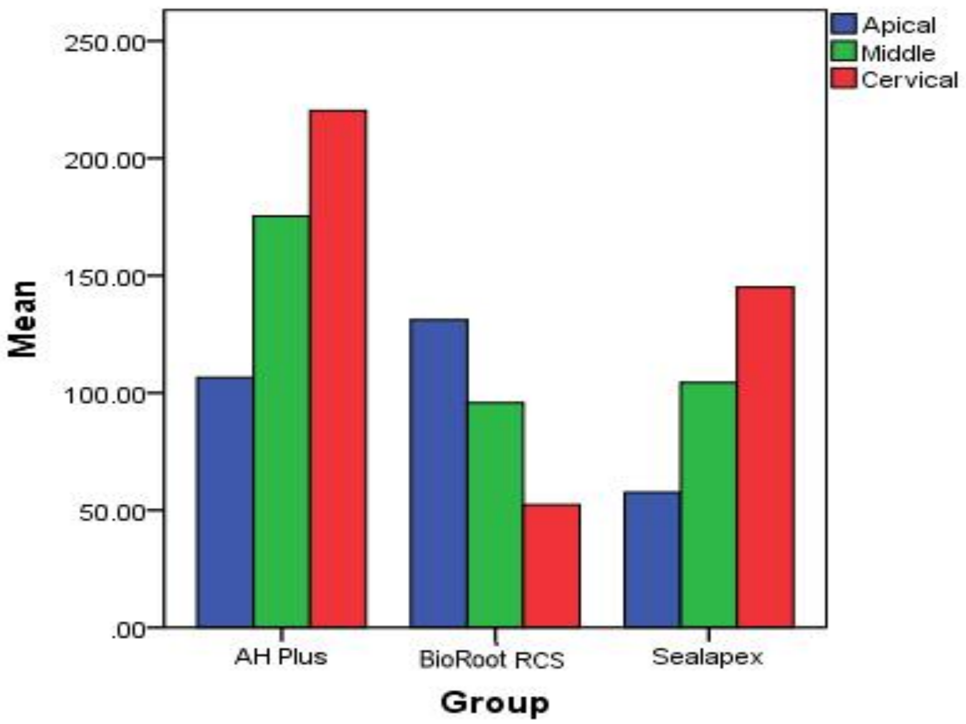
Table 8: One-way ANOVA

From the above table it can be observed that there is significant difference in mean value of sealer penetrability among the materials.

As there is significant difference, pairwise tests are conducted.

	(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.
Apical	AH Plus	BioRoot RCS	-24.522	15.914	0.136
		Sealapex	48.939	15.914	0.005
	BioRoot RCS	AH Plus	24.522	15.914	0.136
		Sealapex	73.461	15.914	0.000
	Sealapex	AH Plus	-48.939	15.914	0.005
		BioRoot RCS	-73.461	15.914	0.000
Middle	AH Plus	BioRoot RCS	79.652	16.842	0.000
		Sealapex	71.001	16.842	0.000
	BioRoot RCS	AH Plus	-79.652	16.842	0.000
		Sealapex	-8.651	16.842	0.612
	Sealapex	AH Plus	-71.001	16.842	0.000
		BioRoot RCS	8.651	16.842	0.612
Cervical	AH Plus	BioRoot RCS	167.924	15.231	0.000
		Sealapex	75.267	15.231	0.000
	BioRoot RCS	AH Plus	-167.924	15.231	0.000
		Sealapex	-92.658	15.231	0.000
	Sealapex	AH Plus	-75.267	15.231	0.000
		BioRoot RCS	92.658	15.231	0.000

Table 9: Post-hoc tests



Difference of gap formation among materials

One-way ANOVA is used to test the null hypothesis that there is no significant difference in mean value of gap formation among the materials. The descriptive statistics is given below.

		N	Mean	Std. Deviation	Std. Error
Apical	AH Plus	9	5.367	1.626	0.542
	BioRoot RCS	9	1.788	1.075	0.358
	Sealapex	9	5.390	1.188	0.396
	Total	27	4.181	2.140	0.412
Middle	AH Plus	9	3.987	1.068	0.356
	BioRoot RCS	9	1.752	0.796	0.265
	Sealapex	9	3.324	1.753	0.584
	Total	27	3.021	1.550	0.298
Cervical	AH Plus	9	2.051	1.142	0.381
	BioRoot RCS	9	4.432	1.621	0.540
	Sealapex	9	2.592	0.963	0.321
	Total	27	3.025	1.604	0.309

Table 10: Descriptive statistics

The results of the one-way ANOVA are given below.

