MICROLEAKAGE ASSESSMENT OF NEW ZINC OXIDE NANO PARTICLE SEALERS, BY GLUCOSE PENETRATION MODEL - AN IN VITRO STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

APRIL 2016

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"MICROLEAKAGE ASSESSMENT OF NEW ZINC OXIDE NANO PARTICLE SEALERS, BY GLUCOSE PENETRATION MODEL- AN IN VITRO STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. Anil Kumar M.D.S.,** Professor, Department of Conservative Dentistry and Endodontics, Ragas Dental College and Hospital, Chennai.

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CERTIFICATE

This is to certify that this dissertation titled **"MICROLEAKAGE ASSESSMENT OF NEW ZINC OXIDE NANO PARTICLE SEALERS, BY GLUCOSE PENETRATION MODEL- AN INVITRO STUDY"** is a bonafide record work done by **Dr. SANDEEP NULI** under our guidance during his postgraduate study period between 2013 - 2016.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICALUNIVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY – CONSERVATIVE DENTISTRY AND ENDODONTICS, BRANCH IV**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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ACKNOWLEDGEMENT

I take this opportunity to express my gratitude to my post graduate teacher, mentor and guide ***Dr. R. Anil Kumar, M.D.S., Professor*** *Department of Conservative Dentistry and Endodontics, Ragas Dental College and Hospital, for his support throughout my postgraduate curriculum. I thank him for his guidance without which this dissertation would not have come true.*

My sincere thanks to ***Dr. R. Indira, M.D.S., Professor*** *and* ***HOD****, Department of Conservative Dentistry and Endodontics, Ragas Dental College and Hospital, who have helped me with her guidance, support and constant encouragement throughout my study period wherever and whenever needed.*

My sincere thanks to ***Dr. S. Ramachandran, M.D.S., Professor & Principal****, Department of Conservative Dentistry and Endodontics, Ragas Dental College and Hospital, who has helped me with his advice and immense support throughout my post graduate curriculum.*

I extend my sincere thanks to ***Dr.P.Shankar, M.D.S., Professor,*** *Ragas Dental College and Hospital, for his guidance, and constant encouragement throughout my study period.*

*I extend my sincere thanks to**, **Dr. C.S. Karumaran, M.D.S., Professor,** for his constant encouragement throughout my postgraduation course.*

*My sincere thanks to **Dr. M. Rajasekaran, M.D.S., Professor,** Ragas Dental College and Hospital, for his encouragement, support and guidance all throughout my study period.*

*I would like to solemnly thank **Dr. Veni Ashok, M.D.S., Associate professor,** for all the help during my study period.*

*I would also like to thank **Dr. S.M. Venkatesan, M.D.S., Dr. Shankar Narayan, M.D.S., Readers** for guidance during my study period.*

*I would also like to thank **Dr. M. Sabari, M.D.S., Dr. Aravind Vikram, M.D.S., Dr. B. Venkatesh, M.D.S., Senior Lecturers** for their friendly guidance and support.*

*I would like to thank my friend **Dr. Ramesh M.V. M.D.S.** for his guidance and help in biostatistics.*

*I would also like to thank **Dr. Chitra Shankar M.D.S. professor** department of prosthodontics for her guidance with my write up.*

*I would also like to thank **Mr. S.Robert raj BSc., DMLT.,** Shivani Diagnostic Centre for his help in my study for SPECTROPHOTOMETRIC analysis.*

I also wish to thank the management of Ragas Dental College and Hospital, Chennai for their help and support.

*I also wish to thank my juniors **Dr. Amit Kumar, Dr. Shalini,** for their support throughout my dissertation work.*

*I remain ever grateful to all **my batch mates, colleagues and friends** for their support.*

*I would like to especially thank **my father Mr. Prakash B.E., and mother Mrs. Lalitha** for their love and affection, encouragement throughout my life, without which I would be nowhere.*

*I would like to thank **my brothers Mr. Swaroop, M.Tech., Mr.Sanjay C.A.,** for their constant support to join in this course.*

*My sincere thanks to **Mr. K. Thavamani** and **Miss. R. Sudha** for their guidance and support in DTP and Binding works.*

*Above all, I am thankful to **God**, who always has his protective hand over me and has given these wonderful people in my life.*

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Introduction

INTRODUCTION

Endodontic treatment is a combination of mechanical instrumentation of the root canal space, chemical debridement and filling the space with an inert material, which aids to maintain or restore the health of periradicular tissues.[43] The three phases of the endodontic treatment are diagnostic phase, preparatory phase and filling phase.[71] For a better prognosis of the endodontic treatment, all the three phases should be followed meticulously. Failure of the endodontic treatment may be due to microleakage (apical or coronal) that have occurred during or after root canal therapy (Dow and Ingle 1955; Madison and Wilcox 1988).[52] Apical leakage is considered as one of the common reason for failure of the endodontic treatment.[60]

The aim of root canal treatment is to obtain a "hermetic seal". The word Hermetic seal is inappropriate; instead *fluid-tight*, *fluid-impervious*, or *bacteria-tight* seal are more relevant and contemporary. Historically various filling materials were used for the filling of the root canal space. Currently, root canal filling materials are available as solid-core filling material (silver points), semi-solid core filling materials (gutta-percha), and paste filling materials (zinc oxide containing paste systems). Gutta-percha, a semi solid core material is the most popular and commonly used root canal filling material. It meets most of the ideal requirements of a root canal filling material; however, major disadvantage of it is that it does not adhere with the canal wall. This problem can be overcome to a certain extent with the help of

sealers, which helps in the adhesion of the core material with the root canal wall, and aids in providing a fluid tight seal.[74]

"Root canal sealer is a radiopaque dental cement used usually in combination with a solid or semi-solid core material, to fill voids and to seal root canals during obturation" (glossary of endodontic terms). These root canal sealers available were classified according to Ingle as, Zinc Oxide containing sealers, Calcium Hydroxide containing sealers, Resin sealers, Glass Ionmer based sealers, Silicone-Based sealers, Solvent based sealers, Urethane Methacrylate Sealers, Paraformaldehyde based sealers.[74]

Zinc oxide-eugenol sealer have been used most commonly for sealing root canals and was introduced by Rickert and Dixon, later, Grossman modified the formulation.[69] Zinc oxide is a valuable antimicrobial component in the sealer and provides cytoprotection to tissue cells.[56] For patients allergic to eugenol, few eugenol free formulations are available.

Even the resin based sealers have a good track of success in obturating the root canal treated teeth. These sealers are known for their adhesive property to the root canal wall. AH 26 and its successor AH Plus sealer are epoxy resin based sealers that has high radiopacity, low solubility, slight shrinkage and tissue compatibility. However, AH 26 sealer had few drawbacks like formaldehyde release and extended setting time. It led to the introduction of new AH plus sealer that has overcome the problem of long setting time and formaldehyde release.[36]

Numerous studies[66] have been carried out to compare the sealing property of various sealers, but there was hardly any consensus. Some studies showed that resin-based sealer provided better seal than other sealers (Timpawat S et al 2001, Miletić I et. al. 1999)[59,39] and others indicated that there was no significant difference in leakage of different types of sealers. (Chailertvanikul P et al. 1996, Kataoka H, et al 2000).[7,30] Hence in the present study, zinc oxide based sealer and resin based sealers were compared.

The physical and chemical properties of the sealers such as the film thickness, microleakage, and antibacterial activity can play an important role in sealing of the root canals(Wu et al. 2000).[52] According to ISO 6876-2001 ADA 57 requirements, the film thickness of a sealer should be <50µm. The particle sizes of the sealer plays an important role in the manipulation of the sealer and its flow. Smaller the particle size, easier the manipulation of the cement with less time needed for mixing, and the resultant cement can be smoother and have a better flow. [20, 21] The nano grade particle size sealers can have a better sealing ability than the conventional counterpart.[26]

'Nano' is a Greek word for 'dwarf little old man'. A nanometer is 10^{-9} or one billionth of a meter.[78] Nano technology deals with the manipulation of matter at nano level sizes. Nanotechnology is used in manufacturing of various dental materials like Light polymerization composite resins and bonding systems, imprint materials, ceramics, coatings for dental implants, bioceramics, mouthwashes containing fluoride and fissure sealant materials.[26]

These nano particles have superior activity because of the higher surface area that enables it to achieve a greater degree of interaction (Kishen, *et al*., 2008).[24] and the nano particles can penetrate into the dentinal tubules to provide 'nano retention'. (Mitra et al., 2003)[46] Many attempts have been made to utilize these nano particles in the endodontic treatment to enhance the treatment outcome by enhancing the beneficial properties of these materials, to act with better antimicrobial activity, radiopacity, flow, film thickness, cytotoxicity and the sealing ability[26] and to counter act the microleakage in the obturated root canals.

Microleakage (apical or coronal) may cause failure of root canal therapy (Dow and Ingle 1955; Madison and Wilcox 1988)[52]. In some instances, persistence of bacteria in the root canal system, or leakage of the bacteria into the canal (U.Sjogren, 1997) can cause root canal treatment failure. Failures can also be due to over extension of the filling material, quality of seal, or procedural errors like over instrumentation, over filling, under filling, perforations.[3]

Many experimental models are available to detect and measure leakage along the root fillings. Dye leakage, fluid transport and bacterial penetration models were used. Xu et al. (2005) discussed a model that measures the leakage using glucose molecule as a tracer.[54] With the glucose penetration model it was possible to continuously quantify the endodontic microleakage over time by analyzing the cumulative value of leaked glucose.

Hence the glucose penetration model was selected for the evaluation of microleakage in the present study.

Zinc oxide sealer has been selected in the present study for comparison. To evaluate the performance of smaller particle size of same materials in similar conditions, the nano powders were selected as new experimental sealers[26] and was also compared with AH Plus sealer, an AH group material with long successful record.

(In this present study the terms particle size and powder size denotes the same)

Aim and Objectives

AIM AND OBJECTIVES

AIM:

Evaluation and comparison of the microleakage for Nano zinc oxide powder sealers, with the conventional zinc oxide eugenol sealer and AH Plus sealer using glucose penetration model.

OBJECTIVE:

1. To evaluate the microleakage of Nano zinc oxide powder sealers compared with that of conventional zinc oxide eugenol sealer, AH Plus sealer, along with a positive control group and negative control group.
2. To evaluate the progression of microleakage of these sealers for a duration of 30 days.

Review of Literature

REVIEW OF LITERATURE

Younis O et al (1976)[68] Investigated the sealing ability of various root canal sealer. One hundred five single-rooted teeth were prepared and filled with ten commercially available root canal paste sealers. Five teeth were filled with only gutta-percha points, fifty teeth were filled with sealer alone, and the rest of the teeth were filled with sealer in conjunction with gutta-percha points. It was found that the gutta-percha point alone is not sufficient for sealing the periapical foramen; nor are N2 (Sargenti), Riebler's paste, and the iodoform paste. In general, it was found that the combination of gutta-percha and cement is more effective in sealing the periapical foramen.

Sandra Madison, (1984)[50] Evaluated the effect of a chelating agent on the apical seal of endodontically treated teeth. The root portions of freshly extracted, single-rooted, human teeth were instrumented while irrigating with a 2.5% solution of NaOCl. Step back flaring was done using either NaOCl alone or an aqueous solution of REDTA in combination with NaOCl for irrigation. The teeth were obturated with gutta-percha and Grossman's sealer. Apical leakage was evaluated by measuring the linear penetration of a 1% solution of methylene blue dye. The results showed no significant differences in dye penetration between the groups regardless of the irrigating solution used.

Kennedy WA et al (1986)[31] Evaluated the effect of smear layer on the apical leakage. Thirty-four teeth with smear layers and 34 teeth without smear layers were tested in vitro to evaluate the effects on apical leakage. These teeth were obturated using Hydron or chloroform-softened gutta-percha master cones with sealer and lateral condensation. Apical leakage was significantly increased ($p<0.001$) in gutta-percha-filled canals with intact smear layers. Smear removal had no effect on leakage in Hydron-obturated teeth. Moreover, Hydron-filled canals showed significantly less apical leakage than the best of the gutta-percha groups.

Cergneux M et al (1987)[6] Evaluated the effect of smear layer on the sealing ability of obturation. 60 single rooted teeth were used and were divided into group I (control group); in group II (ultrasound group), and in group III (EDTA group). All speciments were then subjected to dye infiltration before being transversely sectioned at various levels from the apex. The results were: EDTA-treated canals showed the least infiltration, while those treated with ultrasound showed significantly less compared with the control group.

Fogel HM et al (1988)[19] Reported the hydraulic conductance of radicular dentin. Dentin slabs prepared from human third molar teeth were placed in a split-chamber device to permit quantitation of fluid filtration rate. Dentinal tubule numbers and diameters were recorded using SEM. The results indicated that hydraulic conductance decreased with distance from the pulp

and with increasing dentin thickness. Tubule density and diameter correlated well with the measured hydraulic conductance. The relatively low hydraulic conductance of outer root dentin makes it a significant barrier to fluid movement across root structure.

Fan B et al (1999)[18] Investigated the seal compromise caused to root canal filling by post space preparation. Eighty human mandibular premolars each with a single canal were obturated with laterally condensed gutta-percha cones and a sealer. Immediate post space preparation was carried out on half the number of teeth and delayed post space preparation on the remaining 40 teeth. Leakage along the apical root fillings was determined using a fluid transport device under a head space pressure of 30 kPa (0.3 atm). More leakage was found after delayed preparation than after immediate preparation ($P = 0.0059$).

Abarca AM et al (2001)[2] Compared the apical sealing ability and extrusion between thermafil and lateral condensation. The experimental group was obturated using the Thermafil technique and the control group was obturated using the lateral condensation technique. Topseal sealer was used in both groups. Apical extrusion was recorded. All specimens were stored in 100% humidity for 1 week, and were suspended in black India ink for 48 hours. Molars were decalcified, rendered transparent, and linear dye penetration was measured. Linear dye leakage and apical extrusion between the techniques were not statistically different (Mann-Whitney U test).

Wu MK et al (2002)[63] Determined the long-term sealing ability of root canal sealer RSA RoekoSeal. With the use of a fluid transport model, leakage along entire root fillings was measured before post space preparation. After post space preparation, leakage along the remaining apical root fillings was measured repeatedly at 1 week, 1, 2, 6, 12, and 18 months respectively and recorded in microliters per day. RSA used in combination with either cold laterally compacted or warm vertically compacted gutta-percha provided a consistent seal during a period of 18 months.

Huang FM (2002)[23] Evaluated the cytotoxicity of three different types of root canal sealer on human periodontal ligament (PDL) cells and a permanent hamster cell line (V79 cells). Set specimens from two resin based sealers (AH26 and AHPlus), three zinc oxide–eugenol based sealers (Canals, Endomethansone and N2) and one calcium hydroxide-based sealer (Sealapex) were eluted with culture medium for 1, 2, 3 and 7 days. Cytotoxicity was judged using tetrazolium bromide. The results showed that elute from sealers were cytotoxic to primary human PDL cultures and V79 cells. Calcium hydroxide-based sealer was the least toxic sealer.

Wu MK et al (2003)[64] Compared the fluid movement (FM) along the coronal two-thirds of gutta percha, sealer root fillings. Three groups obturated by cold lateral compaction (LC), warm vertical compaction (VC) or the single-cone technique (SC), using RoekoSeal Automix (RSA) as the sealer. The apical 4 mm of each root filling was removed, and FM along the remaining

7 mm was measured. The VC group displayed more FM than the other two groups (P = 0.023). No significant difference in FM was found between the LC and SC groups (P = 0.629). The coronal two-thirds of the VC root fillings did not prevent FM when RSA sealer was used.

Clark-Holke D et al (2003)[13] Investigated if the smear layer affects the passage of bacteria through or around obturating material. Removal of the smear layer was accomplished by rinsing with 17% EDTA. Standardized suspensions containing Fusobacterium nucleatum, Campylobacter rectus, and Peptostreptococcus micros were inoculated. Models were incubated anaerobically at 37 °C. Leakage results were: (1) smear layer present-6/10 leaked; (2) smear layer removed-0/10 leaked; (3) negative control-0/10 leaked. F. nucleatum was the predominant microorganism and removal of the smear layer reduced the leakage of bacteria through the root canal system.

Cobankara FK et al (2004)[14] Evaluated the effect of the smear layer on apical and coronal leakage in root canals obturated with AH26 or RoekoSeal sealers. Eight groups were created by all possible combinations of three factors: smear layer (present/absent), leakage assessment (apical/coronal), and sealer used (AH26/RoekoSeal). A fluid filtration method was used for evaluating leakage. The results proved that, the smear (+) groups displayed higher apical and coronal leakage than those smear (-) groups for both root canal sealers ($p < 0.05$) and apical leakage was significantly higher than coronal leakage for both root canal sealers used in this study ($p < 0.05$).

Ozturk B (2004)[45] Compared the sealing properties of five different dentine adhesives: Prime&Bond NT (PBNT); Prompt L-Pop (PLP); Clearfil SE Bond (CSEB); Scotchbond Multi-Purpose Plus (SMPP); EBS-Multi (EBSM) inside the pulp chamber. The samples were connected to Plexiglass plates, and a fluid filtration method and the resin-dentine interfaces were observed under a scanning electron microscope. PBNT and PLP had the least leakage during immediate measurements, after 1 month, leakage of all groups was not significantly different ($P < 0.05$). None of the materials had created a perfect seal to the pulp chamber walls.

Camps J et al (2004)[5] Investigated the effects of modifications of the powder/liquid ratio by endodontists on the physical properties and other clinically relevant properties of zinc oxide-eugenol-based root canal sealers according to ISO standards. On increased powder/liquid ratio led to a decreased flow, an increased radiopacity and a decreased amount of eugenol released. The variations in the powder/liquid ratio did not influence the dimensional changes and the apical leakage. Variations in the powder/liquid ratio of zinc oxide-eugenol-based root canal sealers have a limited influence on the properties of the sealers.

Kokkas AB et al (2004)[33] Analyzed the effect of the smear layer on the penetration depth of three different root canal sealers into the dentinal tubules. 64 extracted single-rooted teeth were selected. Ten roots from each group were obturated with laterally condensed gutta-percha points and sealers

AH Plus, Apexit, and Roth 811, respectively. Examination in scanning electron microscope revealed that the smear layer obstructed all the sealers from penetrating dentinal tubules suggesting that smear layer plays an important role in sealer penetration into the dentinal tubules, as well as in the potential clinical implications.

Kishor Gulabivala et al (2005)[32] Evaluated the condition of the root canal wall surface. It may vary from that of an intact pulp-dentine complex, through partially degraded pulp tissue with infection, to a dentine surface coated with a mature bacterial biofilm. Subsequent treatment procedures will alter the surface in ways that depend upon the root canal anatomy, the instruments used, the strategy and mode of their use, and the chemicals used to facilitate debridement. These changes may have a profound effect on the survival of the tooth, both in terms of progression of apical periodontitis and the long-term integrity of the tooth. Therefore, a balance has to be achieved in delivering antibacterial agents effectively to the apical anatomy while maintaining tooth strength and integrity.

