EVALUATION OF MICROLEAKAGE OF THREE DIFFERENT SINGLE-CONE OBTURATION SYSTEMS BY QUANTITATIVE GLUCOSE LEAKAGE 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 **"EVALUATION OF MICROLEAKAGE OF THREE DIFFERENT SINGLE-CONE OBTURATION SYSTEMS BY QUANTITATIVE GLUCOSE LEAKAGE MODEL - AN *IN VITRO* STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. M. RAJASEKARAN, 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 **"EVALUATION OF MICROLEAKAGE OF THREE DIFFERENT SINGLE-CONE OBTURATION SYSTEMS BY QUANTITATIVE GLUCOSE LEAKAGE MODEL - AN *IN VITRO* STUDY"** is a bonafide record work done by **Dr. SREEKANTH KANDEPU** under our guidance during his postgraduate study period between 2013-2016.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**, 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 sincerely thank my post graduate teacher and my guide ***Dr.M. Rajasekaran M.D.S., Professor,*** *Department of Conservative Dentistry and Endodontics, Ragas Dental College and Hospital, for his perseverance in motivating and supporting me throughout my study period.*

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 have 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 during the completion of my study.*

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

I extend my sincere thanks to ***Dr. C.S. Karumaran, M.D.S., Professor,*** *for his constant encouragement throughout the completion of this work.*

I would like to solemnly thank ***Dr. Veni Ashok, M.D.S., Dr. Shankar Narayan, M.D.S., Dr.S.M. Venkatesan, M.D.S., Readers,*** *for all the help during my study period.*

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

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

My sincere thanks ***Ganeshan, Omega labs, guindy*** *for their help and guidance in laboratory testing.*

My sincere thanks to ***Mr. Ravanan.,*** *for his guidance in biostatistics.*

I remain ever grateful to all ***my batchmates, juniors and friends*** *for their support.*

I would like to especially thank ***my parents, my sister and my wife*** *for their love, understanding, support and encouragement throughout these years without which, I would not have never reached so far.*

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 guides me and has given these wonderful people in my life.*

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Introduction

INTRODUCTION

Success of endodontic treatment largely depends on the three dimensional obturation of the root canal system. The main objective of the obturation is to achieve a hermatic seal, to be more precise it should have a fluid impervious or bacterial tight seal.[18,10]

Adequate obturation of the root canal system following intracanal preparation is a major objective of endodontic treatment. Different endodontic filling materials and techniques have been introduced to the dental community in an attempt to improve the apical seal. It is, therefore important to assess the sealing quality of obturation materials.[43]

Ideally an obturating material should have good sealing ability to prevent micro-leakage between the root canal filling and the canal walls. Thus preventing bacterial invasion, that will adversely affect the outcome of root canal treatment.[3,18]

When filling the root canal system, the sealer plays an important role in reducing microleakage. To achieve this property it is important that the root canal filling material should adhere to the root canal dentin. Good adhesion eliminates any space that would allow any penetration and inhibition of bacteria between the sealer and the wall thus, preventing failure of the obturation.[43]

According to Sousa-Neto et al. (2005), Adhesion of an endodontic sealer is defined as its capacity to adhere to the root canal walls and promote the union of the Gutta-percha cones to each other and to the dentine.

The most commonly used obturating technique is cold lateral condensation because of its advantages of controlled placement of gutta-percha in the root canal and low cost. The disadvantage of this technique was its poor adaptation and inability to achieve a homogenous mass. This technique is not suitable for curved canals.[40,42]

Various researchers attempted to overcome the drawback of this technique, thus newer obturation techniques have been introduced. One such obturation is the thermoplastic obturation technique which was introduced by Schilder in 1967. This technique has shown better adaptation to the root canal walls as compared to lateral condensation and at the same time it could successfully obturate the lateral canals. However this new technique still could not fulfill the three basic requisites of obturation.[40,42]