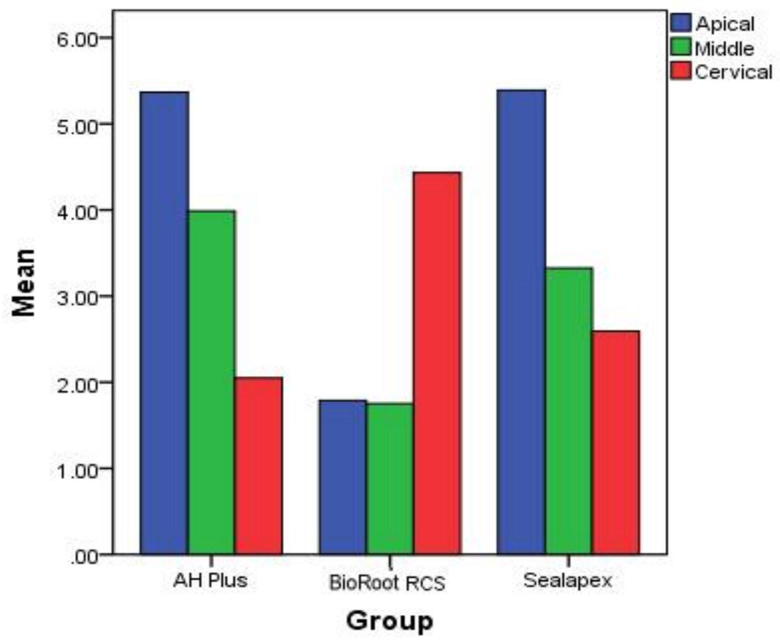
		Sum of Squares	df	Mean Square	F	Sig.
Apical	Between Groups	25178.667	2	12589.334	11.047	.000
	Within Groups	27351.954	24	1139.665		
	Total	52530.621	26			
Middle	Between Groups	34381.428	2	17190.714	13.467	.000
	Within Groups	30635.184	24	1276.466		
	Total	65016.612	26			
Cervical	Between Groups	127347.462	2	63673.731	60.995	.000
	Within Groups	25054.173	24	1043.924		
	Total	152401.635	26			

Table 11: One-way ANO VA

As there is significant difference, pairwise tests are conducted.

	(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.
Apical	AH Plus	BioRoot RCS	3.579	0.621	0.000
		Sealapex	-0.023	0.621	0.970
	BioRoot RCS	AH Plus	-3.579	0.621	0.000
		Sealapex	-3.602	0.621	0.000
	Sealapex	AH Plus	0.023	0.621	0.970
		BioRoot RCS	3.602	0.621	0.000
Middle	AH Plus	BioRoot RCS	2.234	0.599	0.001
		Sealapex	0.662	0.599	0.280
	BioRoot RCS	AH Plus	-2.234	0.599	0.001
		Sealapex	-1.572	0.599	0.015
	Sealapex	AH Plus	-0.662	0.599	0.280
		BioRoot RCS	1.572	0.599	0.015
Cervical	AH Plus	BioRoot RCS	-2.381	0.600	0.001
		Sealapex	-0.541	0.600	0.376
	BioRoot RCS	AH Plus	2.381	0.600	0.001
		Sealapex	1.840	0.600	0.005
	Sealapex	AH Plus	0.541	0.600	0.376
		BioRoot RCS	-1.840	0.600	0.005

Table 12: Post-hoc tests



Difference in sealer penetrability among Irradiated and Non irradiated group

Independent sample t-test is used to test the null hypothesis that there is no significant difference in mean value of sealer penetrability among the Group 1 and Group 2.

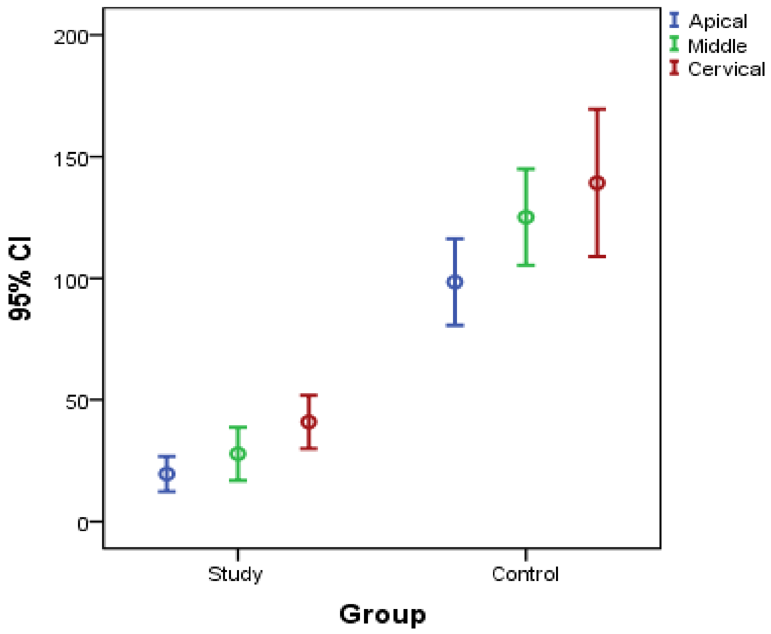
Group		N	Mean	Std. Deviation	Std. Error Mean
Apical	Group 1	27	19.537	18.333	3.528
	Group 2	27	98.449	44.949	8.650
Middle	Group 1	27	27.824	27.726	5.336
	Group 2	27	125.158	50.006	9.624
Cervical	Group 1	27	40.961	27.746	5.340
	Group 2	27	139.205	76.561	14.734

Table 13: Descriptive statistics

The results of the t-test are given below.

	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Apical	-8.447	34.418	0.000	-78.912	9.342
Middle	-8.845	40.605	0.000	-97.334	11.004
Cervical	-6.269	32.714	0.000	-98.244	15.672

Table 14: Independent sample t-test



Error bar Graph

The results suggest that there is significant difference in apical, middle and cervical values among Group1 and Group 2.

Difference in gap formation among Irradiated and Non irradiated group.