Chu CH et al (2005)[12] Evaluated the outcome of root canal treatment (RCT) using either Thermafil (TF) or lateral condensation (LC) as filling technique, and to compare the time required for the treatment. Post-treatment there was no statistically significant difference ($P>0.05$) for the presence of disease. Irrespective of the filling method, teeth restored with extracoronal restorations had a lower association with disease than intracoronal restorations

(7% vs. 30%; P=0.037). RCT took, on average, 20 min less when TF was used for filling compared with LC (98 min vs. 78 min, P = 0.003). The type of post endodontic restoration had a significant association with the presence of post-treatment disease.

Takatsuka T et al (2005)[58] Investigated the inhibitory effect of zinc oxide on dentine demineralization in vitro and in situ. Dentine specimens treated with a zinc oxide suspension were demineralized in a pH 5 solution and subjects wore dentine specimens on their teeth and instructed to rinse with zinc-containing toothpaste slurry three times a day for 14 days. Microradiography revealed that the dentine surfaces treated with distilled water had a lower mineral content than those treated by zinc. Toothpaste with zinc had a statistically significant, 49% greater inhibitory efficacy on dentine demineralization over the control group and proved that zinc oxide may be effective in the prevention of root caries.

Li P et al (2005)[35] Evaluated the cytotoxicity of a new nano-hydroxyapatite (n-HA) root canal sealer. MTT assay in vitro has been used, and culture medium F12 as control. Three concentrations of the soaking material cultured with mouse osteoblast separately, to test the cell relative growth rate (RGR) of every group. The toxicity graduation of the n-HA root canal sealer tends to 0 with the culture time increasing. The cell survival rate of n-HA root canal sealer group was relatively high. The result indicated that n-HA root canal sealer was compatible with the test cells.

Xu Q et al (2005)[66] Investigated a new method for quantitative testing of endodontic leakage. The samples were obturated with gutta-percha and Pulp Canal Sealer EWT, Sealapex, or AH Plus sealer. With the leakage test device, coronal 1 mol/L glucose solution was forced under a hydrostatic pressure of 1.5. Leakage was measured in apical reservoir at 1, 2, 4, 7, 10, 15, 20, and 30 days with the enzymatic glucose oxidase method. No significant difference of sealing ability was found among 3 test groups at 1, 2, 4, and 7 days. From the tenth day, Pulp Canal Sealer EWT showed the highest leakage, and the leakage was not significantly different between Sealapex and AH Plus. The quantitative method is sensitive, nondestructive, and clinically relevant. Pulp Canal Sealer EWT showed more leakage than Sealapex and AH Plus in most observation time.

Shemesh H et al (2006)[54] Compared two different experimental models by measuring leakage along root fillings with or without smear layer. Leakage of glucose was evaluated for a total period of 56 days using a glucose penetration model. Fluid transport was evaluated using a fluid transport model, 1 and 8 weeks after canal filling. Removing the smear layer before filling did not improve the sealing of the apical 4 mm of filling. Resilon allowed more glucose penetration but the same amount of fluid transport as the gutta-percha root fillings.

Karagenç B et al (2006)[28] Compared four different micro leakage tests used for the evaluation of the coronal seal of teeth obturated using Thermafil

or lateral condensation techniques. Coronal microleakage was assessed after exposing one group of specimens (n -15 teeth) from each of the obturation techniques to each of the four microleakage tests. In the fluid filtration test, lateral condensation showed statistically less leakage than the Thermafil technique (*P* - .05). Electrochemical and dye leakage test results showed no difference between the two obturation techniques (*P* -.05). However, in the bacterial leakage test, Thermafil showed less leakage than lateral condensation (*P* -.05). There is poor correlation between various methods to evaluate hydraulic leakage. The clinical significance of leakage tests in vitro is questionable.

Shemesh H M et al (2007)[53] Compared the glucose penetration and fluid transport through coronal root structure in leakage along the coronal region of root fillings. Ten roots were sectioned longitudinally and the apical portion was removed leaving a length of 9 mm. The groups presented significantly different glucose penetration ($P < 0.05$). No significant difference in leakage existed between the two vertically compacted filling materials, Resilon with Epiphany sealer and gutta-percha with AH26. Both models used samples of coronal root structure did not show any leakage.

Xu Q et al (2007)[67] Evaluated the sealing ability of 4 different obturation techniques by using a glucose leakage test. Samples were filled with gutta-percha and sealer by using either cold lateral compaction, warm vertical compaction, Thermafil, or the E & Q Plus system. No significant

difference in the cumulative amount of leakage was found among the 4 groups at 24 hours and 1 week. Lateral compaction showed significantly more leakage than the other 3 techniques at longer intervals. No significant difference was found between vertical compaction, Thermafil, and E & Q Plus at all observation times and showed a better sealing result than cold lateral compaction of gutta-percha at extended observation periods.

Hanley C et al (2008)[22] Investigated the potential utility of nanoparticles in biological applications including Nano medicine. ZnO nanoparticles exhibit a strong preferential ability to kill cancerous T cells compared to normal cells. Mechanisms of toxicity are by the generation of reactive oxygen species, with cancerous T cells producing higher inducible levels than normal T cells. In addition, nanoparticles were found to induce apoptosis and the inhibition of reactive oxygen species was found to be protective against nanoparticle induced cell death.

Javidi M et al (2008)[27] Evaluated the fluid filtration system for quantitative evaluation of microleakage in dental materials. The roots were connected to a tube filled with an underwater pressure supply. A bubble was introduced into the water to measure endodontic leakage. A digital camera and professional software were utilized to record and measure the bubble displacement. This system was efficient for the evaluation of micro leakage of dental materials.

Sun Let al (2008)[57] Discussed the preparation of nano-sized calcium fluoride (CaF_2) that could be used as a labile Fluoride reservoir for more effective Fluoride regimens and as an agent for use in the reduction of dentin permeability. Nano-sized CaF_2 powders were prepared using a spray-drying system with a two-liquid nozzle. The CaF_2 ion activity product (IAP) of the solution in equilibrium with the nano-CaF_2 was four times greater than macro CaF_2. The Fluoride deposition by the nano-CaF_2 rinse was greater than the Sodium Fluoride solution. The nano-CaF_2 can be used as an effective anticaries agent, tooth remineralizing agent and for reduction of dentin permeability.

Moradi S et al (2009)[41]Compared the apical leakage of roots obturated with gutta-percha using either an epoxy resin sealer (AH26) or a dual cure dentin binding agent (Excite DSC) as sealer in the presence or absence of smear layer with fluid filtration method. After 3 days and 3 months, the samples were connected to a fluid filtration system. Micro leakage in AH26 groups decreased significantly at 3 months compared to 3 days; however, in the DBA groups, the amount of micro leakage at 3 days and 3 months was not significantly different. DBA had better apical sealing ability and could be applied clinically.

Nair S et al (2009)[42] Investigated the role of nano size scale, surface capping, and aspect ratio of zinc oxide (ZnO) particles on toxicity toward prokaryotic and eukaryotic cells. Cytotoxicity was studied using a human

osteoblast cancer cell line and antibacterial activity using Gram-negative bacteria and Gram-positive bacteria. Scanning electron microscopy was conducted to characterize any visual features of the biocidal action of ZnO. The author observed that antibacterial activity increased with reduction in particle size and found to be consistent with a membrane-related mechanism for nanoparticle toxicity toward microbes.

Scarparo RK et al (2009)[51]Investigated the reaction of the subcutaneous connective tissue of rats to methacrylate resin-based sealer (EndoREZ), epoxy resin-based sealer (AH Plus), and zinc oxide– eugenol sealer (EndoFill). Polyethylene tubes containing the test materials were implanted in 18 rats. After 7, 30, and 60 days, tissues were collected for biopsy and fixed and processed for histologic evaluation. EndoREZ and EndoFill sealers showed a more intense and longer-lasting inflammation. With AH Plus, the inflammatory reaction showed a tendency to diminish over time. The only group to show a statistically significant reduction in inflammation during the 60-day period was the control group. None of the materials tested proved to have ideal characteristics for biocompatibility.

Rasmussen JW et al (2010)[47]Analyzed the biomedical applications of metal oxide and ZnO nanomaterials under development at the experimental, preclinical, and clinical levels. The author discussed on ZnO, and other metal oxide nanomaterial systems, and their proposed mechanisms of cytotoxic action, as well current approaches to improve their targeting and cytotoxicity

against cancer cells. The author concluded that the inherent toxicity and selectivity of ZnO nanoparticles against cancer may be further improved to make them attractive new anti-cancer agents.

Wong RH et al (2011)[62] Determined the effect on the physical properties of two commercially available zinc oxide non-eugenol temporary luting cements with incorporation of up to 8% (w/w) CPP-ACP. 0, 0.5, 1.0, 2.0, 3.0, 4.0 and 8.0% (w/w) CPP-ACP incorporated into Freegenol™ and Temp-Bond NE. Compressive and diametral tensile strengths progressively decreased with increasing concentrations of up to 8.0% (w/w) CPP-ACP incorporated into both Freegenol™ and Temp-Bond NE. Setting time was delayed beyond ISO requirements. Film thickness was not adversely affected. Increased solubility of Temp-Bond NE with 8.0% (w/w) CPP-ACP incorporation suggested an effect of the CPP-ACP on this property for this cement. Solubility investigations suggest that CPP-ACP leaches out of the zinc oxide non-eugenol luting cements into an aqueous environment.

Chandrasekhar V (2011)[10] Evaluated the three-dimensional expansion of gutta-percha at various powder/liquid ratios of ZOE-based sealer by using spiral computed tomography. The teeth were obturated with gutta-percha cones with Pulp Canal Sealer EWT with powder/liquid ratio of 1:1, 1:2, 1:3, and 1:4, and gutta-percha alone in control group. The filled volume in each canal was measured at 1 day, 7 days, and 1 month after obturation. The groups ZE 1:2 and ZE 1:3 gave the highest mean volume

values during a 1-month period and were significantly different in comparison with groups ZE 1:1 and ZE 1:4 ($P < .05$). Increasing the ratio of eugenol in sealer resulted in volumetric increase of gutta-percha.

Aal-Saraj AB et al. (2012)[1] Evaluated the antimicrobial activity of nano-hydroxyapatite epoxy resin-based sealer (Nanoseal) AH26, Tubliseal, Sealapex and Roekoseal against Enterococcus faecalis, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus sobrinus and Escherichia coli for up to 7 days. Agar diffusion was used in this study. The results of this study showed that all the test materials exhibited inhibition zones towards the tested micro-organisms for 7 days except for Roekoseal, which showed no inhibition zones. Nanoseal and AH26 exhibited similar zones of inhibition. Significant difference was found between Nanoseal and the other tested sealers ($P < 0.001$).

Collares FM et al (2012)[15] Evaluated the effect of different concentrations of nanostructured hydroxyapatite on the radiopacity, flow and film thickness of an experimental root canal sealer. A dual-cured root canal sealer was produced with a methacrylate-based co-monomer blend. Nanostructured hydroxyapatite/calcium tungstate solutions (ratios 10:90, 20:80, 30:70 and 40:60) were added to produce the sealer. All groups had levels of radiopacity and film in accordance with ISO 6876. The flow of the experimental sealers was not significantly different. The addition of up to 40%

HA (nano) to root canal sealers did not alter their radiopacity and film thickness.

De-Deus G et al (2012)[16] Determined the correlation between leakage and sealer penetration into dentinal tubule. Teeth were placed into a device to assess glucose penetration using 15 psi pressure application. After 1 h, glucose concentrations in the lower chamber were measured. Each specimen was then sectioned horizontally at 3, 6 and 8 mm from the apex, and a standard metallographic preparation was performed. The coronally facing surface of each slice was examined in a high-resolution stereomicroscope and under Confocal Laser Scanning Microscope. The Spearman correlation test revealed no significant correlation between the two factors analyzed (P = 0.082). There was no significant correlation between sealability of a sealer and its ability to penetrate into dentinal tubles.

Mirhashemi AH (2013)[40] Evaluated the antimicrobial effects of ZnO-NP and CS-NP-containing orthodontic composite against Streptococcus mutans, Streptococcus sanguis and Lactobacillus acidophilus which were grown in planktonic and as a biofilm forms on composites. One control group and three groups consisting ZnO-NPs and CS-NPs mixture: 1%, 5% and 10% (1:1 w/w). Disc agar diffusion (DAD) test was done to determine antimicrobial effects. Viable counts of microbes on days 3, 15 and 30. In biofilm formation test, a reduction in bacterial counts was observed with 10% nanoparticle-containing composites. ZnO-NPs and CS-NPs has induced

an antibacterial activity in resin composite; especially in 10% weight concentrations.

Ilic DV (2013)[25] Investigated the flow of two zinc-oxide eugenol sealers Roth 801 and Endomethasone in regard to the applied force and a variation of sealer's components. The control group results displayed Roth 801 as less viscous than Endomethasone sealer ($p < 0.01$). Application of 1 or 2 kg pressure on the samples of both sealers does not significantly affect the flow values. The flow values comparison of the regular to thick consistency of Endomethasone were not statistically significant while comparison of its regular to thin mass shows a significant difference.

Javidi M et al (2014)[26] Evaluated the sealing ability of new experimental nano‐ZOE‐based sealer. Three types of nano‐ZOE‐based sealer (calcined at 500, 600 and 700°C) with commercially available sealers (AH26 and micro‐sized zinc oxide eugenol sealer) were used for obturation with gutta percha.After 3, 45 and 90 days, the samples were connected to a fluid filtration system. The synthesized powders an average particle size of about 30 ‐60 nm at different calcination temperatures. Microleakage in AH26 and Zno-micro powders groups was significantly more than Zno nano powder sealer. But the sealing ability did not differ significantly.

Samy Ali Hussein et al (2014)[49] Reported the protective effect of zinc oxide nanoparticles (ZnONPs), on oxidative stress in experimental induced

diabetes in rats were evaluated. The blood glucose,serum insulin, malondialdehyde (MDA) and serum nitric oxide (NO) levels were determined after 15 and 30 days of ZnONPs and/ or insulin treatment. The results indicated that the blood glucose, serum insulin, MDA and NO levels were increased while serum insulin levels were decreased in diabetic rats, while they are significantly modified in rats that administrated ZnONPs and/or insulin in a dose dependent manner. The author concluded that the zinc oxide nanoparticles act as potent antidiabetic through decreasing of blood glucose and increasing of serum insulin as well inhibition of lipid and protein free radicals.

Materials and Methods

MATERIALS AND METHODS

ARMAMENTARIUM:

- Extracted mandibular premolar teeth
- Satelec X-ray unit (70kVp, 8mA) Class 1 Type 2
- Sopix 2 RVG unit (ACTEON)
- Sopro imaging software
- K- files (10# to 80# MANI, INC)
- Gates-Glidden drills (2# to 6# MANI, INC)
- Finger spreaders (15# to 40# MANI,INC)
- Hand plugger (Dispodent)
- 3% sodium hypochlorite for irrigation & cleaning
- Chelating agent – (Endo prep R C- Annabond)
- #2 Endoaccess bur (Dentsply)
- Aerotor hand piece (NSK PANAAIR)
- Contra angle micro motor hand piece (NSK)
- Diamond disc and lab micro-motor
- 2% Gutta-percha (DENTSPLY)
- Paper points (dentsply)
- Nano Zinc oxide powder 30nm (SRL CHEM)
- Nano Zinc oxide powder 240nm (SRL CHEM)
- Conventional zinc oxide powder (DEEPAK ENTERPRISES)

- Eugenol liquid (DEEPAK ENTERPRISES)
- AH plus sealer (DENTSPLY)
- Magnifying loupes
- 0.2% Sodium azide solution (CHENCHEMS)
- 0.2% Sodium azide solution with 1mol/L glucose (CHENCHEMS)
- 10 microliter pipette
- Self cure acrylic resin (DPI)
- Glass pipette (16 cm)
- 15ml glass bottles
- Rubber tube
- Sticky wax
- Cyano acrylate glue
- Stainless steel ligature wire (DENTSPLY)
- 2ml syringe (unolok)

SPECIAL EQUIPMENT:

- Spectrophotometer (PRIMACHEM V-2)
- Glucose analysis kit (ASPEN LABORATORIES)
- Incubator (TECHNICO)

SPECIMEN SELECTION:

90 extracted human mandibular premolars were selected.

Inclusion Criteria:

- Tooth with mature apices
- Single rooted

Exclusion Criteria :

- Tooth with root caries
- Tooth with cracks and fractures

SPECIMEN PREPRATION:

90 extracted human mandibular premolar teeth (figure 1)with intact roots were taken and immersed in sodium hypochlorite(3%) and their external surfaces were carefully cleaned of calculus and debris with an ultrasonic scaler and washed under running water. The teeth were radiographed from the bucco-lingual and mesio-distal directions using satelec x-ray unit and RVG digital sensor. The Xray tube was angulated at 0° angulation and radiographed. Teeth with single root canal were selected and stored in 0.2% sodium azide solution and were decoronated horizontally below the cemento-enamel junction using a diamond disc mounted and lab micro motor to uniform lengths of 15mm (figure:10). The coronal 4mm of the root sample were embedded in acrylic resin to form an acrylic cylinder around the root (figure: 21) and enable contact with the rubber tube to connect with the glucose leakage apparatus during leakage phase of the study. All the

procedures were performed by a single operator. The teeth were divided into 4 experimental groups and 2 control groups with each group containing 15 samples.

PREPARATION OF THE SAMPLES:

GROUP A : NANO ZINC OXIDE POWDER SEALER (30nm)

Access cavity was prepared using a #2 Endoaccess bur and the patency of the canal was checked with a #10 size K file (figure: 11) and the working length was determined. The root canals were shaped using Step-Back technique and the apical portion was enlarged with #15 size K file using 15% EDTA with 10 % carbamide peroxide gel during the instrumentation followed by irrigation with 3% sodium hypochlorite. Then #20 size K file was again coated with 15% EDTA with 10% carbamide peroxide gel and the apical region was enlarged and irrigated with 3% sodium hypochlorite. The canals were recaptulated with #15 size K file till the working length and irrigated with 3% sodium hypochlorite, followed by which the canal was enlarged using #25, 30, 35, 40, 45, 50 size K file in similar fashion using 15% EDTA with 10% carbamide peroxide gel and 3% sodium hypochlorite along with subsequent recaptulation using previous size file. After the apical preparation of the canal till size 50 K file (figure: 12); subsequent enlargement is done using #55K file by reducing 1mm from the working length and canal was recaptulated with size 50 file and irrigated with 3% sodium hypochlorite and

15 % EDTA gel as chelating agent. In similar way #60, 70, 80 K files were used 1mm short to the length of the previous size file and recaptulation and irrigation was done.

After the preparation with hand files, the coronal third of the canal was enlarged using Gates Glidden drills sizes #2 - #6 (figure: 13). The initial preparation was started with size #2 Gates Glidden drill till 5mm into the canal followed by irrigation with 3% sodium hypochlorite solution and then size #3 Gates Gliden drill was used at a length 1 mm short of size #2 and irrigated with 3% sodium hypochlorite. In similar fashion, enlargement was done using #4, #5, #6 Gates Glidden drill. Between each change of instrument, irrigaton was done with 3% sodium hypochlorite. Finaly circumferential filing was done using the master apical file to ensure a smooth taper of the preparation and then irrigated with 3% sodium hypochlorite. Then the canals were dried using paper points.