Advances in adhesive technology have reinforced the search for newer means to minimize apical and coronal marginal leakage by improving sealer adhesion to root canal walls. Therefore the mono-block concept has emerged, where the core material, sealer and dentinal tubules becomes a single solid structure. A thermoplastic synthetic polymer based root canal filling material was introduced. The resin core filling material, Resilon (Resilon Research

LLC, Madison, CT), handles like gutta-percha. Obturation with Resilon cones is accomplished by use of Epiphany primer (Pentron Clinical Technologies, LLC, Wallingford, CT) and Epiphany resin-based sealer (Pentron Clinical Technologies).[3]

The thermoplasticity of Resilon is because of polycaprolactone, a biodegradable polyester with a relatively low melting point, while its ability to bond is derived from the inclusion of resin with methacryloxy groups. This filling material also contains glass fillers and barium chloride as fillers, and is capable of coupling to resin sealers, an example of which is Epiphany (Pentron Clinical Technologies, Wallingford CT). Epiphany Root Canal Sealant is a dual-curable resin composite containing a new redox catalyst, that enables optimal auto-polymerization under acidic environments. [36]

With further progress in dentistry yet another new material found its way into Endodontics namely the Smart Seal System. The system consists of obturation points (C-points) containing a polyamide core with an outer bonded hydrophilic polymer coating and an accompanying bio-ceramic sealer.

The endodontic points are designed to expand laterally without expanding axially by absorbing residual water from the instrumented root canal space and the naturally present moisture in the dentinal tubules. The inner core of C-points is a mix of two nylon polymers, Trogamid T and Trogamid CX. The polymer coating is a cross-linked copolymer of acrylonitrile and vinylpyrrolidone which has been polymerised and cross-linked using allyl methacrylate and a thermal initiator.(8)

A great deal of attention has been given to the evaluation of sealing ability of root canal filling materials and associated obturation techniques. Various laboratory based experimental models are used to detect and measure leakage along root fillings. Dye leakage, fluid transport and bacterial penetration are currently the methods commonly used.

However, there was no standardization of methods, such as measurement of time, the applied pressure, the diameter of the tube containing bubble and the length of the bubble which might influence the results.(43)

Recently, Xu et al (2005) discussed a new model that measures the leakage of glucose molecules and checks penetration of different tracers through the root canal, assuming it travels along the canal and reaches the apical region. Glucose has a low molecular size (MW=180 Da), and may be used as an indication for toxins that might penetrate the canal. Shemesh et al. (2006) described this model as a further development of the fluid transportation concept that might be more sensitive than the measurement with an air bubble.(22,43)

Therefore the aim of the present study was to evaluate microleakage along root canal fillings using the said glucose leakage model by comparing three single-cone filling systems at different time intervals.

Aims and Objectives

AIM AND OBJECTIVES

AIM: The aim of the present study was to compare the microleakage and the sealing ability of three single cone obturating systems using a glucose leakage model.

OBJECTIVES:

1. To evaluate the microleakage of the three obturating systems at different time intervals.
2. To check the quantity of the glucose concentration leaked in each group.
3. To compare the sealing ability of commonly used Gutta-percha/AH Plus versus the recently introduced C-points/Bio ceramic sealer and Resilon/ Epiphany systems.

Review of Literature

REVIEW OF LITERATURE

Wu et al (1993)[41] in their review on leakage studies, compared some data on linear measurement of dye penetration following the cold lateral condensation of gutta-percha. They evaluated various techniques and the cold lateral condensation technique has been used as a standard control for comparison. They concluded that more research should be done on leakage study methodology, instead of continuing to evaluate the sealing ability of different materials and techniques by methods that may give little relevant information.

von Fraunhofer et al (2000)[10] in their study evaluated the effects of smear layer and canal instrumentation on leakage in root-filled teeth in an in-vitro study on six groups of freshly extracted human canines and premolars concluded that smear layer removal is beneficial to root canal sealing and obturation with thermo-plasticized gutta-percha provides a superior seal whilst canal instrumentation with engine-driven Ni-Ti files reduces the extent of micro-leakage in root canals.