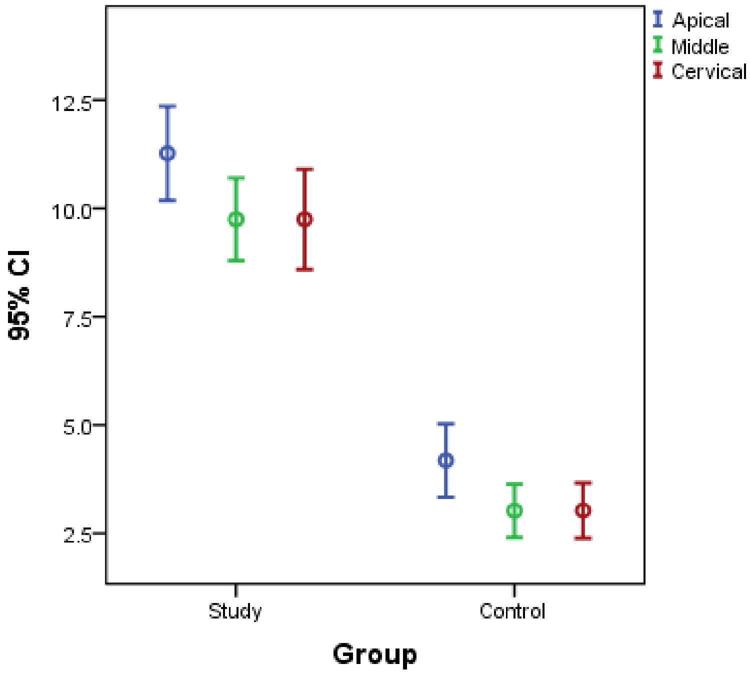
Independent sample t-test is used to test the null hypothesis that there is no significant difference in mean value of gap formation among Group 1 and Group 2.

Group		N	Mean	Std. Deviation	Std. Error Mean
Apical	Group 1	27	11.270	2.745	0.528
	Group 2	27	4.181	2.140	0.412
Middle	Group 1	27	9.747	2.415	0.465
	Group 2	27	3.021	1.550	0.298
Cervical	Group 1	27	9.747	2.930	0.564
	Group 2	27	3.025	1.604	0.309

Table 15: Descriptive statistics

	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Apical	10.584	52.000	0.000	7.089	0.670
Middle	12.178	44.312	0.000	6.726	0.552
Cervical	10.455	40.300	0.000	6.722	0.643

Table 16: Independent sample t-test



Error bar Graph

The results suggest that there is significant difference in apical, middle and cervical values among Group 1 and Group 2

From the statistical analysis it is observed that;

- **Difference Sealer penetrability among two groups**

  - Group 2 (Non irradiated) > Group 1 (Irradiated)

- **Difference in sealer penetrability among two groups at different levels of root**
  Group 2 cervical > Group 2 middle > Group 2 apical > Group 1 cervical > Group 1 middle > Group 1 apical

  One-way ANOVA was conducted among subgroup 1, 2 and 3 of Group 1 and Group 2 and statistically significant difference was seen ($p<0.05$)

- **Difference in sealer penetrability among materials in group1**
  Group 1 subgroup 1a (AH Plus) > Group 1 subgroup 1c (BioRoot RCS) > Group 1 subgroup 1b (Sealapex)

  As there was statistically significant difference post hoc was conducted to check the difference among materials at different levels of root

- **Difference in sealer penetrability in group1 among materials at different levels of root**
  Group 1 Subgroup 1a Cervical > Group 1 Subgroup 1b Middle > Group 1 subgroup 1c Cervical > Group 1subgroup 1c Apical > Group 1 subgroup 1a Apical > Group 1 subgroup 1c Middle > Group 1 subgroup 1b cervical > Group 1 subgroup 1b middle > Group 1 subgroup 1b Apical
  And statistically significant difference was observed between

  At the apical level

  Group 1 subgroup 1c > Group 1 subgroup 1a

  Group 1 subgroup 1c > Group 1 subgroup 1b

  At the middle level

  Group 1 subgroup 1a > Group 1 subgroup 1c

  Group 1 subgroup 1a > Group 1 subgroup 1b

  Group 1 subgroup 1c > Group 1 subgroup 1b

  At the cervical level

  Group 1 subgroup 1a > Group 1 subgroup 1c

  Group 1 subgroup 1a > Group 1 subgroup 1b

  Group 1 subgroup 1c > Group 1 subgroup 1b

- **Difference in sealer penetrability among materials in Group 2**

  Group 2 subgroup 2a (AH Plus) > Group 2 subgroup 2c (BioRoot RCS) > Group 2 subgroup 2b (Sealapex)

  As there was statistically significant difference post hoc was conducted to check the difference among materials at different levels of root

- **Difference in sealer penetrability in Group 2 among materials at different levels of root**

  Group 2 subgroup 2a Cervical > Group 2 subgroup 2a Middle > Group 2 subgroup 2b Cervical > Group 2 subgroup 2c Cervical > Group 2 subgroup 2a Apical > Group 2 subgroup 2b Middle > Group 2 subgroup 2c Middle > group 2 subgroup 2b Apical > group2 subgroup 2c Cervical

  And statistically significant difference was observed between

  At the apical level

  Group 2 subgroup 2a > Group 2 subgroup 2b

  Group 2 subgroup 2c > Group 2 subgroup 2b

  At the middle level

  Group 2 subgroup 2a > Group 2 subgroup 2c

  Group 2 subgroup 2a > Group 2 subgroup 2b

  At the cervical level

  Group 2 subgroup 2a > Group 2 subgroup 2c

  Group 2 subgroup 2a > Group 2 subgroup 2b

  Group 2 subgroup 2c > Group 2 subgroup 2b

- **Difference in Gap Formation among two groups**

  Group 2 (Non irradiated) < Group 1 (Irradiated)

- **Difference in Gap Formation among two groups at different levels of root**

  Group1 apical > Group1 middle = Group1 cervical > Group 2 apical > Group 2 cervical > Group 2 middle

  One-way ANOVA was conducted among subgroup 1, 2 and 3 of group 1 and group 2 and statistically significant difference was seen ($p<0.05$)