The master cone size 50 was selected which had a snug fit (figure: 15) and the sealer was mixed with conventional eugenol liquid to the desired thick and creamy consistency. Canal wall was coated with the nano zinc oxide powder sealer (30 nm) using K file by rotating in anti clock-wise direction(figure: 14). Additionally the pumping action of the sealer was performed with the master gutta percha cone.

The master cone (size 50 - 2% gutta percha cones) was coated with the sealer and placed into the root canal till the working length. The measured spreader is inserted between the master cone and the canal wall using firm pressure within 1 to 2 mm from working length, creating a space for an accessory cone. Accessory cone tips were coated with sealer and inserted. Cold Lateral condensation was done until no more accessory cones could be placed to a length of 10 mm into the canal. The coronal gutta percha was removed with plugger to leave 4mm of apical obturating material (figure: 17, 18) and subjected to leakage test. The specimens are examined under magnification and illumination to detect vertical root fractures using magnifying loupes (figure: 19). The samples with fractures were discarded.

GROUP B : NANO ZINC OXIDE POWDER SEALER (240nm)

Teeth were cleaned and shaped in the similar way as Group A samples were treated and were obturated in similar pattern except the Nano zinc oxide powder sealer (240 nm) was used during the obturation and then subjected to leakage test.

GROUP C : CONVENTIONAL ZINC OXIDE EUGENOL SEALER (45 µm)

Teeth were cleaned and shaped in the similar way the Group A samples were treated and were obturated in similar pattern except the

conventional zinc oxide sealer (45µm) was used during the obturation and then subjected to leakage test.

GROUP D : AH PLUS SEALER

Teeth were cleaned and shaped in the similar way the Group A samples were treated and were obturated in similar pattern except the AH plus was used during the obturation and then subjected to leakage test.

POSITIVE CONTROL : WITHOUT SEALER

Teeth were cleaned and shaped in the similar way the Group A samples were treated and were obturated to the full length without sealer by cold lateral condensation using 2% gutta-percha. The samples were subjected to glucose leakage test.

NEGATIVE CONTROL: CONVENTIONAL ZINC OXIDE EUGENOL SEALER (45µm)

Teeth were cleaned and shaped in the similar way the Group A samples were treated and were obturated to the full length of the root by cold lateral condensation using conventional zinc oxide sealer (45µm) and 2% gutta-percha. The external root surface was entirely coated with sticky wax including the root canal orifice and the apical foramen (figure:20) The samples were subjected to glucose leakage test.

All the specimens were incubated at 37°C for 2 weeks to allow the complete setting of the sealer.

GLUCOSE PENETRATION MODEL – PREPARATION AND MEASUREMENTS

The glucose penetration model was made in accordance with the model introduced by Xu et al (2005) which was modified by Shemesh et al (2006). The coronal part of each root was connected to a 16cm long glass pipette with the help of a rubber tube with stainless steel wires. The assembly was placed in a glass bottle with the screw cap and sealed with sticky wax where a uniform hole was drilled in the screw cap to assure an open system.(figure: 22) Two millilitres of 0.2% NaN_3 (sodium azide) solution was injected into the glass bottle upto the level of the apical third of the root sample. NaN_3 (sodium azide) solution inhibits microbial growth which can alter the glucose readings and as glucose has low molecular weight and is hydrophilic and chemically stable, 1mol L^{-1} glucose solution was used as tracer with pH 7.0

About 4.5ml of glucose solution containing 0.2% NaN_3 was injected into the pipette until the top of the solution was 14cm higher than the top of gutta-percha in the canal, which created a hydrostatic pressure1.5 kPa or 15cm H_2O (Xu et al 2005), density of 1.09×10^3 g/L and viscosity 1.18×10^{-3} Pa-s at 37°C. Glucose which is hydrophilic and chemically stable also has a low

molecular weight of 180 Da. The specimens were placed in an incubator at 37°C for the duration of 1 month observation period.

Measurement of microleakage:

A 10 microlitre of solution was drawn from the glass bottle using micropipette. The same amount of fresh 0.2 % NaN_3 was added to the glass bottle reservoir to maintain the constant volume. The collected solution was added to 1000 micro litre glucose reagent solution and were held at room temprature for 10 minutes. The samples were analyzed using a spectrophotometer at 505 nm wavelength (ultraviolet) (figure: 23). The leakage in all the groups was calculated as mmol/L at particular time after obturation (1, 10, 20, and 30 days).

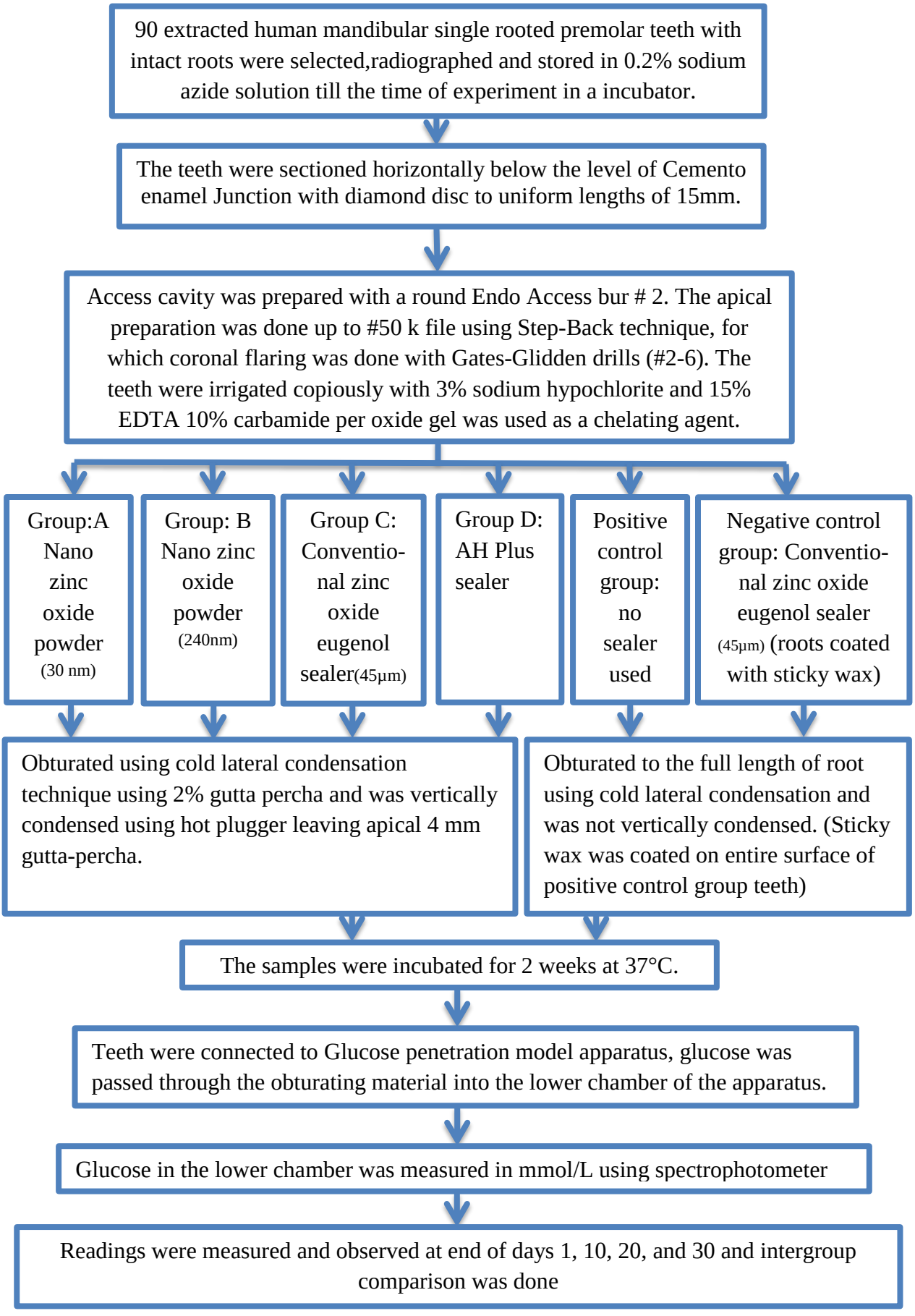
The colorimetric indicator is quinoneimine, which is generated from 4 aminoantipyrine and phenol by hydrogen peroxide under the catalytic reaction of peroxidase. Glucose is oxidized by the enzyme glucose oxidase in the presence of oxygen to gluconic acid with formation of hydrogen peroxide. Then in the presence of a peroxidase enzyme, a chromogenic oxygen acceptor (4-aminoantipyrine and phenol) is oxidized by the hydrogen peroxide, resulting in the formation of a red product (oxidized chromogen). The quantity of this oxidized chromogen is proportional to the glucose present initially in the first reaction, whose quantity is determined by spectrophotometry.

The reaction is as follows:

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Oxidized chromogen} + H_2O$$

Methodology Overview



Figures

Figure1: Extracted mandibular premolar teeth

Figure 2: Satelec X- ray unit (70kVp, 8mA) Class 1 type

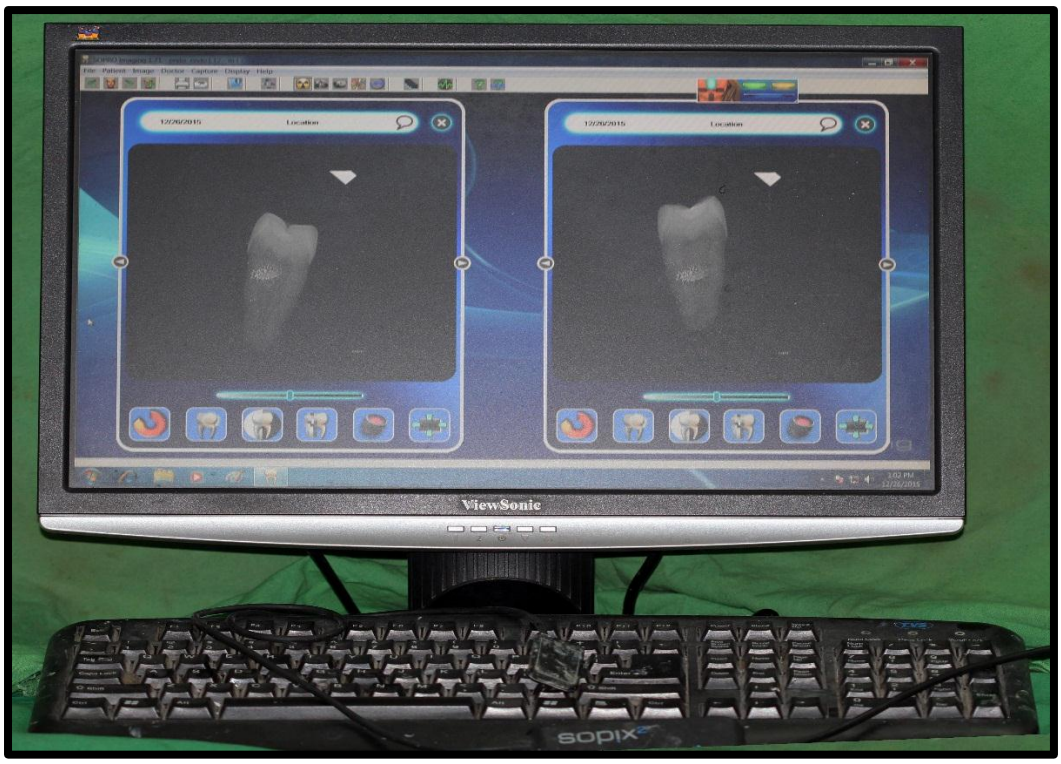


Figure 3: Sopix 2 RVG unit (ACTEON) and Sopro imaging software

Figure 4: Armamentarium

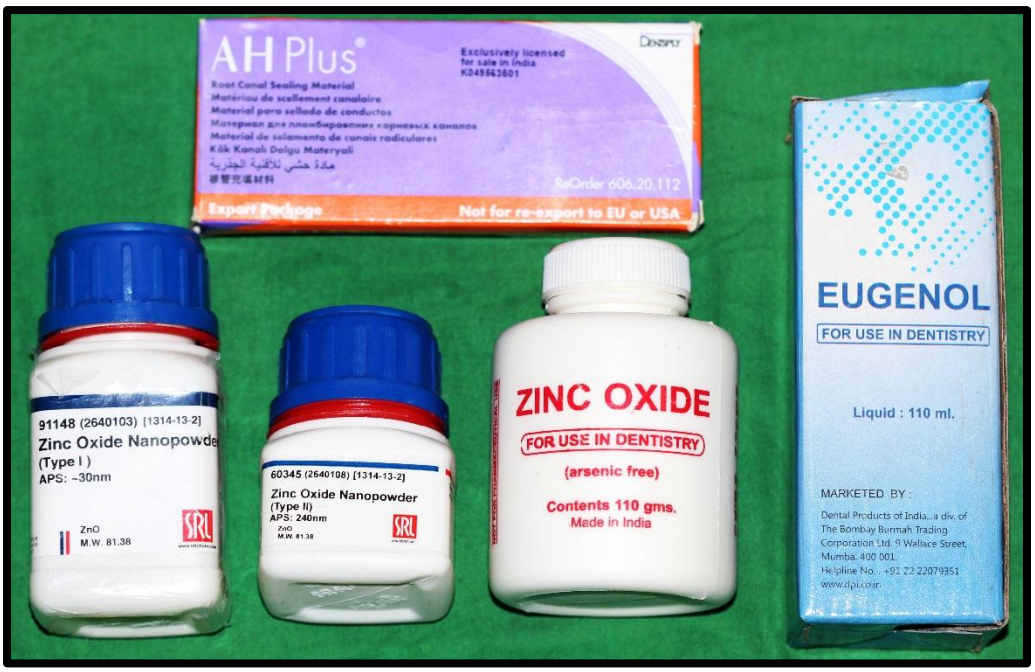


Figure 5: Sealers

Figure 6: Materials used for Micro leakage apparatus

Figure 7: Glucose analysis reagent

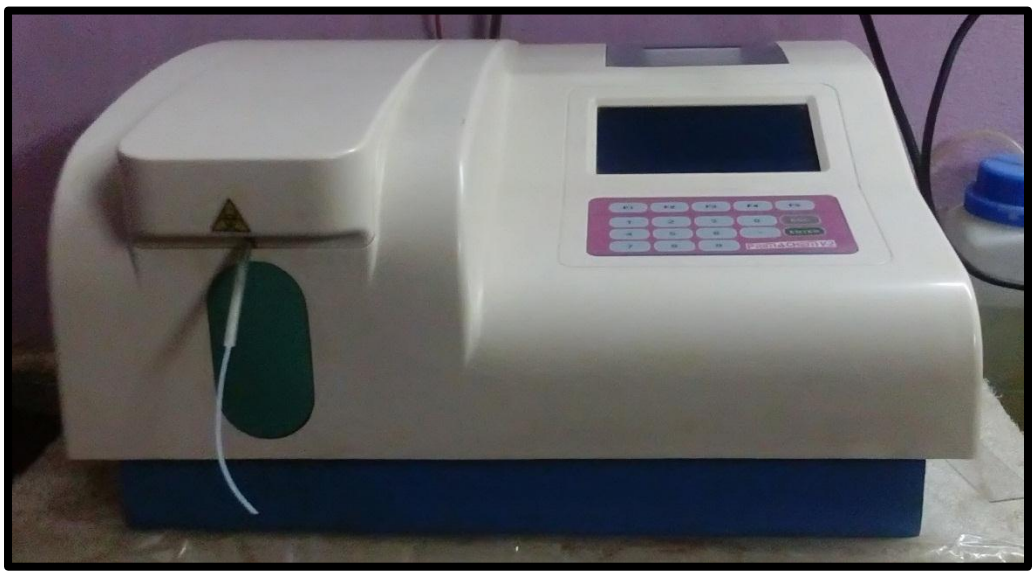


Figure 8: Spectrophotometer (PRIMACHEM V-2)