Kont Cobankara et al (2002)[18] in their in-vitro study evaluated, the micro-leakage of root fillings involving four root-canal sealers including AH Plus , RoekoSeal , Ketac-Endo and Sultan using fluid filtration study on forty extracted human maxillary anterior teeth. Preparation and obturation of the teeth was done and a fluid filtration method was used for quantitative evaluation of apical leakage. They concluded that root fillings with RoekoSeal in combination with cold lateral condensation technique showed better sealing than those with Ketac-Endo, AH Plus and Sultan sealers after 21 days. The fluid filtration test gave quantitative results and allowed nondestructive long term evaluation of specimens.

Pommel et al (2003)[24] did a study to evaluate the sealing properties of four root canal sealers- Sealapex, Pulp Canal Sealer, AH 26, and Ketac on forty eight maxillary central incisors. They measured the apical leakage using fluid filtration method and concluded that the teeth filled with Sealapex displayed a higher apical leakage than those filled with AH 26, Pulp Canal Sealer or Ketac-Endo.

Tagger et al (2003)[37] conducted a study to verify whether an interaction existed between some sealers and different brands of gutta-percha cones. Three brands of cones (Kerr,UDM,Beldent) were tested with three types of endodontic sealers (AH 26, Apexit, Roth's). They concluded that an interaction between sealer and cones is present in some combinations. According to the author it was not possible to ascribe a trend of greater effect to a certain sealer, but Roth's 811 had the least effect on flow.

Weis et al (2004)[40] compared the average sealer cement film thickness and the extent and pattern of sealer penetration into dentinal tubules in association with four obturation techniques in curved root canals. Mesial canals of 44 teeth were randomly divided among SimpliFill, continous wave, Thermafill and 0.04 matched taper lateral compaction obturation groups. They concluded that sealer thickness was strongly dependent on obturation technique. Assuming that minimal sealer thickness and fewer voids are good measures of long-term sealing ability, Thermafil resulted in the best outcome. Consistent, extensive sealer penetration into dentinal tubules was seen and was unrelated to the obturation technique.

Tay et al (2005)[36] compared the ultra-structural quality of the apical seal achieved with Resilon/Epiphany an Gutta-percha/AH Plus. They examined for gaps along canal walls using SEM, and for apical leakage using transmission electron microscopy (TEM). SEM revealed both gap-free regions, and gap-containing regions in canals filled with both materials. TEM revealed the presence of silver deposits along the sealer-hybrid layer interface in Resilon/Epiphany, and between the sealer and gutta-percha in the controls. It was concluded that a complete hermetic apical seal cannot be achieved with either root filling materials.

Xu et al (2005)[43] did a study to introduce a new method for quantative testing of endodontic leakage. Eighty straight maxillary anterior teeth were divided into 3 groups. The conclusions drawn were that 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.

Stratton et al (2006)[34] compared the sealing ability of gutta-percha and AH Plus sealer versus Resilon and Epiphany Resin Root Canal sealer using three different final irrigants (5.25% NaOCl, 0.012% chlorhexidine (CHX), or 2% CHX) with the fluid filtration model using 140 teeth. The two-way ANOVA analysis indicated significantly less leakage using Resilon with Epiphany sealer compared to gutta-percha and AH Plus sealer. There was no statistical significance between any of the irrigants used for either obturation group.

Shemesh et al (2006)[35] conducted a two month longitudinal study to compare the leakage along apical root fillings with and without smear layer using two different leakage models. 120 single rooted teeth were used in this study. Under the conditions of this study, the glucose penetration model was more sensitive in detecting leakage along root fillings. 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.

Veríssimo et al (2007)[38] compared the level of apical leakage between canals filled with gutta-percha/ AH-Plus (GP) and the Resilon/Epiphany System (RES), when submitted to two filling techniques [lateral condensation and Hybrid technique (HT)]. 70 extracted teeth were instrumented and randomly divided into four groups in accordance with the materials and techniques used. After 7 days in an oven the teeth were immersed in India ink and cleared. Leakage was measured by the NIH image program. The comclusion was that there was no difference between the filling techniques, but there was a statistically significant difference when RES was compared with GP, which leaked more than RES. With RES, leakage was confined to the apical third and HT could be used to thermoplasticize RES with satisfactory results.