- **Difference in Gap Formation among materials in group1**

  Group 1 subgroup 1c (BioRoot RCS) < Group1 subgroup 1a (AH Plus) < Group1 subgroup 1b (Sealapex)

  As there was statistically significant difference post hoc was conducted to check the difference among materials at different levels of root

- **Difference in Gap Formation in group1 among materials at different levels of root**

  Group 1 Subgroup 1c Middle < Group 1 Subgroup 1c Cervical < Group 1 subgroup 1c Apical < Group 1 subgroup 1a Cervical < Group1 subgroup 1a Middle < Group 1 subgroup 1a Apical < Group 1 subgroup 1b Middle < Group 1 subgroup 1b Cervical < group 1 subgroup 1b Apical

  And statistically significant difference was observed between

  At the apical level

  Group1 subgroup 1a > Group 1 subgroup 1c

  Group 1 subgroup 1a< Group 1 subgroup 1b

  Group 1 subgroup 1c < Group 1 subgroup 1b

  At the middle level

  Group 1 subgroup 1a > group 1 subgroup1c

  Group 1 subgroup 1a< Group 1 subgroup 1b

  Group 1 subgroup 1c< Group 1 subgroup 1b

  At the cervical level

  Group 1 subgroup 1a > Group 1 subgroup 1c

  Group 1 subgroup 1a < Group1 subgroup 1b

  Group 1 subgroup 1c < Group 1subgroup 1b

- **Difference in Gap Formation among materials in Group 2**

  Group 2 subgroup 2c (BioRoot RCS) < Group 2 subgroup 2a (AH Plus) < Group 2 subgroup 2b (Sealapex)

  As there was statistically significant difference post hoc was conducted to check the difference among materials at different levels of root

- **Difference in Gap Formation in group2 among materials at different levels of root**

  Group 2 subgroup 2c Middle < Group 2 subgroup 2c Apical < Group 2 subgroup 2a Cervical < Group 2 subgroup 2b Cervical < Group 2 subgroup 2b Middle < Group 2 subgroup 2a Middle < Group 2 subgroup 2c Cervical < Group 2 subgroup 2a Apical < Group 2 subgroup 2b Apical

And statistically significant difference was observed between

At the Apical level

Group 2 subgroup 2a > Group 2 subgroup 2c

Group 2 subgroup 2c < Group 2 subgroup 2b

At the Middle level

Group 2 subgroup 2a > Group 2 subgroup 2c

Group 2 subgroup 2a < Group 2 subgroup 2b

Group 2 subgroup 2c < Group 2 subgroup 2b

At the Cervical level

Group 2 subgroup 2a > Group 2 subgroup 2c

Group 2 subgroup 2c < Group 2 subgroup 2b

RESULTS

- Group 1 showed more gaps between sealer dentin interface and lower sealer penetrability compared to Group 2

- For both Group 1 and Group 2 statistically significant increase in the penetrability of sealer was observed at the cervical area

- Group 1 subgroup 1c showed increased penetrability at apical third compared to Group 1 subgroup 1a and 1b which was statistically significant

- Group 2 subgroup 2a showed increased penetrability at middle and cervical level compared to 2b and 2c

- Statistically significant lower penetrability was observed for 1b and 2b compared to 1a, 1c and 2a, 2c respectively at apical, middle and cervical levels

- For both Group 1 and Group 2 statistically significant increase in gap formation was observed in apical area

- Subgroup 1c and 2c shows statistically significant lesser mean values of gap formation compared to subgroup 1a, 1b and subgroup 2a, 2b respectively at apical, middle and cervical levels.

- More statistically significant gaps formation was observed for Groups 1b and 2b compared to Groups 1a, 1b and 2a, 2b respectively.

DISCUSSION

Cancer of the head and neck ranks the seventh among the most common neoplasms worldwide, with an annual incidence of approximately 640,000 new cases.[49]

The treatment of head and neck cancer depends upon several factors such as the type of cancer, the stage the cancer is at, it's location and if it can be treated successfully by surgery, radiation therapy, chemotherapy or a combination of these.[50]

Radiation therapy acts directly on the DNA by inhibiting cell division, or indirectly by producing free radicals resulting in cellular necrosis. Conventional radiation fractionation is the most commonly used regimen to minimize the side effects of radiation therapy on healthy tissues, favouring their repair.[51]

Adjacent tissues are rarely preserved during head and neck radiation therapy and this may vary according to the patient's age, the dose given and the location of the ionizing radiation.[52]

The use of radiotherapy in the treatment of certain carcinomas of the oral cavity, pharynx and larynx can cause caries-like destruction in the hard dental tissues namely the enamel and the dentin.[53]

Structural changes in enamel, dentin and direct damages to collagen as well as reduction of dentin microhardness, favour the development and progression of radiation caries, which may lead to pulpal changes resulting in the need for endodontic treatment.[37]

Since the survival rate of patients with cancer of the head and neck is higher nowadays, the chances of radiation caries occurring is high too. Therefore, it is important to evaluate different protocols for the endodontic treatment of teeth that have been subjected to radiation therapy.[8] After radiation, when caries is established, most of the teeth do not respond normally to thermal pulp tests. They give delayed responses due to reduced pulpal blood supply that is related to fibrosis of the inner layer of the blood vessels. This fibrosis occurs after radiation and may be responsible for producing calcifications or irregular dentin. At this phase, a periapical radiograph is recommended, to verify alterations in the dentin or thickening of the pericemental membrane.[54]

- **According to Rosales**, approximately 41% of patients who did not have a dental evaluation performed before radiotherapy were in need of endodontic treatment. On the other hand, only 10.8% who had dental evaluation prior to radiotherapy had to undergo endodontic treatment in the post-radiotherapy period.[55]

There are few studies that propose to establish a safe time for initiation of endodontic therapy after radiotherapy.