Figure 9: Incubator

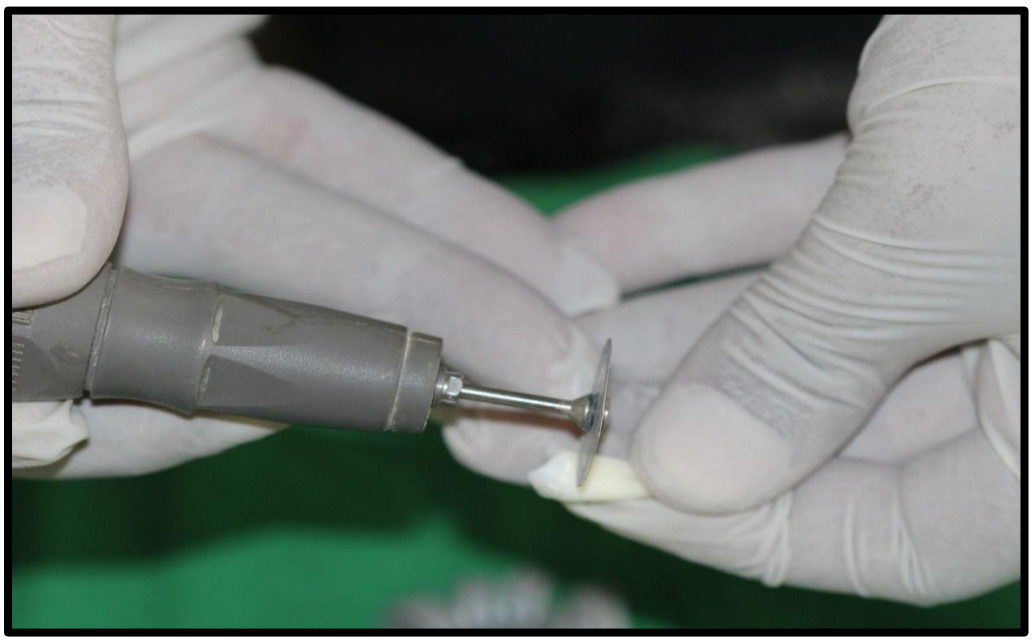


Figure 10: Decoronation of the tooth

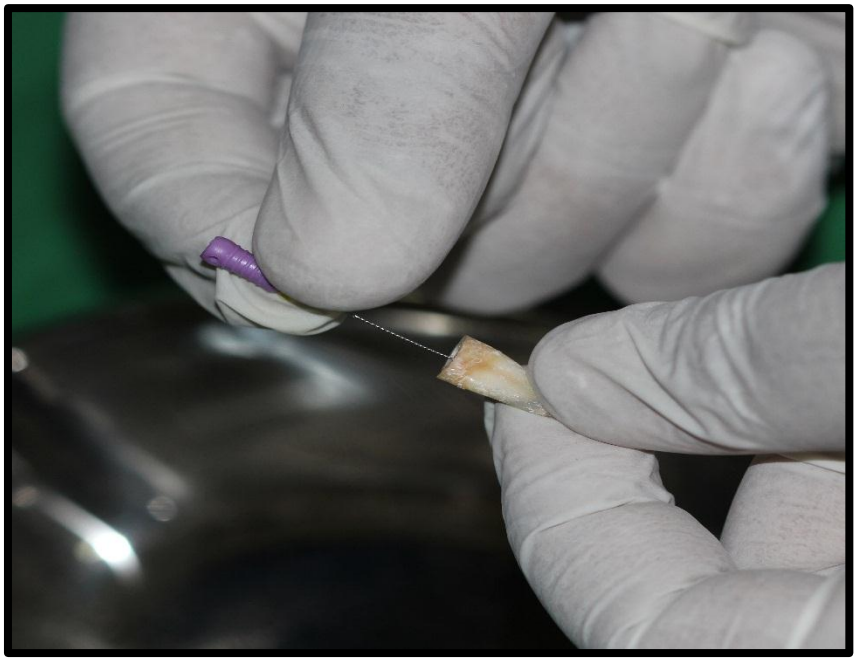


Figure 11: Canal patency assessment using #10 k File

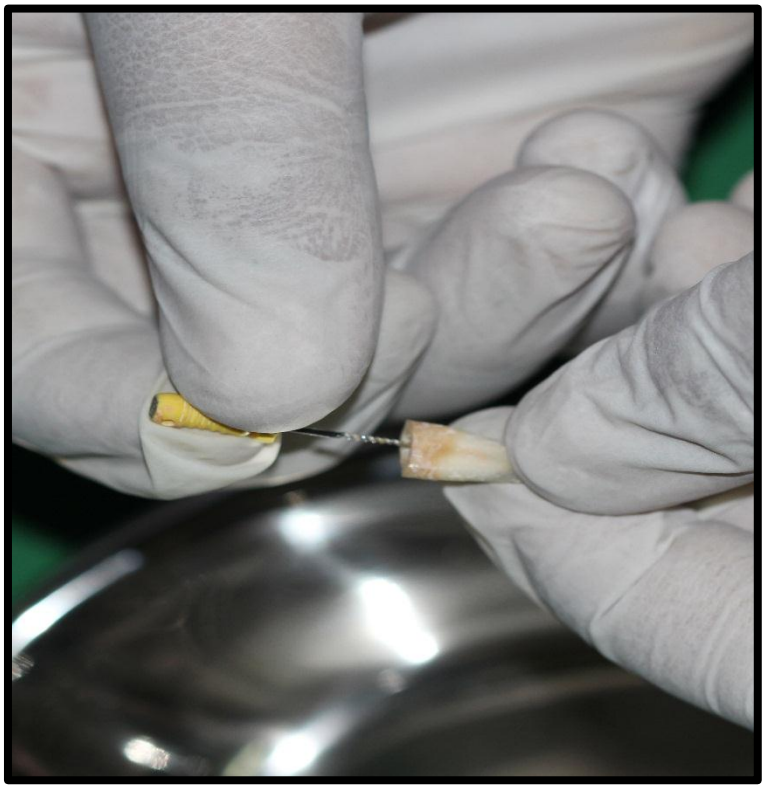


Figure 12: Master apical file #50

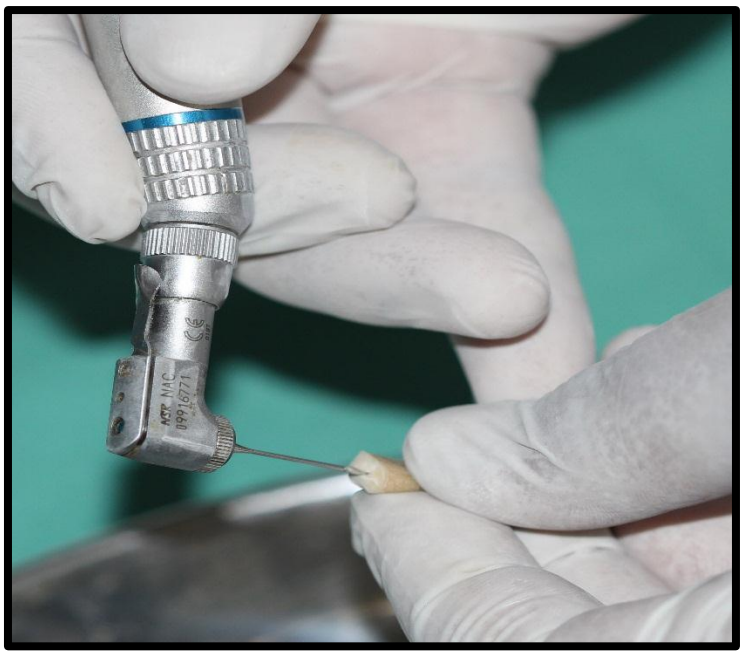
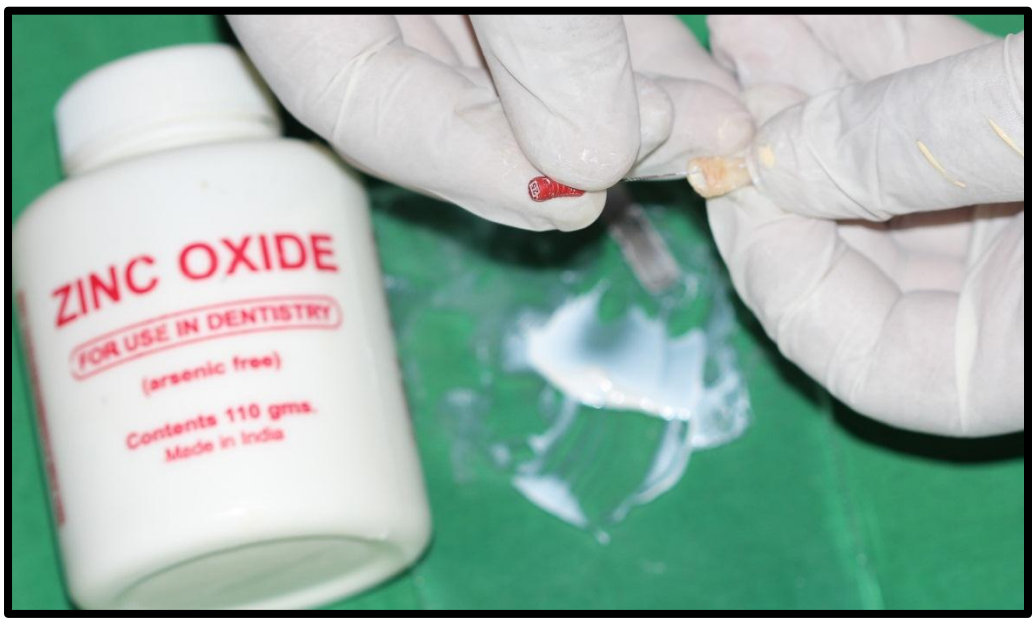