- **Shafer** stated that the ideal period for endodontic treatment would be from 60 to 120 days after radiotherapy ends, at which time any bone alterations would be less present.

According to these authors, the Endodontists would have to evaluate the oral cavity conditions as well as the patient's systemic health before starting any treatment.[56]Several studies have shown that radiation therapy does not induce pulpal damage.

- **Hutton and Nickens** noted no histological differences in pulp tissue after being submitted to 70 Gy of irradiation.[57, 58]
- **Knowles** observed that decreased pulpal sensitivity was noticed only in teeth within or adjacent to an irradiated field, while Kataoka noted a time dependent decrease in oxygen saturation levels in pulpal tissues submitted to radiation.[59, 60]
- **Cox** (1976) stated that these complications may be reduced by dose fractionation, use of radiation protection devices, dental assessment and treatment prior to radiation therapy.[61]

The present study aimed to simulate the radiation doses used to treat cancer patients subjected to fractionated doses of 2 Gy for 5 consecutive days with 30 cycles every 6 weeks, totalling to 60 Gy. This protocol has been used to study the changes radiation therapy produces in the dental structure.[37]

In this study artificial saliva for tooth storage. Although the exact qualities of natural saliva is not reproduced like in the case of saliva of irradiated patients who have altered salivary flow and secretion, this was still the best option to show the clinical condition that best resembles the natural saliva of patients not subjected to radiation therapy.[63]

- **Kignel et al** stated that the endodontic filling step should be performed with the least irritating materials and extra care should be applied in order to not overfill the canal. Because of the fragility of dentin structure of the irradiated patients, compression performed during lateral condensation must be delicate.[64]
- According to a study conducted by **W Qu et al**, a significant reduction in the flow property was observed in the bioceramic tricalcium silicate–based sealer, which could negatively affect the treatment and quality of warm vertical compaction obturation technique. [65]

In this study, single cone obturation was done in order to standardize the obturation and to avoid compression forces on dentin of irradiated samples.

In this study, irradiated teeth had lower sealer penetrability and increased gap formation than non-irradiated samples. This finding is in agreement with the study conducted by

- **Martini et al** suggested that radiation was associated with a decrease in the marginal adaptation to intraradicular dentin and formation of more gaps and fewer tags at the sealer/dentine interface[37]

This is probably associated with changes in the dentin ultrastructure such as obliteration of dentinal tubules, alterations in the intertubular, peritubular and intratubular dentin as well as with the fragmentation of the network of collagen fibers of the dentinal tissue and its deprotenization.

- Dentin is characterized as a heterogeneous substrate due to its constitution, which has about 70% of inorganic material, 20% of organic material and 10% of water wherein the organic material consists mainly of collagen fibers. According to **Soares et al,** due to the fragmentation of the collagen fiber network, radiation has been shown to be more damaging to the organic components than to the inorganic components. This may add to the explanation of why there is reduction in the marginal adaptation of the sealer after irradiation, regardless of the sealer type.[28]
- Study conducted by **Goncalves et al** suggested that head and neck radiation therapy may lead to alterations in the amide III group present in the collagen structure resulting in the disorganization of the secondary structure of the protein unit that forms the collagen fibers, modifying the natural arrangement between mineral and organic contents of dentin, changing its physical and mechanical properties.[5] This finding can be considered as justification for the reduction in marginal adaptation of sealers following radiation therapy.

Qualitative SEM analysis showed the presence of gaps at the dentin-filling material interface more expressively in the specimens after irradiation, corroborating the decrease of marginal adaptation and increase in the prevalence of adhesive failures in dentin and in the filling material, respectively. The interface integrity is crucial for the sealing and minimizing the chances of recontamination, which could lead to failure of endodontic treatment.[66] Besides, the influence of irradiation reduces the resin tag formation, probably due to the obliteration of dentinal tubules and alterations in the intertubular, peritubular, and intratubular dentin at 60 Gy cumulative doses.

Short, asymmetric and low-density resin tags were observed in SEM when bioceramic sealer was used.

In the present study the sections analyzed showed greatest penetration depth at cervical third in both irradiated and non-irradiated samples except bioceramic sealer

- This finding is in accordance to a study conducted by **Weis et al**. This study noted a significant deeper penetration of an epoxy resin sealer into tubules 5 mm from the anatomical apex compared to the 3 and 1 mm.[24]
- Study conducted by **Carrigen et al** reported that apical dentine displays less tubule density while some areas are completely devoid of tubules.[19]
- Study conducted by **Sen et al** proved that the effectiveness of smear layer removal techniques is also reduced closer to the apex.[67]

The diameter and density of the dentinal tubules is more at the coronal and middle third of the root canal system whereas it is minimal at the apical third and this factor plays a major role in sealer penetration.[68]

- **Balguerie et al** in his study stated that both coronal and middle thirds showed higher penetration than the apical part.[69.]
- **Mjor et al** In his study, the tubule number decreased from 40,000 - 14,400 (coronal to apical). This meant that lesser tubules were found available for sealer penetration.[70]

Gutta-percha is the most commonly used core material and offers the advantages such as inertness, bio-compatibility, less technique sensitivity, ease of manipulation and reinforces the root canal system. The major drawback of gutta-percha is its lack of inherent bonding to the root dentin which is balanced by using a root canal sealer which enhances its adaptation to the root canal wall.[71]

A fluid-tight seal is the main requisite to achieve a successful obturation. Several types of endodontic sealers have been recommended to achieve this goal which includes silicon-based sealers, epoxy resin-based sealers, mineral trioxide aggregate-based sealer, calcium silicate-phosphate-based bioceramic sealer and methacrylate resin-based sealer.[72]

Among all the tested groups used in this study, BioRoot RCS sealer showed greater penetrability at the apical third and minimal gap formation for both irradiated and non irradiated samples even though the sealer penetrability and gap formation of irradiated group was considerably lower than non irradiated group.