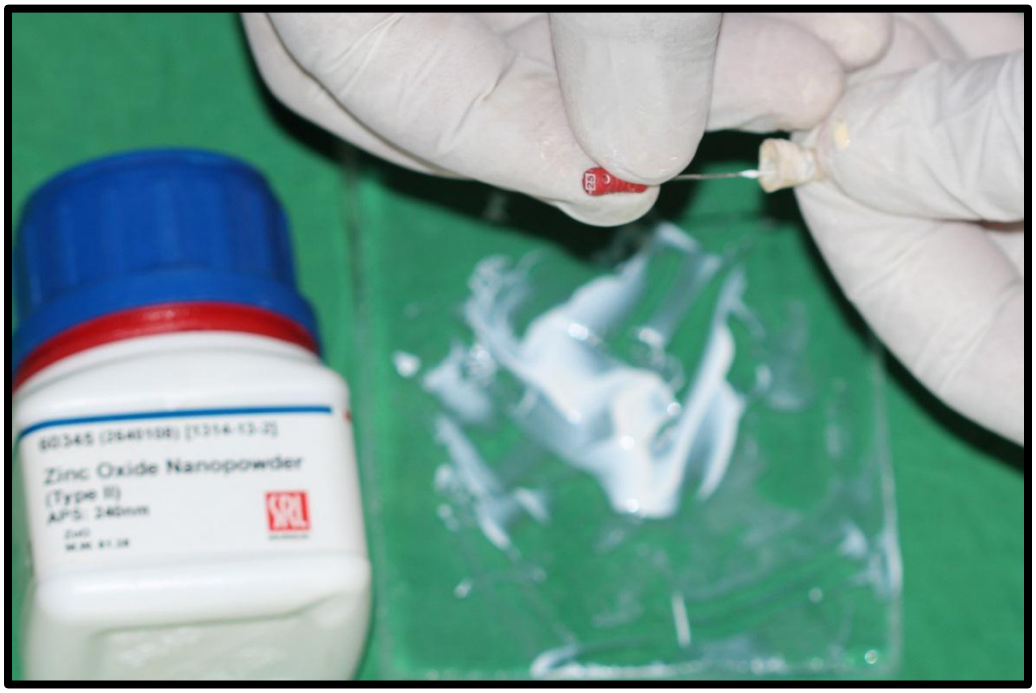
Figure 13: Coronal flaring

Figure 14: Sealer application

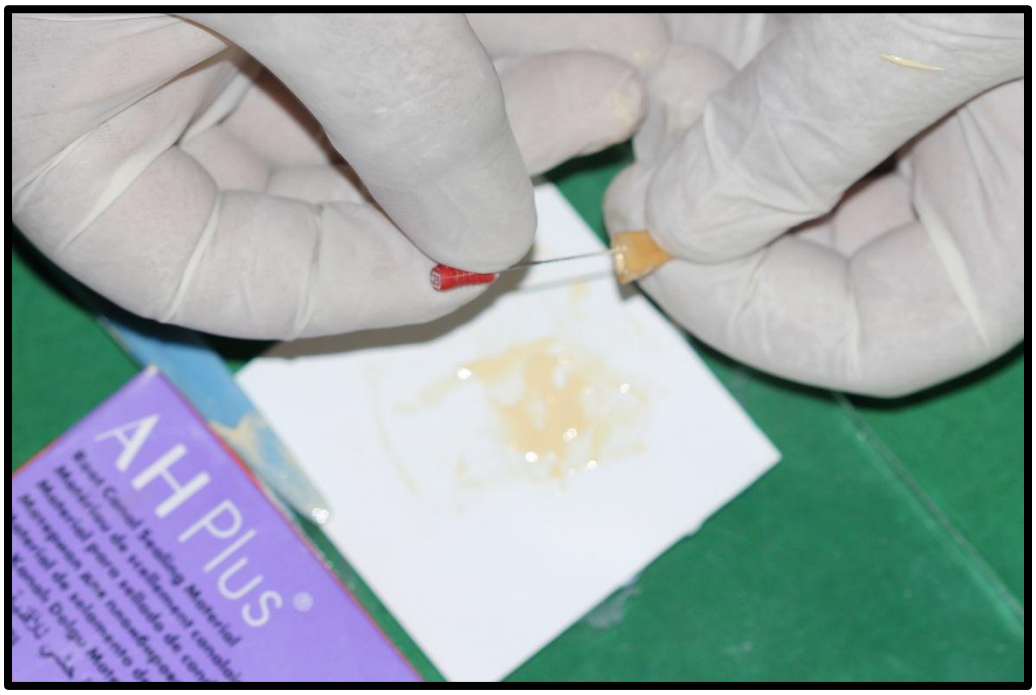


14 (A): Sealer application – conventional zinc oxide powder

14 (B): Sealer application – Nano Zinc oxide powder 30nm



14 (C): Sealer application – Nano Zinc oxide particle 240 nm



14 (D): Sealer application – AH Plus

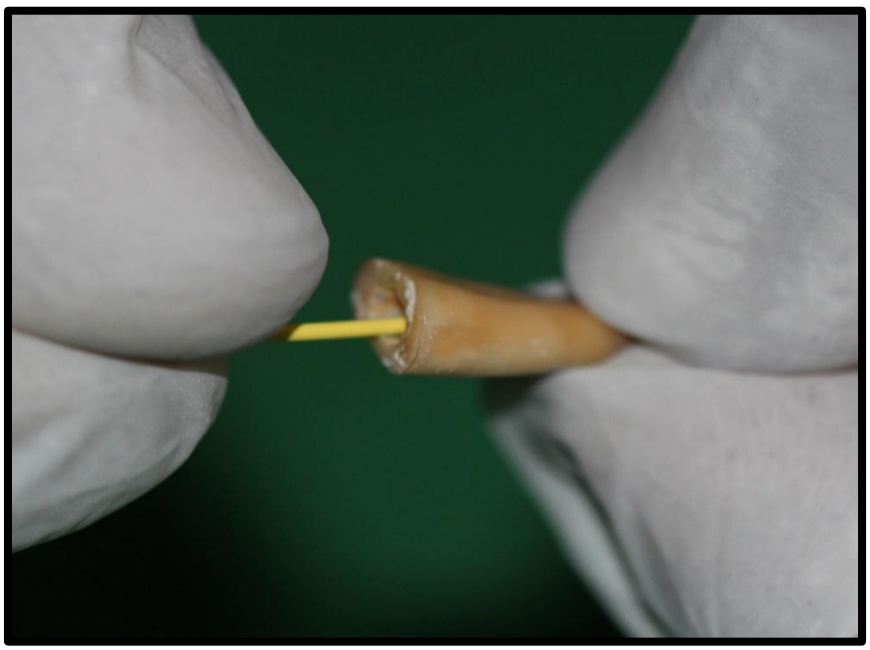


Figure 15: Master cone # 50

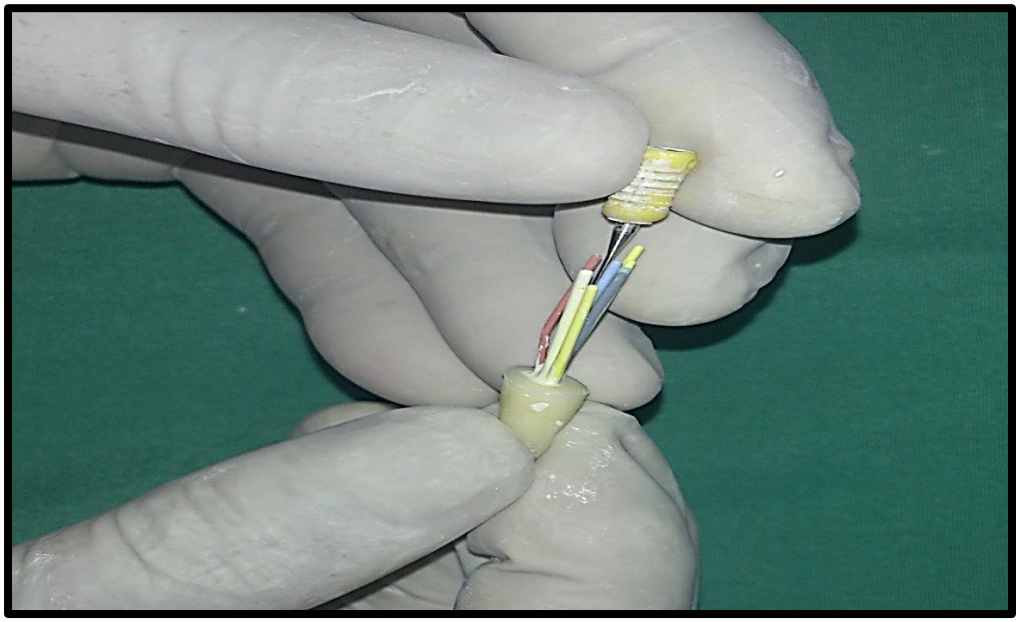


Figure 16: Lateral condensation

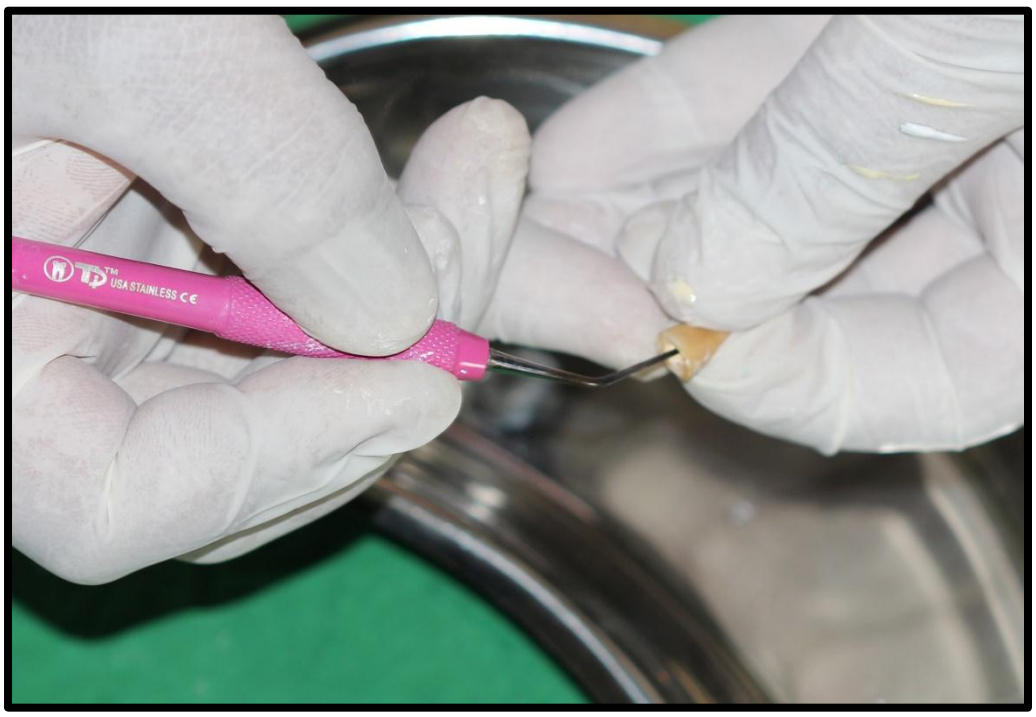


Figure 17: Warm compaction with plugger

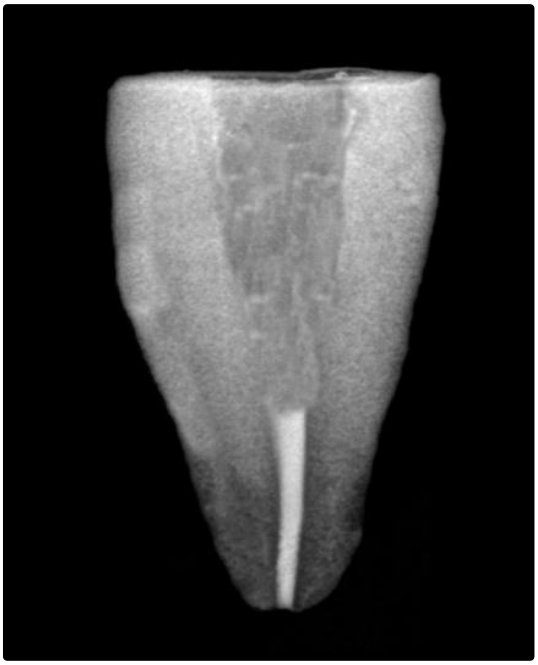


Figure 18: Post obturation Radiograph in mesiodistal direction

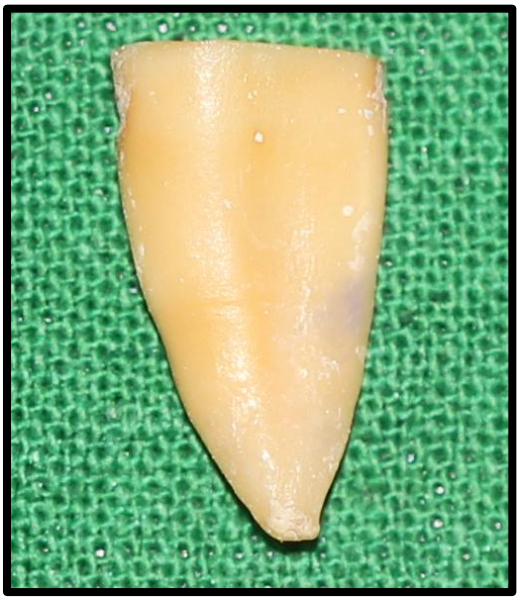


Figure 19: Inspection of microcracks at 2.5X magnification using loupes

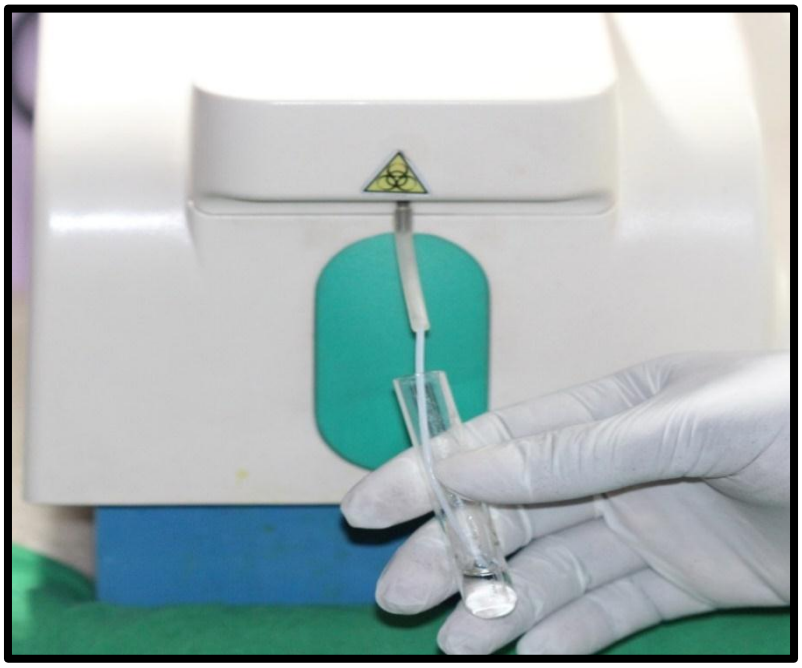
Figure 20: Negative control specimen

Figure 21: Cylindrical self cure acrylic arround specimen

Figure 22: Glucose microleakage model

Figure 23: Spectrophotometer for analysis

23 (A): Addition of sodium azide solution to the glucose reagent



23 (B): Loading of the reacted sample into the spectrophotometer for analysis

Results

RESULTS

The results of the present study were subjected to statistical analysis to interpret the significance of microleakage among various root canal sealers used for obturation. Data entry and data base management was done in SPSS (Statistical Package for Social Sciences) 17.0 version for windows.

One way ANOVA was used for intergroup comparison of mean microleakage values at same interval of time.(Tables 2,5,8,11) Tukey's post hoc analysis was used to pairwise comparison of mean microleakage values at the same interval of time (Tables 3,6,9,12).

Repeated measures ANOVA was used for intragroup comparison of mean microleakage values at different intervals of time (Tables 13,15,17,19,21). LSD Bonferroni analysis was used for pair wise comparison of mean microleakage values of each group (Tables 14,16,18,20,22).

The one-way analysis of variance (ANOVA) is used to determine whether there are any significant differences between the means of two or more independent groups.

A post-hoc test is needed after an ANOVA is completed in order to determine which groups differ from each other. For the Tukey's post-hoc test the differences between the means of all of our groups are found. Difference of score is compared to a critical value to see if the difference is significant.

The critical value is the HSD (honestly significant difference) and it must be computed. It is the point when a mean difference becomes honestly significantly different.

An ANOVA with repeated measures is used to compare three or more group means where the participants are the same in each group.

LSD stands for Least Significant Difference t test. This test does not control the overall probability of rejecting the hypothesis that some pairs of means are different, while in fact they are equal. The Bonferroni adjustment is the simplest. It basically multiplies each of the significance levels from the LSD test by the number of tests performed.[76]

Various sealers used in the groups are as follows:

- Group A : Zinc Oxide Nano Powder Sealer (30nm)
- Group B : Zinc Oxide Nano Powder Sealer (240nm)
- Group C : Conventional Zinc Oxide Eugenol Sealer (45µm)
- Group D : AH plus sealer
- Positive Control: without sealer
- Negative Control: Conventional Zinc Oxide Eugenol Sealer (45µm) (root surface of all these samples were coated with sticky wax)

Table 1 denotes the values of individual samples of each group on day 1 and their mean value

Table 2 denotes the significance of p value of intergroup comparison on day 1 of the test conducted. The mean value of negative control group is least (0.00) followed by Group A (0.25±0.02), Group B (0.33±0.03), Group D (0.34±0.03), Group C (0.52±0.02) and highest value was for positive control group (18.14±0.46). The p value was 0.001 which was statistically significant.

Table 3 denotes the significance of p value for the pairwise comparisons of the mean difference on day 1 of the test conducted.

Group A and Group B had a mean difference of -0.07800 with a p value of 0.873 which was statistically insignificant. Group A and Group C had a mean difference of -0.27067 with a p value of 0.003 which was statistically significant. Group A and Group D had a mean difference of -0.09000 with a p value of 0.791 which was statistically insignificant. Group A and Positive control had a mean difference of -17.81800 with a p value of 0.000 which was statistically significant. Group A and Negative control group had a mean difference of 0.25133 with a p value of 0.007 which was statistically significant.

Group B and Group C had a mean difference of -0.19267 with a p value of 0.075 which was statistically insignificant. Group B and Group D had a mean difference of -0.01200 with a p value of 1.000 which was statistically

insignificant. Group B and Positive control group had a mean difference of -17.89600 with a p value of 0.000 which was statistically significant. Group B and Negative control group had a mean difference of 0.32933 with a p value of 0.000 which was statistically significant.

Group C and Group D had a mean difference of 0.18067 with a p value of 0.112 which was statistically insignificant. Group C and Positive control group had a mean difference of -17.62533 with a p value of 0.000 which was statistically significant. Group C and Negative control group had a mean difference of 0.52200 with a p value of 0.000 which was statistically significant.

Group D and Positive control group had a mean difference of -17.80600 with a p value of 0.000 which was statistically significant. Group D and Negative control group had a mean difference of 0.34133 with a p value of 0.000 which was statistically significant.

Positive control group and Negative control group had a mean difference of 18.14733 with a mean value of 0.000 which was statistically significant.

Table 4 denotes the values of individual samples of each group on day 10 and their mean value

Table 5 denotes the significance of p value of intergroup comparison on day 10 of the test conducted. The mean value of negative control group is

least (0.00) followed by Group A (1.55±0.57), Group B (2.27±0.25), Group D (2.52±0.26), Group C (3.42±0.31) and highest value was for positive control group (21.85±1.13). The p value was 0.001 which was statistically significant.

Table 6 denotes the significance of p value for the pairwise comparisons of the mean difference on day 10 of the test conducted.

Group A and Group B had a mean difference of -0.72000 with a p value of 0.008 which was statistically significant. Group A and Group C had a mean difference of -1.87333 with a p value of 0.000 which was statistically significant. Group A and Group D had a mean difference of -0.96733 with a p value of 0.000 which was statistically significant. Group A and Positive control had a mean difference of -20.30200 with a p value of 0.000 which was statistically significant. Group A and Negative control group had a mean difference of 1.55333 with a p value of 0.000 which was statistically significant.

Group B and Group C had a mean difference of -1.15333 with a p value of 0.000 which was statistically significant. Group B and Group D had a mean difference of -0.24733 with a p value of 0.827 which was statistically insignificant. Group B and Positive control group had a mean difference of -19.58200 with a p value of 0.000 which was statistically significant. Group B and Negative control group had a mean difference of 2.2733 with a p value of 0.000 which was statistically significant.

Group C and Group D had a mean difference of 0.90600 with a p value of 0.000 which was statistically significant. Group C and Positive control group had a mean difference of -18.42867 with a p value of 0.000 which was statistically significant. Group C and Negative control group had a mean difference of 3.42667 with a p value of 0.000 which was statistically significant.

Group D and Positive control group had a mean difference of -19.33467 with a p value of 0.000 which was statistically significant. Group D and Negative control group had a mean difference of 2.52067 with a p value of 0.000 which was statistically significant.

Positive control group and Negative control group had a mean difference of 21.85533 with a mean value of 0.000 which was statistically significant.

Table 7 denotes the values of individual samples of each group on day 20 and their mean value

Table 8 denotes the significance of p value of intergroup comparison on day 20 of the test conducted. The mean value of negative control group is least (0.00) followed by Group A (7.33±0.31), Group B (8.40±0.37), Group D (8.66±0.55), Group C (12.02±1.35) and highest value was for positive control group (33.41±2.27). The p value was 0.001 which was statistically significant.

Table 9 denotes the significance of p value for the pairwise comparisons of the mean difference on day 20 of the test conducted.

Group A and Group B had a mean difference of -1.06600 with a p value of 0.107 which was statistically significant. Group A and Group C had a mean difference of -4.69000 with a p value of 0.000 which was statistically significant. Group A and Group D had a mean difference of -1.32733 with a p value of 0.020 which was statistically significant. Group A and Positive control had a mean difference of -26.07667 with a p value of 0.000 which was statistically significant. Group A and Negative control group had a mean difference of 7.33533 with a p value of 0.000 which was statistically significant.

Group B and Group C had a mean difference of -3.62400 with a p value of 0.000 which was statistically significant. Group B and Group D had a mean difference of -0.26133 with a p value of 0.988 which was statistically insignificant. Group B and Positive control group had a mean difference of -25.01067 with a p value of 0.000 which was statistically significant. Group B and Negative control group had a mean difference of 8.40133 with a p value of 0.000 which was statistically significant.

Group C and Group D had a mean difference of 3.36267 with a p value of 0.000 which was statistically significant. Group C and Positive control group had a mean difference of -21.38667 with a p value of 0.000 which was statistically significant. Group C and Negative control group had a mean

difference of 12.02533 with a p value of 0.000 which was statistically significant.

Group D and Positive control group had a mean difference of -24.74933 with a p value of 0.000 which was statistically significant. Group D and Negative control group had a mean difference of 8.66267 with a p value of 0.000 which was statistically significant.

Positive control group and Negative control group had a mean difference of 33.41200 with a mean value of 0.000 which was statistically significant.

Table 10 denotes the values of individual samples of each group on day 30 and their mean value

Table 11 denotes the significance of p value of intergroup comparison on day 30 of the test conducted. The mean value of negative control group is least (0.00) followed by Group A (11.27±0.47), Group B (11.63±0.53), Group D (13.06±0.93), Group C (19.62±1.88) and highest value was for positive control group (55.97±2.71). The p value was 0.001 which was statistically significant.

Table 12 denotes the significance of p value for the pairwise comparisons of the mean difference on day 30 of the test conducted.

Group A and Group B had a mean difference of -0.35933 with a p value of 0.983 which was statistically insignificant. Group A and Group C had a mean difference of -8.34867 with a p value of 0.000 which was statistically significant. Group A and Group D had a mean difference of -1.79400 with a p value of 0.011 which was statistically significant. Group A and Positive control had a mean difference of -44.70333 with a p value of 0.000 which was statistically significant. Group A and Negative control group had a mean difference of 11.27133 with a p value of 0.000 which was statistically significant.

Group B and Group C had a mean difference of -7.98933 with a p value of 0.000 which was statistically significant. Group B and Group D had a mean difference of -1.43467 with a p value of 0.076 which was statistically insignificant. Group B and Positive control group had a mean difference of -44.34400 with a p value of 0.000 which was statistically significant. Group B and Negative control group had a mean difference of 11.63067 with a p value of 0.000 which was statistically significant.

Group C and Group D had a mean difference of 6.55467 with a p value of 0.000 which was statistically significant. Group C and Positive control group had a mean difference of -36.35467 with a p value of 0.000 which was statistically significant. Group C and Negative control group had a mean difference of 19.62000 with a p value of 0.000 which was statistically significant.

Group D and Positive control group had a mean difference of -42.90933 with a p value of 0.000 which was statistically significant. Group D and Negative control group had a mean difference of 13.06533 with a p value of 0.000 which was statistically significant.

Positive control group and Negative control group had a mean difference of 55.97467 with a mean value of 0.000 which was statistically significant.

Table 13 denotes the significance of p value of intra group comparison of mean microleakage values at different time intervals in Group A. The mean value was least on Day 1 (0.25 ±0.02) followed by Day 10 (1.55±0.57), Day 20 (7.33±0.31) and highest on day 30 (11.27±0.47). The p value was 0.001 which was statistically significant.

Table 14 denotes the significance of p value of pair wise comparisons of mean microleakage values of Group A at different time intervals.

The mean difference on Day 1 and Day 10 was -1.302 with a p value of 0.000 which was statistically significant. The mean difference on Day 1 and Day 20 was -7.084 with a p value of 0.000 which was statistically significant. The mean difference on Day 1 and Day 30 was -11.020 with a p value of 0.000 which was statistically significant.

The mean difference on Day 10 and Day 20 was -5.782 with a p value of 0.000 which was statistically significant. The mean difference on Day 10

and Day 30 was -9.718 with a p value of 0.000 which was statistically significant.

The mean difference on Day 20 and Day 30 was -3.936 with p value of 0.000 which was statistically significant.

Table 15 denotes the significance of p value of intra group comparison of mean microleakage values at different time intervals in Group B. the mean value was least on Day 1 (0.33 ±0.03) followed by Day 10 (2.27±0.25), Day 20 (8.40±0.37) and highest on day 30 (11.63±0.53). The p value was 0.001 which was statistically significant.

Table 16 denotes the significance of p value of pair wise comparisons of mean microleakage values of Group B at different time intervals.

The mean difference on Day 1 and Day 10 was -1.944 with a p value of 0.000 which was statistically significant. The mean difference on Day 1 and Day 20 was -8.072 with a p value of 0.000 which was statistically significant. The mean difference on Day 1 and Day 30 was -11.301 with a p value of 0.000 which was statistically significant.

The mean difference on Day 10 and Day 20 was -6.128 with a p value of 0.000 which was statistically significant. The mean difference on Day 10 and Day 30 was -9.357 with a p value of 0.000 which was statistically significant.

The mean difference on Day 20 and Day 30 was -3.229 with p value of 0.000 which was statistically significant.

Table 17 denotes the significance of p value of intra group comparison of mean microleakage values at different time intervals in Group C. the mean value was least on Day 1 (0.52 ±0.02) followed by Day 10 (3.42±0.31), Day 20 (12.02±1.35) and highest on day 30 (19.62±1.88). The p value was 0.001 which was statistically significant.

Table 18 denotes the significance of p value of pair wise comparisons of mean microleakage values of Group C at different time intervals.

The mean difference on Day 1 and Day 10 was -2.905 with a p value of 0.000 which was statistically significant. The mean difference on Day 1 and Day 20 was -11.503 with a p value of 0.000 which was statistically significant. The mean difference on Day 1 and Day 30 was -19.098 with a p value of 0.000 which was statistically significant.

The mean difference on Day 10 and Day 20 was -8.599 with a p value of 0.000 which was statistically significant. The mean difference on Day 10 and Day 30 was -16.193 with a p value of 0.000 which was statistically significant.

The mean difference on Day 20 and Day 30 was -7.595 with p value of 0.000 which was statistically significant.

Table 19 denotes the significance of p value of intra group comparison of mean microleakage values at different time intervals in Group D. the mean value was least on Day 1 (0.34 ±0.03) followed by Day 10 (2.52±0.26), Day 20 (8.66±0.55) and highest on day 30 (13.06±0.93). The p value was 0.001 which was statistically significant.

Table 20 denotes the significance of p value of pair wise comparisons of mean microleakage values of Group D at different time intervals.

The mean difference on Day 1 and Day 10 was -2.179 with a p value of 0.000 which was statistically significant. The mean difference on Day 1 and Day 20 was -8.321 with a p value of 0.000 which was statistically significant. The mean difference on Day 1 and Day 30 was -12.724 with a p value of 0.000 which was statistically significant.

The mean difference on Day 10 and Day 20 was -6.142 with a p value of 0.000 which was statistically significant. The mean difference on Day 10 and Day 30 was -10.545 with a p value of 0.000 which was statistically significant.

The mean difference on Day 20 and Day 30 was -4.403 with p value of 0.000 which was statistically significant.

Table 21 denotes the significance of p value of intra group comparison of mean microleakage values at different time intervals in Positive control group the mean value was least on Day 1 (18.14 ±0.46) followed by Day 10

(21.85±1.13), Day 20 (33.41±2.27) and highest on day 30 (55.97±2.71). The p value was 0.001 which was statistically significant.

Table 22 denotes the significance of p value of pair wise comparisons of mean microleakage values of Positive control Group at different time intervals.

The mean difference on Day 1 and Day 10 was -3.708 with a p value of 0.000 which was statistically significant. The mean difference on Day 1 and Day 20 was -15.265 with a p value of 0.000 which was statistically significant. The mean difference on Day 1 and Day 30 was -37.827 with a p value of 0.000 which was statistically significant.

The mean difference on Day 10 and Day 20 was -11.557 with a p value of 0.000 which was statistically significant. The mean difference on Day 10 and Day 30 was -34.119 with a p value of 0.000 which was statistically significant.

The mean difference on Day 20 and Day 30 was -22.563 with p value of 0.000 which was statistically significant.

From the results it was clear that the negative control group had minimum leakage among all the groups followed by group A, group B, group D and group C, with the highest leakage in positive control groups at all the time intervals. The micro leakage on the first day was least followed by day 10, day 20 and 30 highest leakage in all the groups, except in negative control group were no leakage was observed right through the experimental period.

Tables and Graphs

Table 1: Microleakage values of samples in various groups on Day 1

SAMPLE NO	GROUP A (mmol/L)	GROUP B (mmol/L)	GROUP C (mmol/L)	GROUP D (mmol/L)	POSITIVE CONTROL (mmol/L)	NEGATIVE CONTROL (mmol/L)
1	0.25	0.31	0.48	0.32	18.52	0
2	0.22	0.32	0.54	0.28	18.22	0
3	0.23	0.29	0.51	0.36	18.51	0
4	0.24	0.36	0.54	0.33	17.89	0
5	0.27	0.33	0.55	0.32	18.62	0
6	0.26	0.34	0.52	0.39	17.32	0
7	0.28	0.33	0.48	0.37	17.56	0
8	0.27	0.36	0.56	0.36	17.88	0
9	0.23	0.35	0.55	0.38	18.73	0
10	0.24	0.29	0.52	0.36	18.66	0
11	0.28	0.26	0.51	0.32	17.56	0
12	0.27	0.33	0.49	0.29	18.53	0
13	0.28	0.38	0.55	0.31	18.45	0
14	0.22	0.32	0.52	0.36	17.75	0
15	0.23	0.37	0.51	0.37	18.01	0
Mean value	0.25	0.33	0.52	0.34	18.14	0.00

Group A: ZINC OXIDE NANO POWDER SEALER (30nm)

Group B: ZINC OXIDE NANO POWDER SEALER (240nm)

Group C:CONVENTIONAL ZINC OXIDE EUGENOL SEALER (45 µm)

GROUP D : AH PLUS SEALER

POSITIVE CONTROL : WITHOUT SEALER

NEGATIVE CONTROL: CONVENTIONAL ZINC OXIDE EUGENOL SEALER (45µm) (external surface coated with sticky wax)

Table 2: Intergroup comparison of mean micro leakage values among different groups on Day 1 using one-way analysis of variance (ANOVA)

Groups	Mean	SD	One way ANOVA	p-value
Group A	0.25	0.02	F = 217.94	0.001*
Group B	0.33	0.03		
Group C	0.52	0.02		
Group D	0.34	0.03		
Positive Control	18.14	0.46		
Negative Control	0.00	0.00		

* p value < 0.05 is considered as statistically significant.