This finding is in accordance with the study conducted by

- **Wang et al** (2018) which shows greatest penetration of bioceramic sealer at apical level compared to resin based sealer.[73]
- The better performance of the Bioceramic sealer can be explained on the basis of its small particle size, hydrophilicity and low contact angle which enables the cement to spread easily over the dentin walls of the root canal and fill the lateral micro canals.
- Bioceramic root canal sealers also exhibit chemical bonding to root canal dentin walls as well as its corresponding bioceramic particle impregnated gutta-percha.[74]

It also exhibits a significant expansion of 0.20%. All these features result in a gap-free chemical bond between the sealer and dentinal walls, thus making it an effective sealer.

- Study conducted by **Husnan et al** showed comparable penetrability of bioceramic sealer with resin based sealer[75]

Bioceramic based sealers denature the collagen present in the dentin providing a 'mineral infiltrated zone'. They are found to have better penetration and dimensional stability.

- Study conducted by **Savaris et al** reveals that AH plus has good biocompatibility, tissue tolerance, long-term dimensional stability and sealing ability but silicone oil content of AH plus increases the surface tension. As a result, shrinkage occurs at the sealer-dentin interface. This finding correlates with the decrease in sealer penetration at the apical aspect compared to bioceramic sealer in this study.[76]

Present study shows that resin based sealer revealed more gaps in sealer dentin interface compared to bioceramic sealer

- During the curing of resin materials, shrinkage stress increases and the root canal sealer tends to dislodge from the sealer-dentin interface, forming gaps and which validate the increased gaps seen in the samples filled with resin-based sealer compared to bioceramic sealer[77]

In the present study AH Plus had good penetration at the cervical third than the middle and apical third in both radiated and non irradiated samples

- This can be because of its low particle size and film thickness which is in the range of 20 - 25µm.
- Low solubility of AH Plus on exposure to tissue fluids aids in better penetration. The results of this present study are in agreement with the previous study conducted by Borges et al.[78]
- According to Messer et al tubular penetration of resin-based sealers does not depend on hydraulic forces formed during obturation; rather the sealer is sucked into the tubules by capillary action.[41]

AH Plus is considered as a material which provides good marginal adaptation as it has better penetration into micro irregularities because of its creep capacity and long setting time which increases the mechanical interlocking between sealer and root dentin.

Moreover, it has low solubility, small expansion while setting and bonds to the root dentin through adamantine.[79]

Many studies have been evaluated to assess the sealing ability of the endodontic sealers through various methods such as dye penetration method, electrical methods, fluid filtration technique, radioisotope tracing, and scanning electron microscopy.[80]

In this study, the scanning electron microscope was utilized to estimate the mean penetration of root canal sealers. The advantage of using SEM over other sealing methods is that in SEM the defects at the submicron level can be observed at required magnification and a final evaluation can be done by preserving the microphotographs.[81]

In the present study Sealapex showed decreased penetrability and more gaps compared to BioRoot RCS and AH Plus.

In the present study Sealapex shows significant reduction in sealer penetration and increased gap formation compared to AH Plus and BioRoot RCS

- According to a study conducted by **Kontakiotis**, Sealapex presents volumetric expansion during setting due to water sorption caused by the presence of calcium oxide; this characteristic may increase its solubility, thus raising the risk of formation of gaps over time.[82]
- But the study conducted by **Ordinola sapata et al** contradicts this finding and shows a higher tubular penetration for Sealapex compared to other sealers.[25]

The ability of any one particular sealer cement to penetrate dentinal tubules consistently and effectively and to produce less gaps between sealer and dentin will be one of the many factors which will influence the choice of material considered for root canal filling. This study was an attempt select a sealer with better performance in terms of marginal adaptation with regards to the patients undergoing or have completed radiation treatment. Therefore, it is important to validate the results from in vitro studies with findings from clinical cases.

CONCLUSION

- Root canal treatment after therapeutic cancer radiation resulted in significant decrease in marginal adaptation of sealers (decreased penetration of sealers into the dentinal tubules and increased gap between sealer and dentin) irrespective of the material used compared to the non irradiated group.
- In both irradiated and non irradiated groups, Epoxy resin based AH Plus sealer revealed better penetrability.
- Minimum Gap formation was observed for bioceramic sealer in both irradiated and non irradiated groups.
- Better penetration for Epoxy resin based sealer and Calcium hydroxide based sealer were revealed at coronal third than the middle third.
- Higher penetration for bioceramic sealer was revealed at the apical third in both irradiated and non irradiated groups.
- Root canal treatments can be performed using bioceramic sealers after therapeutic cancer radiation but it is preferable to perform root canal treatment before the irradiation protocol to guarantee better marginal adaptation of the sealer to the dentin.

SUMMARY

Radiotherapy is one of the primary treatments for head and neck cancers. The risk of developing caries increases substantially after radiotherapy because radiation causes direct alterations in the enamel and dentine ultrastructure. Considering the changes in the oral tissue, radio-xerostomia and radiation caries occur following radiotherapy. These individuals are thus more susceptible to develop pulpal alterations with a higher chance of requiring endodontic treatment. According to Martins et al., radiation therapy performed before endodontic treatment reduced the marginal adaptation of the filling material to root dentin, regardless of the sealer type used, once it damages the dentin collagen fiber network. The ability of any one particular sealer cement to penetrate dentinal tubules consistently and to reduce marginal gaps effectively will be one of the many factors influencing the choice of material for filling. It is therefore important to evaluate the behaviour of different types of root canal sealers before root canal filling of teeth in patients who have undergone radiation therapy.