Table 3: Pairwise comparisons of mean micro leakage values on Day 1 using Tukey's post hoc analysis

Groups		Mean Difference	p-value
Group A	Group B	-.07800	.873
	Group C	-.27067	.003*
	Group D	-.09000	.791
	Positive Control	-17.89600	.000*
	Negative Control	.25133	.007*
Group B	Group C	-.19267	.075
	Group D	-.01200	1.000
	Positive Control	-17.81800	.000*
	Negative Control	.32933	.000*
Group C	Group D	.18067	.112
	Positive Control	-17.62533	.000*
	Negative Control	.52200	.000*
Group D	Positive Control	-17.80600	.000*
	Negative Control	.34133	.000*
Positive Control	Negative Control	18.14733	.000*

* p value < 0.05 is considered as statistically significant.

Table 4: Microleakage values of samples in various groups on Day 10

SAMPLE NO	GROUP A (mmol/L)	GROUP B (mmol/L)	GROUP C (mmol/L)	GROUP D (mmol/L)	POSITIVE CONTROL (mmol/L)	NEGATIVE CONTROL (mmol/L)
1	1.12	2.12	3.56	2.48	22.36	0
2	1.32	2.15	3.11	2.56	22.12	0
3	1.11	2.61	3.54	2.11	21.03	0
4	1.24	2.31	3.48	2.65	20.45	0
5	1.31	1.78	3.44	2.48	22.56	0
6	1.22	2.44	3.45	2.75	23.45	0
7	1.56	1.84	3.47	2.88	24.01	0
8	2.4	2.47	3.54	2.11	21.77	0
9	2.98	2.54	3.21	2.77	22.05	0
10	2.45	2.62	3.89	2.87	21.45	0
11	1.12	2.21	3.54	2.63	22.78	0
12	1.55	2.43	3.97	2.54	22.12	0
13	1.22	2.12	3.45	2.55	19.78	0
14	1.26	2.34	2.98	2.12	20.54	0
15	1.44	2.12	2.77	2.31	21.36	0
Mean value	1.55	2.27	3.42	2.52	21.85	0.00

Table 5: Intergroup comparison of mean microleakage values among different groups on Day 10 using one-way analysis of variance (ANOVA)

Groups	Mean	SD	One Way ANOVA	p-value
Group A	1.55	0.57	F = 326.83	0.001*
Group B	2.27	0.25		
Group C	3.42	0.31		
Group D	2.52	0.26		
Positive Control	21.85	1.13		
Negative Control	0.00	0.00		

* p value < 0.05 is considered as statistically significant.

Table 6: Pairwise comparisons of mean micro leakage values on Day 10 using Tukey's post hoc analysis

Groups		Mean Difference	p-value
Group A	Group B	-.72000	.008*
	Group C	-1.87333	.000*
	Group D	-.96733	.000*
	Positive Control	-20.30200	.000*
	Negative Control	1.55333	.000*
Group B	Group C	-1.15333	.000*
	Group D	-.24733	.827
	Positive Control	-19.58200	.000*
	Negative Control	2.27333	.000*
Group C	Group D	.90600	.000*
	Positive Control	-18.42867	.000*
	Negative Control	3.42667	.000*
Group D	Positive Control	-19.33467	.000*
	Negative Control	2.52067	.000*
Positive Control	Negative Control	21.85533	.000*

* p value < 0.05 is considered as statistically significant.

Table 7: Microleakage values of samples in various groups on Day 20

SAMPLE NO	GROUP A (mmol/L)	GROUP B (mmol/L)	GROUP C (mmol/L)	GROUP D (mmol/L)	POSITIVE CONTROL (mmol/L)	NEGATIVE CONTROL (mmol/L)
1	7.25	8.84	11.21	8.67	31.05	0
2	7.35	7.44	12.45	8.12	30.45	0
3	7.48	8.45	14.11	8.54	34.15	0
4	7.84	8.77	11.24	7.52	32.45	0
5	7.44	8.12	11.54	8.45	29.84	0
6	7.12	8.45	11.33	9.01	34.04	0
7	7.88	8.61	12.03	8.12	36.87	0
8	7.45	8.21	11.56	9.23	34.62	0
9	7.12	8.56	16.01	8.12	35.89	0
10	7.45	8.45	11.12	8.59	31.45	0
11	7.66	8.01	12.33	9.45	34.89	0
12	6.87	8.12	11.24	9.48	31.09	0
13	7.22	8.45	11.45	9.01	36.45	0
14	6.79	8.65	11.09	8.65	35.12	0
15	7.11	8.89	11.67	8.98	32.82	0
Mean value	7.33	8.40	12.02	8.66	33.41	0.00

Table 8: Intergroup comparison of mean micro leakage values among different groups on Day 20 using one-way analysis of variance (ANOVA)

Groups	Mean	SD	One Way ANOVA	p-value
Group A	7.33	0.31	F = 154.63	0.001*
Group B	8.40	0.37		
Group C	12.02	1.35		
Group D	8.66	0.55		
Positive Control	33.41	2.27		
Negative Control	0.00	0.00		

* p value < 0.05 is considered as statistically significant.

Table 9: Pairwise comparisons of mean micro leakage values on Day 20 using Tukey's post hoc analysis

Groups		Mean Difference	p-value
Group A	Group B	-1.06600	.107
	Group C	-4.69000	.000*
	Group D	-1.32733	.020*
	Positive Control	-26.07667	.000*
	Negative Control	7.33533	.000*
Group B	Group C	-3.62400	.000*
	Group D	-.26133	.988
	Positive Control	-25.01067	.000*
	Negative Control	8.40133	.000*
Group C	Group D	3.36267	.000*
	Positive Control	-21.38667	.000*
	Negative Control	12.02533	.000*
Group D	Positive Control	-24.74933	.000*
	Negative Control	8.66267	.000*
Positive Control	Negative Control	33.41200	.000*

* p value < 0.05 is considered as statistically significant.

Table 10: Microleakage values of samples in various groups on Day 30

SAMPLE NO	GROUP A (mmol/L)	GROUP B (mmol/L)	GROUP C (mmol/L)	GROUP D (mmol/L)	POSITIVE CONTROL (mmol/L)	NEGATIVE CONTROL (mmol/L)
1	11.56	11.77	21.56	12.05	56.87	0
2	10.89	11.09	19.48	12.47	55.12	0
3	12.03	11.37	18.45	12.79	58.45	0
4	11.45	11.51	19.12	11.48	57.09	0
5	10.75	11.37	19.78	12.89	51.64	0
6	11.05	11.09	19.88	13.61	57.78	0
7	11.11	11.69	19.46	11.79	56.78	0
8	10.64	11.45	18.44	13.65	54.78	0
9	11.45	11.35	25.45	12.76	53.45	0
10	11.03	10.79	18.45	14.87	50.12	0
11	11.87	11.49	17.45	13.88	58.65	0
12	11.45	12.45	19.69	13.63	56.77	0
13	11.89	12.54	18.08	13.79	60.12	0
14	11.45	12.45	19.56	13.87	57.45	0
15	10.45	12.05	19.45	12.45	54.55	0
Mean value	11.27	11.63	19.62	13.06	55.97	0.00

Table 11: Intergroup comparison of mean micro leakage values among different groups on Day 30 using one-way analysis of variance (ANOVA)

Groups	Mean	SD	One Way ANOVA	p-value
Group A	11.27	0.47	F = 275.83	0.001*
Group B	11.63	0.53		
Group C	19.62	1.88		
Group D	13.06	0.93		
Positive Control	55.97	2.71		
Negative Control	0.00	0.00		

* p value < 0.05 is considered as statistically significant.

Table 12: Pairwise comparisons of mean micro leakage values on Day 30 using Tukey's post hoc analysis

Groups		Mean Difference	p-value
Group A	Group B	-.35933	.983
	Group C	-8.34867	.000*
	Group D	-1.79400	.011*
	Positive Control	-44.70333	.000*
	Negative Control	11.27133	.000*
Group B	Group C	-7.98933	.000*
	Group D	-1.43467	.076
	Positive Control	-44.34400	.000*
	Negative Control	11.63067	.000*
Group C	Group D	6.55467	.000*
	Positive Control	-36.35467	.000*
	Negative Control	19.62000	.000*
Group D	Positive Control	-42.90933	.000*
	Negative Control	13.06533	.000*
Positive Control	Negative Control	55.97467	.000*

* p value < 0.05 is considered as statistically significant.

Table 13: Intragroup comparison of mean micro leakage values at different time intervals in Group A using Repeated measures ANOVA

Time Interval	Mean	SD	Repeated Measures ANOVA	p-value
Day1	0.25	0.02	F = 504.53	0.001*
Day10	1.55	0.57		
Day20	7.33	0.31		
Day30	11.27	0.47		

* p value < 0.05 is considered as statistically significant.

Table 14: Pairwise comparisons of mean micro leakage values of Group A at different time intervals using LSD Bonferroni analysis

Groups		Mean Difference	p-value
Day 1	Day 10	-1.302	.000*
	Day 20	-7.084	.000*
	Day 30	-11.020	.000*
Day 10	Day 20	-5.782	.000*
	Day 30	-9.718	.000*
Day 20	Day 30	-3.936	.000*

* p value < 0.05 is considered as statistically significant.

Table 15: Intragroup comparison of mean micro leakage values at different time intervals in Group B using Repeated measures ANOVA

Time Interval	Mean	SD	Repeated Measures ANOVA	p-value
Day1	0.33	0.03	F = 385.03	0.001*
Day10	2.27	0.25		
Day20	8.40	0.37		
Day30	11.63	0.53		

* p value < 0.05 is considered as statistically significant.

Table 16: Pairwise comparisons of mean micro leakage values of Group B at different time intervals using LSD Bonferroni analysis

Groups		Mean Difference	p-value
Day 1	Day 10	-1.944	.000*
	Day 20	-8.072	.000*
	Day 30	-11.301	.000*
Day 10	Day 20	-6.128	.000*
	Day 30	-9.357	.000*
Day 20	Day 30	-3.229	.000*

* p value < 0.05 is considered as statistically significant.

Table 17: Intragroup comparison of mean micro leakage values at different time intervals in Group C Repeated measures ANOVA

Time Interval	Mean	SD	Repeated Measures ANOVA	p-value
Day1	0.52	0.02	F = 111.33	0.001*
Day10	3.42	0.31		
Day20	12.02	1.35		
Day30	19.62	1.88		

* p value < 0.05 is considered as statistically significant.

Table 18: Pairwise comparisons of mean micro leakage values of Group C at different time intervals using LSD Bonferroni analysis

Groups		Mean Difference	p-value
Day 1	Day 10	-2.905	.000*
	Day 20	-11.503	.000*
	Day 30	-19.098	.000*
Day 10	Day 20	-8.599	.000*
	Day 30	-16.193	.000*
Day 20	Day 30	-7.595	.000*

* p value < 0.05 is considered as statistically significant.

Table 19: Intragroup comparison of mean micro leakage values at different time intervals in Group D Repeated using measures ANOVA

Time Interval	Mean	SD	Repeated Measures ANOVA	p-value
Day1	0.34	0.03	F = 171.83	0.001*
Day10	2.52	0.26		
Day20	8.66	0.55		
Day30	13.06	0.93		

* p value < 0.05 is considered as statistically significant.

Table 20: Pairwise comparisons of mean micro leakage values of Group D at different time intervals using LSD Bonferroni analysis

Groups		Mean Difference	p-value
Day 1	Day 10	-2.179	.000*
	Day 20	-8.321*	.000*
	Day 30	-12.724*	.000*
Day 10	Day 20	-6.142*	.000*
	Day 30	-10.545*	.000*
Day 20	Day 30	-4.403*	.000*

***** p value < 0.05 is considered as statistically significant.

Table 21: Intragroup comparison of mean micro leakage values at different time intervals in Positive Control group using Repeated measures ANOVA

Time Interval	Mean	SD	Repeated Measures ANOVA	p-value
Day1	18.14	0.46	F = 729.62	0.001*
Day10	21.85	1.13		
Day20	33.41	2.27		
Day30	55.97	2.71		

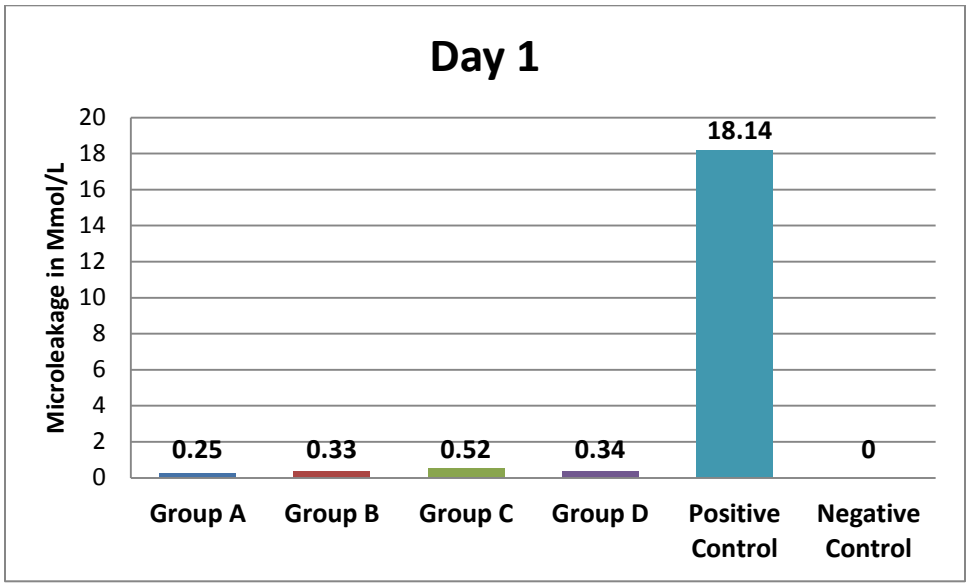
***** p value < 0.05 is considered as statistically significant.

Table 22: Pairwise comparisons of mean micro leakage values of Positive Control group at different time intervals using LSD Bonferroni analysis

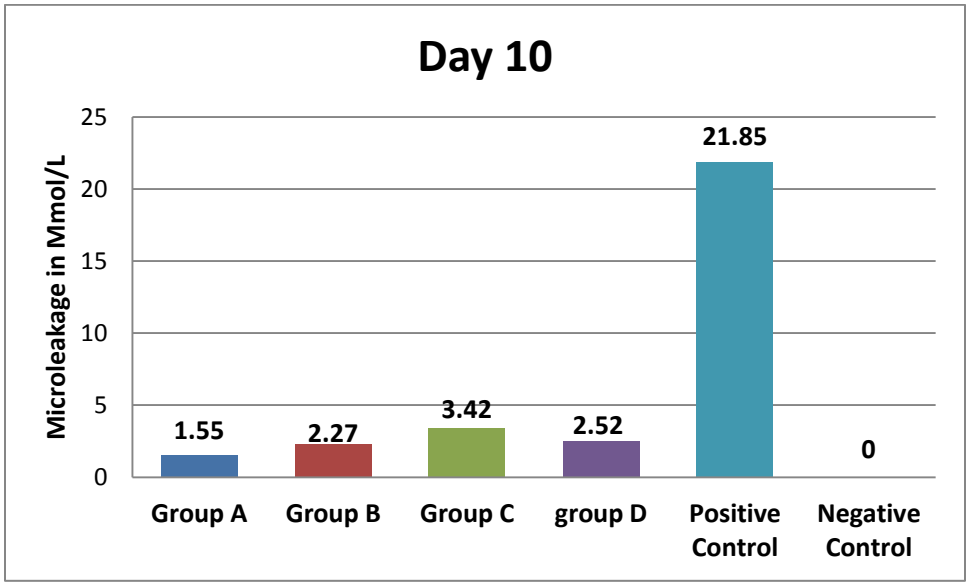
Groups		Mean Difference	p-value
Day 1	Day 10	-3.708*	.000*
	Day 20	-15.265*	.000*
	Day 30	-37.827*	.000*
Day 10	Day 20	-11.557*	.000*
	Day 30	-34.119*	.000*
Day 20	Day 30	-22.563*	.000*

***** p value < 0.05 is considered as statistically significant.

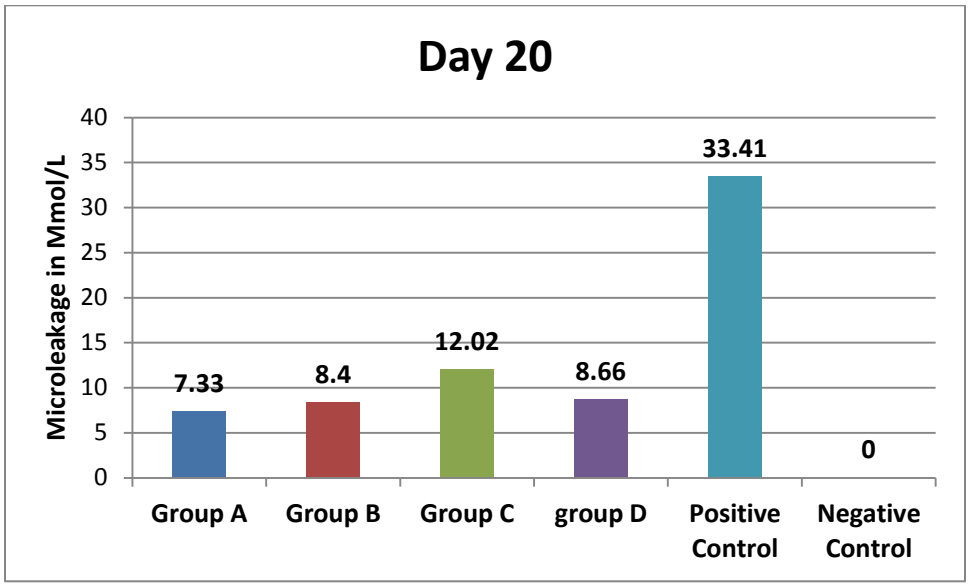
Graph: 1 Intergroup comparison of mean micro leakage values among different groups on Day 1 using one-way analysis of variance(ANOVA)