The aim of this study was to evaluate the Influence of Therapeutic cancer Radiation on marginal adaptation of root canal sealer to dentin in teeth filled with AH-Plus, Sealapex and BioRoot RCS sealers.

Fifty-four human mandibular second premolar were selected for the study based on the selection criteria. The teeth were stored in labelled plastic vials containing artificial saliva and were randomly assigned to 2 Groups, Group 1- Irradiated (n=27), Group 2 - Non irradiated (n=27). 27 samples were irradiated with 60 Gy cumulative radiation for 5 consecutive days per week for 6 weeks over a period of 30 days. The Samples were stored in artificial saliva in between the radiation which was renewed daily. All samples were de-coronated and conventional root canal therapy was done with Protaper gold rotary files. These 27 samples were further divided into three subgroups of 9 samples each. Each subgroup was then filled with AH Plus, Sealapex, BioRoot RCS respectively and obturated with single cone technique. 1mm sections of apical, middle and cervical 1/3rd were taken using water cooled low speed saw. All specimens were evaluated using Scanning Electron Microscopy.

It was found that the AH Plus group had a higher depth of sealer penetration than the other Groups in both irradiated and non irradiated groups.

The BioRoot RCS group revealed minimum gap formation at the apical, middle and cervical levels and the highest penetrability was at the apical third when compared with the other Groups of sealers that were evaluated in the study in both irradiated and non irradiated samples.

The Irradiated Group of teeth showed significantly lower penetration and higher gaps compared to the non irradiated Groups.

Therefore it was seen in this study that endodontic therapy performed before radiation treatment reduced the marginal adaptation of the filling material to the root dentin, regardless of the sealer type used.

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ANNEXURE

ST.GREGORIOS DENTAL COLLEGE

UNDER THE MANAGEMENT OF MJSCE TRUST, PUTHENCRUZ

CHELAD , KOTHAMANGALAM, ERNAKULAM DIST, KERALA - 686681

ETHICAL CLEARANCE CERTIFICATE

SGDC/152/2017/1733/4 Date:- 20-10-2017

To,

Dr. Asha Pius
St. Gregorios Dental College
Chelad, Kothamangalam

Dear Dr. Asha Pius,

Subject:- Ethics Committee Clearance Reg.

Protocol – Evaluation and comparison of the effect of therapeutic cancer radiation on marginal adaptation of epoxy, calcium hydroxide and bioceramic based root canal sealer to root dentin: An *in vitro* study

After the Institutional Ethics Committee (IEC) held on 20th of October, 2017, this study was examined and discussed. After the consideration, the committee had 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.CKK Nair - Former BARC scientist
2. Dr.Ommen Aju Jacob - Dean, St. Gregorios Dental College, Chelad
3. Dr.Cinu Thomas A - Scientist, Senior Lecturer, Department of Pharmaceutical Sciences Centre for Professional and Advanced Studies
4. Rev. Fr. Shanu K. Paulose
5. Lissy Jose – Former Member Women's Welfare Association
6. Adv. Jose Aranjani - Advocate
7. Dr.Sauganth Paul - Senior Lecturer, Department of Biochemistry, St.Gregorios Dental College
8. Dr.Eapen Cherian - Secretary
9. Dr.Jain Mathew - Principal and Head of the Department, Department of Conservative Dentistry and Endodontics.
10. Dr.George Francis - Head of the Department, Department of Prosthodontics Crown & Bridge
11. Dr.Binnoy Kurian - Head of the Department, Department of Orthodontics & Dentofacial Orthopaedics

Dr.CKK Nair
Chairman Institutional Ethics Committee

St. Gregorios Dental College 686 681

Dr.Eapen Cherian
Secretary

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LIST OF ABBREVIATIONS USED

(In alphabetical order)

	Abbreviations	**Descriptions**
1	ANOVA	Analysis of Variance
2	BS	Bond Strength
3	CEJ	Cemento-Enamel Junction
4	DEJ	Dentino-Enamel Junction
5	EDTA	Ethylene Diamine Tetraacetic Acid
6	GP	Gutta Percha
7	KH	Knoop Hardness
8	MTA	Mineral Trioxide Aggregate
9	NaOCl	Sodium Hypochlorite
10	RCS	Root Canal Sealer
11	RT	Radiation Therapy
12	VHN	Vickers Hardness Number
13	ZOE	Zinc Oxide Eugenol

GROUP 1 - SEALER PENETRABILITY

	Subgroup 1a		
	APICAL	MIDDLE	CERVICAL
1	38.79μm	72.42μm	100.40μm
2	60.55μm	115.07μm	87.87μm
3	72.95μm	92.70μm	116.76μm
4	62.40μm	89.26μm	102.39μm
5	65.59μm	83.76μm	94.96μm
6	62.97μm	90.52μm	114.55μm
7	55.40μm	101.67μm	112.09μm
8	82.37μm	95.66μm	93.33μm
9	86.24μm	86.79μm	85.67μm

	Subgroup1b		
	APICAL	MIDDLE	CERVICAL
1	19.78μm	32.19μm	25.87μm
2	29.81μm	20.98μm	47.33μm
3	23.78μm	42.31μm	31.97μm
4	12.82μm	25.78μm	42.31μm
5	9.87μm	20.89μm	33.86μm
6	8.19μm	31.79μm	35.91μm
7	13.28μm	32.97μm	45.13μm
8	19.83μm	21.98μm	34.68μm
9	6.56μm	20.32μm	35.99μm

	Subgroup 1c		
	APICAL	MIDDLE	CERVICAL
1	99.38μm	68.72μm	102.27μm
2	90.68μm	72.69μm	76.87μm
3	58.71μm	74.15μm	103.21μm
4	68.92μm	95.99μm	99.40μm
5	62.19μm	99.62μm	113.82μm
6	63.78μm	88.51μm	111.16μm
7	53.91μm	79.96μm	95.62μm
8	64.38μm	59.32μm	114.78μm
9	65.18μm	58.23μm	112.76μm

GROUP 1 - GAP FORMATION

	Subgroup 1a		
	APICAL	MIDDLE	CERVICAL
1	6.28µm	4.83µm	3.12µm
2	6.63µm	4.54µm	3.42µm
3	6.5 µm	4.95µm	3.45µm
4	6.2 µm	4.73µm	4.40µm
5	7.83µm	3.94µm	3.85µm
6	5.93µm	3.18µm	4.63µm
7	6.81µm	4.19µm	3.02µm
8	7.01µm	3.48µm	3.68µm
9	6.59µm	3.56µm	3.21µm

	Subgroup 1b		
	APICAL	MIDDLE	CERVICAL
1	9.31µm	8.07µm	8.42µm
2	9.60µm	7.43µm	8.00µm
3	10.73µm	7.72µm	9.07µm
4	8.84µm	7.94µm	8.39µm
5	8.35µm	7.29µm	8.72µm
6	9.25µm	7.69µm	8.62µm
7	8.70µm	8.53µm	8.31µm
8	9.56µm	8.15µm	9.09µm
9	9.33µm	7.75µm	8.92µm

	Subgroup 1c		
	APICAL	MIDDLE	CERVICAL
1	2.69µm	2.16µm	2.81µm
2	3.40µm	2.5 µm	1.41µm
3	3.74µm	1.52µm	2.64µm
4	2.39µm	2.53µm	2.36µm
5	2.10µm	2.53µm	1.92µm
6	2.95µm	2.37µm	2.35µm
7	3.25µm	2.76µm	2.46µm
8	2.51µm	2.29µm	2.69µm
9	2.82µm	1.55µm	2.22µm

GROUP 2 - SEALER PENETRABILITY

	Subgroup 2a		
	APICAL	MIDDLE	CERVICAL
1	106.45µm	196.83µm	192.96µm
2	112.42µm	163.74µm	248.59µm
3	82.16µm	167.34µm	207.61µm
4	75.83µm	184.57µm	231.31µm
5	89.02µm	193.56µm	228.59µm
6	93.59µm	129.26µm	248.62µm
7	159.38µm	189.22µm	216.78µm
8	118.29µm	195.57µm	238.54µm
9	122.15µm	158.29µm	169.42µm

	Subgroup 2b		
	APICAL	MIDDLE	CERVICAL
1	154.04µm	199.00µm	231.74µm
2	34.29µm	139.01µm	177.33µm
3	64.81µm	89.69µm	201.53µm
4	26.21µm	49.02µm	112.47µm
5	39.51µm	89.22µm	126.21µm
6	68.00µm	94.14µm	119.36µm
7	44.1µm	75.2µm	108.54µm
8	27.59µm	77.5µm	135.79µm
9	60.29µm	126.59µm	92.05µm

	Subgroup2c		
	APICAL	MIDDLE	CERVICAL
1	132.09µm	69.20µm	41.43µm
2	80.63µm	59.05µm	45.86µm
3	126.61µm	147.07µm	46.83µm
4	154.56µm	62.18µm	59.16µm
5	98.6µm	101.6µm	28.29µm
6	105.1µm	161.82µm	62.52µm
7	132.5µm	98.5µm	53.16µm
8	152.3µm	86.29µm	70.26µm
9	197.6µm	75.8µm	63.59µm

GROUP 2 - GAP FORMATION

	Subgroup 2a		
	APICAL	MIDDLE	CERVICAL
1	7.23µm	4.22µm	0.60µm
2	6.80µm	3.94µm	1.35µm
3	3.87µm	4.96µm	0.63µm
4	2.20µm	5.68µm	1.89µm
5	5.87µm	2.40µm	2.83µm
6	6.14µm	3.38µm	3.92µm
7	4.62µm	2.88µm	3.08µm
8	4.89µm	4.93µm	1.52µm
9	6.68µm	3.49µm	2.64µm

	Subgroup 2b		
	APICAL	MIDDLE	CERVICAL
1	5.49µm	0.62µm	1.80µm
2	2.97µm	0.66µm	2.12µm
3	5.62µm	3.51µm	4.29µm
4	4.32µm	2.59µm	3.75µm
5	6.93µm	5.04µm	2.09µm
6	6.02µm	4.25µm	3.12µm
7	5.32µm	3.25µm	2.59µm
8	5.25µm	4.71µm	2.29µm
9	6.59µm	5.29µm	1.28µm

	Subgroup 2c		
	APICAL	MIDDLE	CERVICAL
1	2.01µm	0.49µm	4.13µm
2	2.71µm	2.97µm	6.62µm
3	1.39µm	1.21µm	6.67µm
4	3.67µm	1.08µm	5.87µm
5	2.53µm	2.37µm	4.39µm
6	0.71µm	2.62µm	3.78µm
7	0.96µm	1.89µm	2.98µm
8	0.29µm	1.45µm	3.32µm
9	1.82µm	1.69µm	2.13µm