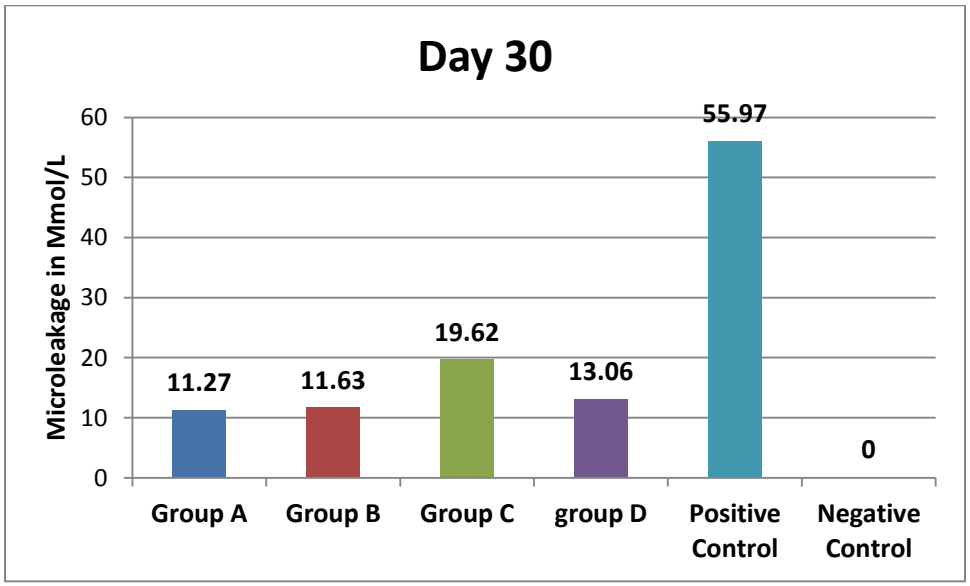
Graph: 2 Intergroup comparison of mean microleakage values among different groups on Day 10 using one-way analysis of variance (ANOVA)



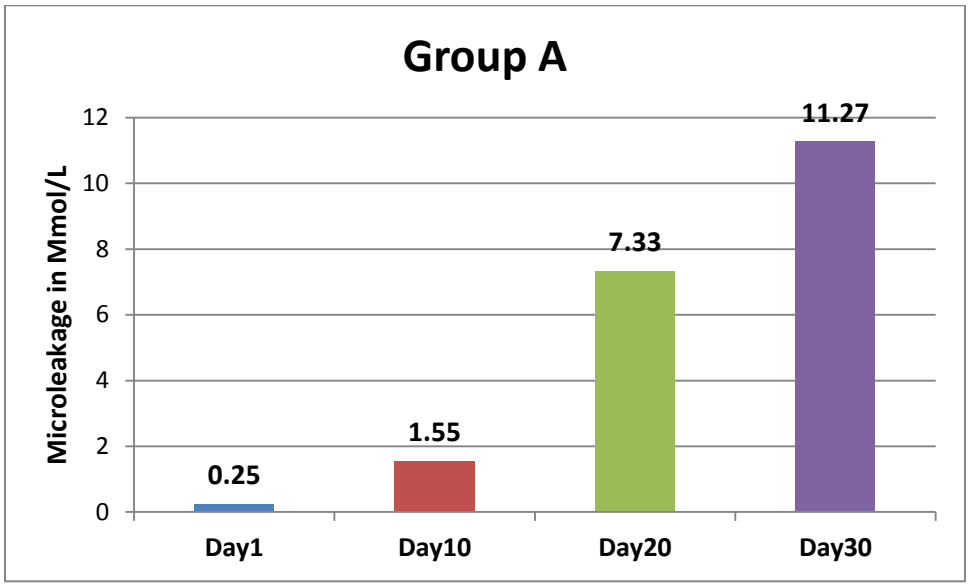
Graph 3: Intergroup comparison of mean micro leakage values among different groups on Day 20 using one-way analysis of variance (ANOVA)



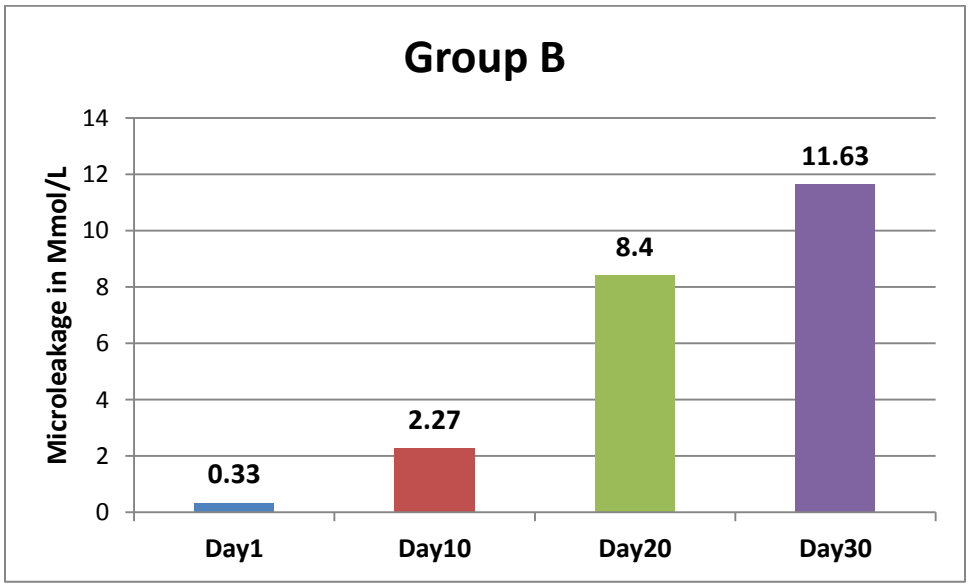
Graph 4: Intergroup comparison of mean microleakage values among different groups on Day 30 using one-way analysis of variance (ANOVA)



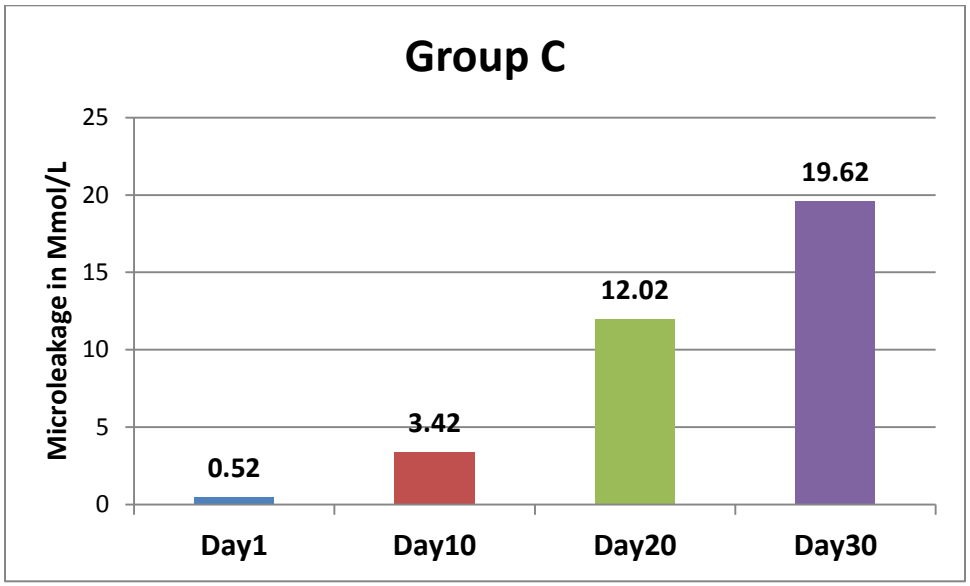
Graph 5: Intragroup comparison of mean microleakage values at different time intervals in Group A Using Repeated measures ANOVA



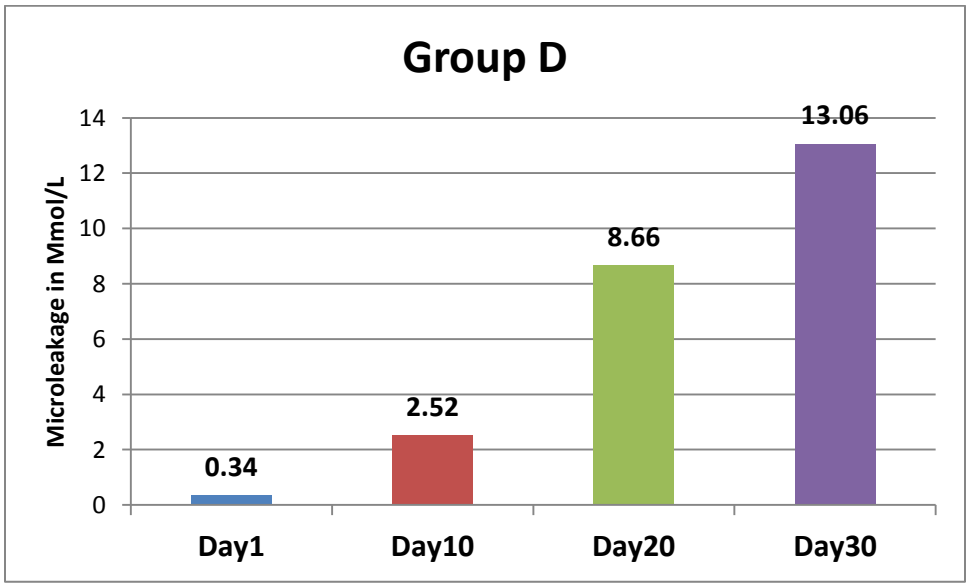
Graph 6: Intragroup comparison of mean microleakage values at different time intervals in Group B using Repeated measures ANOVA



Graph 7: Intragroup comparison of mean microleakage values at different time intervals in Group C using Repeated measures ANOVA



Graph 8: Intragroup comparison of mean microleakage values at different time intervals in Group D using Repeated measures ANOVA



Discussion

DISCUSSION

Endodontic therapy presents the opportunity to save a tooth from a potential extraction. It helps in understanding the internal anatomy, canal shape and contour which indeed facilitates the filling of the canal space available. Endodontic diseases originate from an infected or affected pulp, hence the root canal space must be thoroughly and carefully debrided and obturated. The goal of endodontic treatment is prevention or elimination of microbial infections from the root canal space. Thus the success of endodontic treatment depends on successful infection control.[74]

Instrumentation of the root canal is to remove all necrotic and vital pulp tissues and infected hard tissues, to give the canal system a shape facilitating placement of a permanent root filling material.[74] Instrumentation of the mineralized tissues produces debris. The major part of debris is made up of mineralized collagen matrix and is spread over the surface of the dentinal wall to form smear layer. Smear layer was first reported by Eick et al (1970) and estimated that the particle size can range from 0.5 to 15 µm. Brannstrom & Johnson (1974) estimated the thickness of smear layer produced during cavity preparation can be upto be 2 to 5 µm, with few micrometers extensions into the dentinal tubules during cavity preparations known as smear plugs. McComb and Smith (1975) first described the smear layer on the surface of instrumented root canals, and suggested that the smear layer consisted of dentine and remnants of odontoblastic processes, pulp tissue and bacteria. The

smear layer in the cavity and root canal walls differs from each other due to the different armamentarium used and variation in the dentinal tubule based on the location, number and mineral content. Brannstrom & Johnson (1974) and Mader et al. (1984) concluded that the tubular packing phenomenon of smear layer was due to the action of burs and instruments. Components of the smear layer can be forced into the dentinal tubules to varying distances (Moodnik et al. 1976, Brannstrom et al. 1980, Cengiz et al. 1990) to form smear plugs. However, Cengiz et al. (1990) proposed that the penetration of smear material into dentinal tubules could also be caused by capillary action as a result of adhesive forces between the dentinal tubules and the smear material. The removal of smear layer can be done either by chemical and or mechanical means. A non-instrumental hydrodynamic technique (Lussi et al. 1993) has been proposed, it utilizes sonically driven polymer instruments with tips of variable diameter are reported to disrupt the smear layer in a technique called hydrodynamic disinfection (Ruddle 2007).[61]

Inability of the instruments to reach the crevices, fins and ramifications of the root canal had made the usage of irrigants mandatory. The mechanical objectives of irrigation are to flush out the debris, lubricating the canal and dissolving organic and inorganic tissue. The biological function of irrigants is related to their antimicrobial effect. Ideal irrigant should be an effective disinfectant, non toxic, nonallergenic, have an ability to differentiate between necrotic and vital host tissue, and should be able to retain its effectiveness with

dental hard tissue and when mixed with other irrigants.[69] However none of the irrigants have all the required properties. A combined use of separate irrigants is the clinical protocol recommended to ensure successful outcome of the endodontic treatment. The recommended regime for irrigation is to employ 17% EDTA for 1 minute followed by final rinse with sodium hypochlorite.[69]

Sodium hypochlorite is the most commonly used irrigant, commonly used with 0.5% to 5.25% concentrations. It is an excellent antimicrobial agent, capable of dissolving necrotic tissue, vital pulp tissue, and the organic components of dentin and biofilms. The major disadvantage of the sodium hypochlorite is its inability to dissolve the inorganic component of the smear layer. Chlorine is responsible for the tissue dissolving capacity of sodium hypochlorite, it is stable for only 2 minutes, so it is essential for the continuous replenishment. However the optimal time a hypochlorite irrigant needs to remain in the root canal is an unsolved issue.[69]

Chelating agent like ethylene diamine tetraacetic acid (EDTA) at 15 to 17% can be used for the removal of the smear layer. EDTA helps in softening dentin and usually used during instrumentation.[73] EDTA was first described by Fedinand in 1935 and introduced to endodontics by Nygaard ostby. Demineralization by EDTA increases the dentinal permeability. The minimum time recommended for smear layer removal is 1 to 5 minutes.[69]

The question of keeping or removing the smear layer remains controversial (Drake et al. 1994, Shahravan et al. 2007). Some investigators

believe in retaining the smear layer (Michelich et al. 1980, Pashley et al. 1981, Safavi et al. 1990, Drake et al. 1994, Galvan et al. 1994). Some believe in its removal (McComb & Smith 1975, Goldberg & Abramovich 1977, Wayman et al. 1979, Cunningham & Martin 1982, Yamada et al. 1983). However the data present indicated the removal of the smear layer for thorough disinfection and better adaptation of material to the canal walls.[61]

The objective of obturating the root canal is the substitution of an inert filling in the space previously occupied by the pulp tissue. A Fluid tight seal is required for the successful endodontic treatment. "According to the hollow-tube theory (Rickert & Dixon 1931), stasis of fluid in the apical part of the canal leads to degradation of this fluid and the formation of toxins, and induces and maintains periapical inflammation." The purpose of root canal filling is to prevent microbial penetration from the oral cavity into the periapical tissues through the root canal. Obturation of the root canal space at both the coronal and apical ends prevents the entry of microorganisms. Apical obturation protects the pulp from infection caused by residual microorganisms in the root canal, infection from the peridontium, and infection due to anachoresis, by blocking the portal of exit into the periapex. Reinfection by microorganisms from the oral cavity can be prevented by filling the whole pulp space, to block the dentinal tubules and accessory canals.[65]

Since the 18th century, various materials like tin foil, lead foil, gold foil and other intracanal medicaments like zinc oxide, paraffin, copper points were

used in the endodontic treatment.[74] Currently the root canal filling materials are available in various forms like solid-core materials, semi-solid core materials and paste filling materials. Silver cones, a solid core material were popularly used for root canal obturation. The rigidity made it easy to place into the root canal but the cones were unable to fill irregularities of the canal. Major reason for their failure was due to the leakage and corrosion products. On the other hand, pastes were able to adapt to the complex internal anatomy of the root canal walls. Zinc oxide was the major component of paste system, however, shrinkage and inability to control the flow were major disadvantages of paste system.[69]

Gutta-percha a semi solid core material is the most popular core material used for obturation. The era of the gutta percha began with Asa Hill's development of Hill's stopping in 1847, composed of bleached gutta-percha and carbonate of lime and quartz. Later, in 1867 G. A. Bowman used gutta-percha points to obturate root canals. In 1887 gutta-percha was marketed by S.S. White Company. Gutta-percha is trans-isomer of polyisoprene and available in alpha phase and beta phase (Godman A, 1974). The alpha phase is pliable and tacky which flows on pressure and beta phase is a solid compactable mass. (Schilder, 1985).[74]

Gutta-percha cones are composed of 20% gutta-percha, 65% zinc oxide, 10% radiopacifiers, and 5% plasticizer, (Friedman CE, 1977). Anti-microbial properties are enhanced by adding iodoform (Chogle SC, 2005),

calcium hydroxide (Lohbauer U, 2005) chlorhexidine (Lui JN, 2004) and tetracycline (Melker KB, 2006)[69] Alpha phase is commonly used in warm vertical/ thermo plasticized obturating technique and beta form in the cold lateral condensation technique.[74]

Gutta-percha cones are available in both standardized and non-standardized forms. Standardization specifications are given by International Organization of Standardization (ISO) or ADA American National Standards Institute (ADA ANSI). Gutta percha does not bind or attach to the dentin root canal walls. In order to obtain some form of hydraulic closure of the root canal system, a sealing agent must be employed.[56] Sealer and core material acts synergistically to create impervious seal. The core acts as a piston on the flowable sealer, causing it to spread, fill voids, canal irregularities and to wet the dentin wall.[74]

According to Grossman, an ideal root canal sealer should: [73,74]

- Provide an excellent seal when set.
- Produce adequate adhesion among it, the canal walls and the filling material.
- Be radiopaque.
- Be dimensionally stable.
- Be easily mixed and introduced into the canals.
- Be easily removed if necessary.
- Be insoluble in tissue fluids.

- Be bactericidal or discourage bacterial growth.
- Be non irritating to periradicular tissues.
- Be slow setting to ensure sufficient working.
- It should not provoke an immune response in periradicular tissues
- It should neither be mutagenic nor carcinogenic.

Physical properties, biocompatibility, sealing ability, ease of handling are the important properties which are used to characterize a sealer.[26] Neither shrinkage nor expansion of sealer is considered desirable for a root canal sealing material. Shrinkage produces slits and passageways for bacteria, expansion may create forces which causes infractions and fracture of dentin. Ingle classified sealers, as Zinc Oxide containing sealers, Calcium Hydroxide containing sealers, Resin sealers, Glass Ionomer based sealers, Silicone-Based sealers, Solvent based sealers, Urethane Methacrylate Sealers, Para-formaldehyde based sealers.[74]

Zinc oxide-eugenol sealer has been most commonly used for sealing root canals. Zinc oxide eugenol sealer was introduced by Rickert and Dixon, later Grossman modified the formulation.[69] The basic composition of the zinc oxide sealer is Zinc oxide (42%), Staybelite resin (27%), Bismuth subcarbonate (15%), Sodium borate, anhydrous (1%), and liquid consists of Eugenol (4 allyl 2 methoxyphenol). Some preparations additionally have thymol or thymol iodide to increase the anti-bacterial efficacy. Oil of clove is

used to replace eugenol partially or totally as it is composed of 60 to 80% of eugenol. Many of the zinc oxide- eugenol sealers also contain rosins that increase adhesion and decrease the solubility of the cement. Rosin (colophony) is composed of approximately 90% resin acids. Resin acids are amphiphilic, with the carbon group being lipophilic affecting the lipids in the cell membranes. The resin acids are both antimicrobial and cytotoxic, but the combination with zinc oxide exerts a significant level of cytoprotection.[56] Zinc oxide sealer sets in moist or humid environment by forming chelation compound within 24 hours, this can be altered by addition of resins, calcium phosphates or zinc acetate.[79]

There are several different types of polymer materials used as endodontic sealers. The more common are AH26, AH Plus, Diaket, RSA RoekoSeal, and Endofill.[56] The resin based sealers were proposed by Schröeder, in 1954, and contained epoxy resin and bisphenol[37]. EndoREZ is a dual cure methacrylate resin based sealer which can be used with gutta percha. Diaket is a polyvinyl resin based sealer, consisting of bismuth phosphate and zinc oxide and liquid composed of dichlorophen, triethanolamine, propionyl aceto phenone, and copolymers of vinyl acetate, vinyl chloride, and vinylisobutyl ether. Epoxy resin sealers have an established record in endodontics, especially in the form of AH 26 and its successor AH Plus.[74]

AH26 sealer contains bisphenol A epoxy base and hexamethylene–tetramine as catalyst. AH26 has high radiopacity, low solubility, slight

shrinkage and tissue compatibility. Methenamine (also known as urotropin) one of the constituent of AH 26 produces toxic by-products like formaldehyde. Substitutes which has mixture of amines were introduced to prevent the formation of formaldehyde to modify the sealer.[44]AH plus has the same mechanical properties like AH 26 and also overcame the problem of long setting time and formaldehyde release.[36] AH plus comes in a two paste system unlike AH 26 which was in powder and liquid system. AH Plus improvements are the decreased film thickness and decreased solubility than that of AH 26.[74] AH Plus sealer have fillers of finely ground calcium tungstate with an average particle size of 8 μm and finely ground zirconium oxide of 1.5 μm average particle size. According to ISO 6876-2001 ADA 57 the requirements of the sealer the film thickness should be <50μm.

Numerous studies[66] have been carried out to compare sealing property of various sealers, but there was hardly any consensus. Some studies showed that resin- base sealer provided better seal than other sealers (Timpawat S et al 2001, Miletić I et. al. 1999)[59,39] and others indicated that there was no significant difference in leakage of different types of sealers.(Chailertvanikul P et al. 1996, Kataoka H, et al 2000)[7,30] Hence in the present study zinc oxide based sealer and resin based sealers were compared.

Placement of the sealer in the root canal is done by using various techniques like, coating the sealer on the master cone and pumping the cone up and down in the canal, coating a file with sealer and rotating it in

counterclockwise direction, using a lentulo spiral, or a syringe and using ultrasonic instrument for activation.[69] The master gutta-percha coating technique might be preferable because of its ease of use. A thin layer of the sealer has to be applied evenly to canal walls before the core filling material placement.[46]

Particles of smaller size helps in manipulation of the cement easily in less time and resultant cement mix is smoother and flow better.[21] Grossman (1975) determined the particle size of cement powder indirectly. Microscopic measurement was not used to determine the size because of the linear dimension variations of different components of cement powder. Alternatively, determination of the particle size was done by using a series of sieves, with first particles passing through a sieve of finest dimension (200 mesh or 74µm) openings.[21] The smaller nano powder particle sizes sealers can have a better sealing ability than the conventional counterpart.[26] The Zinc oxide nanostructure enhances antibacterial properties due to surface enhancement.[50]

'Nano' is the Greek derived word for 'dwarf little old man'. A nanometer is 10^{-9} or one billionth of a meter. Nanotechnology deals with manipulating matter, atom by atom. Physicist Richard P Feynman in 1960, had the first notion of how nanotechnology could be applied in medicine.[8] Presently nanomedicine is used for diagnosis, treatment and prevention of disease. Research tools like protein chips in nanotechnology are used for better

understanding of the molecular basis of diseases, it helps to identify new molecular targets for therapy.[78]

Nano technology is used in manufacturing various dental materials: Light polymerization composite resins and bonding systems, imprint materials, ceramics, coatings for dental implants, bioceramics, mouthwashes containing fluoride and fissure sealant materials.[26] These nano particles have higher activity because of the higher surface area that enable to achieve a greater degree of interaction (Kishen, *et al*., 2008).[24] and penetrate dentinal tubules to provide additional 'nano retention'. (Mitra et al., 2003)[46]

Zinc oxide nanoparticles added to composite yielded better results in the reduction of bacterial count in orthodontic cases, and also reduced the incidence of white spot lesions during the treatment.[40] The zinc oxide nano particles have a better penetration than the conventional counterpart, presence of small particle size leads to superior properties of the material. Nano particles are able to diffuse better in the root bone, due to their small size, which results in the decrease of the root canal leakage (shayani et.al. 2013)[52] Hence the zinc oxide nano powder has been selected as an experimental sealer in the present study.

In the present study mandibular premolar teeth with single root were selected and cleansed thoroughly to remove debris, calculus on the external surface. And the teeth were stored in 0.2% sodium azide solution.The cleaning and shaping of the teeth were done using step back technique. Step back

technique also known as telescopic technique was introduced by Clem and Weine. Incremental reduction of working length was done at 1mm steps following the apical enlargement. Gates Glidden drills were used for further enlargement of the coronal third of the canal. 15% EDTA with 10% carbamide peroxide gel was used during instrumentation in the study.[69]

After thorough cleaning and shaping, the canal was irrigated with 3% sodium hypochlorite solution and dried with paper points. Obturation was done by cold lateral condensation. Cold Lateral condensation is a common method used for root canal obturation. It is a relatively uncomplicated technique which requires a simple armamentarium. Sakkal et al demonstrated that lateral condensation produces a three dimensional filling.[71] A major advantage of this technique is length control, and few other advantages like ease of retreatment, adaptation to canal walls, positive dimensional stability and the ability to prepare post space. For the obturation spreader and plugger selection is done during the cleaning and shaping of the canal. Finger spreader and plugger have better tactile sensation over conventional instrument; they have better control and reduction of dentin stress during obturation. They also reduce the incidence of vertical root fractures during obturation. The penetration of spreader within 2 mm of working length will provide a higher quality seal.[77]

In the present study, before the initiation of obturation the master cone was selected and there was a definite stop when the cone fits into place. The

cone is fitted to within 1 mm of working length. A cleared apical area and deep spreader penetration will usually push the gutta-percha and sealer apically to fill the remaining 1 mm.[77] The canal walls were coated with sealer by using K file rotating in anti-clockwise direction. The selected master cone is coated with sealer, then placed in the canal and sealer is further coated with it in pumping motion to the canal walls. Spreaders are selected and inserted up to 1 to 2mm within the working length and space was created for the placement of the accessory cone. The accessory cone tips were coated with the sealer and placed into the space created by the spreader. This procedure is continued till the canal is completely filled with the gutta percha cones.[69] The specimens were examined under magnification and illumination to detect vertical root fractures using magnifying loupes. The samples with fractures were discarded.[38] And the intact samples were subjected to the microleakage test.

Accoring to Timpawat et al., endodontic sealers are used to eliminate the interphase between the gutta percha and the dentinal walls. However leakage occurs at the interfaces between the sealer and dentin; sealer and gutta percha; and in spaces within the sealer itself. Thus the quality of the filling largely depends on the sealing capacity of the sealers.[60] Epley and schilder suggested that the ideal root canal filling should be well adapted to the canal walls and its irregularities, inadequate seal can lead to the microleakage.

Microleakage (apical or coronal) may cause failure of root canal therapy (Dow and Ingle 1955; Madison and Wilcox 1988)[52]

Various tests were used to determine the microleakage from the filled root canal. The hollow-tube theory (Rickert & Dixon 1931), might have been the reason to perform leakage experiments by simply dipping root tips into the dye solution and measured penetration from apical to coronal end of the samples.[65] The dye penetration test was a simple and inexpensive method but often yielded a large variation of the result, (Wu M-K, 1993)[66] Many studies have proved that sterile tissue fluids in tubes are not able to cause long term inflammation, and the primary cause was bacteria and their products (Torneck 1966, Sundqvist 1976, Makkes etfll, 1977). So it is doubtful if the results of this method are of greater clinical significance.

Bacterial leakage test might be more biologically relevant but the results might vary with the bacterial species used. Maintaining aseptic conditions during the experiment can be difficult. Radioisotope labelling and electrochemical technique pose a radiation hazard and require sophisticated materials and apparatus. The fluid filtration method has no standardization of the methods, such as the measurement time, the applied pressure, the diameter of the tube containing the bubble, and the length of the bubble, which might influence the results. (Pommel L, 2001)[66] Quantitative volumetric tests will be more pertinent in analyzing the microleakage. It might be more relevant to evaluate the volume of fluid that can flow through the obturated root canal,

than to evaluate the length of gap in it.[65] With the glucose penetration model, it was possible for quantitative analysis of the endodontic microleakage by calculating the cumulative value of leaked glucose.[66]

The leaked glucose was calculated using spectrophotometer. Glucose was oxidized by the enzyme glucose oxidase in the presence of oxygen to gluconic acid with formation of hydrogen peroxide. Then in the presence of a peroxidase enzyme, a chromogenic oxygen acceptor (4-aminoantipyrine and phenol) was oxidized by the hydrogen peroxide, resulting in the formation of a red product (oxidized chromogen). The quantity of this oxidized chromogen was proportional to the glucose present initially in the first reaction, whose quantity was determined by spectrophotometry.[66]

The reaction is as follows:

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Oxidized chromogen} + H_2O$$

In the present study 1 mol/L glucose solution (pH = 7.0) was used as a tracer, with a density 1.09×10^{-3} g/L and viscosity 1.18×10^{-3} Pa.s at 37°. Molecular weight of glucose is 180 Da, and is hydrophilic and chemically stable.[66] Root canal fillings should be impervious to microorganisms so the tracers used in leakage study should be much smaller molecules (for example: sugar). "Small molecules (for example, water) may leak 100-fold more than

large ones (albumin) (Pashley & Livingstone 1978) and thus the use of small molecular size tracers is indicated.[65] About 4.5 mL of glucose solution, containing 0.2% NaN_3, was injected into the pipette until the top of solution was 14cm higher than the top of gutta-percha in the canal[54]. It created a hydrostatic pressure 15kPa or 15 H_2O.[66] The glass bottle contained 2 mL of 0.2% solution of NaN_3, till the apical third of the root samples are immersed. The leaked glucose from the pipette is collected in the glass bottle and was analysed and recorded in mmol/L.

Sodium azide (NaN_3), have many uses in various fields which include its use as a preservative in aqueous laboratory reagents and biologic fluids and automobile airbags.[11] In the present study sodium azide was used in aqueous solution because of its capacity to inhibit microbial contamination in the glucose solution.[66]

Torabinejad et al.(1990) in his study reported that 50% of root samples with filled root canal were contaminated to the whole length of the canal after 19 and 42 days of exposure to the microorganism. Khayat et al. (1993) in his study reported that the root samples filled laterally or vertically condensed with gutta-percha exposed to human saliva were contaminated within 30 days after exposure.[54] Hence in this present study, the duration of observation was 30 days. The samples were collected on day1, 10, 20, and 30.

The collected samples were analysed under spectrophotometer (PRIMACHEM V-2) at 505 nm. Photometry deals with the light absorption by

molecules in a solution. “When a light at a particular wavelength is passed through solution, some amount of it is absorbed and, therefore, the light comes out is diminished. The nature of light absorption in a solution is governed by Beer-Lambert law.” Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. There are numerous methods for measuring the concentrations of specific substances within body fluids. One commonly used method is called spectrophotometry. A precisely selected wavelength (234nm-610nm) in both ultra violet range and visible range can be used for measurements. The spectrophotometer works on Beer-Lambert law.[72]

In the present study the collected samples were added to the glucose reagent and held for 10 minutes, during which the reaction takes place. The chromogenic product of the reaction is fed to a spectrophotometer and the results indicated the amount of glucose in each sample.

The values were tabulated and were subjected to statistical analysis. One way ANOVA, Tukey’s post hoc analysis, Repeated measures ANOVA, and LSD Bonferroni analysis were used to interpret various inter group and intra group comparisons.

On inter group comparison of the samples showed on day 1 of analysis that; nano zinc powder (30nm) sealer had the least leakage compared to other experimental groups, but statistically insignificant difference was observed with nano zinc oxide powder (240nm) sealer, and AH plus sealer and had a

significant difference with conventional zinc oxide sealer which was in accordance with the results obtained by Maryam Javidi in 2014[26]. However nano zinc powder (240nm) sealer did not have statistically significant difference with AH plus sealer and conventional zinc oxide conventional powder sealer and was in accordance with results obtained by Xu et al(2005)[66].

On day 10 of the analysis nano zinc oxide powder(30nm) sealer had the least leakage compared to all other experimental groups which was a statistically significant difference; and was in accordance with the results obtained by Maryam Javidi in 2014[26]. However, nano zinc oxide powder (240nm) sealer did not have a statistically significant difference with AH plus sealer but a statistically significant difference was seen with conventional zinc oxide powder sealer and was in accordance with results obtained by Xu et al (2005)[66].

On day 20 and day 30 of the analysis nano zinc oxide powder (30nm) sealer had the least leakage compared to other experimental groups but there was no statistically significant difference was observed with nano zinc oxide powder (240nm) sealer. When compared with other experimental groups a statistically significant difference was observed; and was in accordance with the results obtained by Maryam Javidi in 2014[26]. However, nano zinc oxide powder (240nm) sealer did not have a statistically significant difference with AHplus sealer but a statistically significant difference was seen with

conventional zinc oxide powder sealer which was in accordance with results obtained by Xu et al (2005)[66].

With the results obtained, it can be inferred that nano zinc oxide powder (30nm) sealer had a better performance compared to other experimental groups at any point of study (but not statistically significant compared to zinc oxide nano powder 240 nm sealer). Nano zinc oxide powder (240nm) sealer had a better performance compared to AH plus (not statistically significant) and conventional zinc oxide sealer.

The efficacy in reducing microleakage can be graded as Nano zinc oxide powder (30nm) sealer = Nano zinc oxide powder sealer (240nm) = AH plus sealer > Conventional zinc oxide powder sealer.

On intragroup comparison of all the samples at different intervals of time; microleakage increased as the time of observation is progressed, Irrespective of the root canal sealer used the amount of microleakage increased. This observation was in agreement with Torabinejad et al.(1990) Khayat et al. (1993).[54]

However, intergroup comparison in the present study showed that nano zinc oxide powder (30 nm) and nano zinc oxide (240nm) had lesser microleakage (statistically insignificant) when compared with AH Plus and conventional zinc oxide sealer.

The geometry of small particles makes it possible for the material to enter into dentinal tubules. This may be an important mechanism in order to provide a hydraulic seal. (Komabayashi et al)[34]

From the present study it can be inferred that the usage of dental materials with smaller particle size are required for obtaining a better result of the endodontic treatment. But, nano zinc oxide powder sealers of different particle sizes did not have a statistically significant difference in terms of microleakage. Hence there is a need to determine the upper limit of particle size for selection of nano materials in order to obtain desirable properties, as the toxicity caused by the nano particles should also be taken into a prime consideration.[78]

Further studies are required to confirm the results of the present study.

This study did not evaluate the microleakage using various other sizes of nano zinc oxide powders. The possible modifications in the liquid component of the sealer were also not evaluated. The biocompatibility of the nano zinc oxide powder to use as sealer in the endodontic practice should also be evaluated for the patient safety as well as the operator safety. These are the few areas in which further studies can focus for providing better patient care.

Summary

SUMMARY

Microleakage is one of the major factors leading to the failure of the endodontic treatment. Many microleakage tests have been performed on various root canal filling material to find their efficacy in providing an ideal seal to the root canal. None of the root canal filling materials available has the ideal property to provide a bacteria impervious seal. Still there is a constant quest in the field of dentistry to find an ideal material for the obturation of a root canal.

The aim of the present study is to compare the microleakage of various root canal sealers with zinc oxide nano powder sealers.

90 extracted human mandibular premolar teeth with intact roots were selected and radiographed. The teeth with single root canal were selected and decoronated horizontally below the level of the cementoenamel junction to obtain an equal length of 15mm.The samples were divided into 4 experimental groups and 2 control groups, each group containing 15 samples. The samples were cleaned and shaped using a step back technique using K files and Gates Glidden drills. The apical part was enlarged till #50 K file and step back enlargement was done upto #6 Gates Glidden drill. 15% EDTA gel was used as a chelating agent and 3% sodium hypochlorite was used as an irrigant. The prepared canals were obturated with 2% gutta percha cones using a cold lateral condensation, using Nano zinc oxide powder (30nm) sealer in group A, Nano

zinc oxide powder (240nm) in group B, Conventional zinc oxide powder (45µm) in group C, AH plus sealer in group D, the positive control group was obturated without sealer application and negative control group was obturated with Conventional Zinc oxide eugenol sealer. The samples in negative control group were completely covered with sticky wax.

The samples were subjected to Glucose penetration test, using 1 mmol/L glucose as a tracer in a 0.2% sodium azide solution. The amount of glucose passing through the obturating material helps in quantifying the micro leakage. The samples were collected from the glucose leakage apparatus and subjected to spectrophotometric analysis for a duration of one month, in intervals of 1, 10, 20 and 30 days.

The results of the present study suggests that the Nano zinc oxide powder (30nm, 240nm) provides a better sealing against microleakage compared to conventional zinc oxide sealer and AH plus sealer.

Conclusion

CONCLUSION

Results of the present study inferred that:

1. Positive control group had the highest mean micro leakage values compared to all the other groups throughout the experimental period.
2. Negative control group had not shown any micro leakage throughout the experimental period.
3. Nano zinc oxide powder (30nm) sealer showed lesser leakage which was statistically significant when compared with that of the AH Plus sealer and conventional zinc oxide eugenol sealer. The mean leakage was statistically insignificant when compared with that of Nano zinc oxide powder (240nm) sealer.
4. Nano zinc oxide powder (240nm) sealer showed lesser leakage which was statistically significant when compared with that of the conventional zinc oxide eugenol sealer. The mean leakage was statistically insignificant when compared with that of AH Plus sealer.
5. In all the groups the amount of microleakage gradually increased with the progression of time irrespective of the sealer used, which were evaluated at the end of day 1, 10, 20, and 30. The intragroup and inter group comparison of the mean microleakage values were statistically significant, except for the negative control group which did not show any microleakage throughout the experimental period.

Based on the present study, it can be concluded that Nano zinc oxide powder (30nm) sealer showed lesser micro leakage compared to Nano zinc oxide powder (240nm) sealer, Conventional zinc oxide eugenol sealer and AH Plus sealer.

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Annexure

ANNEXURE

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TO WHOM SO EVER IT MAY CONCERN

Date: 07.01.2016
Place: Chennai

From
The Institutional Review Board,
Ragas Dental College & Hospital,
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Chennai – 600119.

The thesis topic **"MICROLEAKAGE ASSESSMENT OF NEW ZINC OXIDE NANO PARTICLE SEALERS,BY GLUCOSE PENETRATION MODEL – AN INVITRO STUDY"** submitted by **Dr. SANDEEP NULI** has been approved by the Institutional Review Board of Ragas Dental College & Hospital on 5th May, 2014.

(Dr. S. RAMACHANDRAN M.D.S.)
Secretary, Institutional Review Board,
Head of the Institution,
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