Wedding et al (2007)[39] in their investigation compared micro-leakage of teeth obturated with gutta-percha and teeth obturated with Resilon by using a fluid filtration model.46 human single rooted mandibular premolars were used. The results showed that Resilon is a suitable replacement for gutta-percha as a root canal filling material on the basis of its increased resistance to fluid micro-leakage.

Paque et al (2007)[22] compared the long term apical sealing ability of Resilon/Epiphany versus gutta-percha/AH Plus. The root canals of 90 single rooted human mandibular premolars were prepared with ProFile 0.4 taper instruments to apical size 40. The teeth were randomly divided into four groups containing 20 teeth each.1o teeth were positive controls. The root canals were filled with respective materials and allowed to set for 7 days at 37c and 100% humidity. Root canal materials were removed and fluid movement was then measured using a fluid transportation model and re-evaluated after 16-months of water storage. The results suggest that initially, Resilon/Epiphany root fillings prevented fluid movement to the same degree as gutta-percha/ AH Plus counterparts, but showed more fluid movement when tested at 16 months.

Patel et al (2007)[23] compared penetration depth into dentinal tubules of RealSeal with that of a well-established endodontic sealer (Tubliseal) by means of confocal microscopy in 20 extracted teeth. Confocal microscopy was used to assess the penetration depths of the sealers at three sites for each specimen. The results are suggestive that the penetration depth of RealSeal into the root dentinal tubules is significantly greater than that of Tubliseal.

Xu et al (2007)[42] evaluated the sealing ability of 4 different obturation techniques by using a glucose leakage test 0n 80 extracted single rooted maxillary incisors. The teeth were de-coronated and the canals were prepared. Then the teeth were randomly divided into4 groups and filled with cold lateral compaction, warm vertical compaction, Thermafil, or the E & Q Plus. A glucose leakage model was used for quantitative evaluation of the coronal-to-apical micro-leakage at 24 hours,1,2,3,5,8 and 12 weeks. The authors concluded that the warm vertical compaction, Thermafil, and the E & Q Plus system showed a better sealing result than cold lateral compaction of gutta-percha at extended observation periods.

Shemesh et al (2007)[33] measured glucose penetration and fluid transport through coronal root structure and compared it with leakage along the coronal region of root fillings in 50 teeth and concluded that no leakage was observed through root structure. Filled canals were associated with penetration of glucose regardless of the material used.

Shemesh et al (2008)[31] evaluated the reactivity of different endodontic materials and sealers with glucose and assessed the reliability of the glucose leakage model in measuring penetration of glucose through these materials ten uniform discs were made of each of the following materials: Portland cement, MTA sealer 26, calcium sulphate, calcium hydroxide, AH 26, Epiphany, Resilon, Gutta-percha and dentine. After storing the discs for1 week at 37̊c and humid condition, they were immersed in 0.2 $mgmL^{-1}$ glucose solution in a test tube. The concentration of glucose was evaluated using an enzymatic reaction after 1 week. They concluded that Portland cement, MTA, Ca(OH)2 and Sealer 26 react with a 0.2 mg/ml glucose solution and therefore should not be evaluated for sealing ability with the glucose leakage model.

Souza et al (2008)[30] conducted a study to check whether leakage results of the same specimens measured by 2 different leakage models are similar. Canine root canals were prepared and filled with cold gutta-percha cones and 1 of 4 sealers. The 80 specimens were first connected to a fluid transport model where air-bubble movement was measured. The same specimens were later connected to glucose penetration model where glucose concentration was measured. They concluded that the leakage results recorded in the fluid transport model and glucose penetration model were similar.

Slutzky et al (2008)[32] did a study to evaluate the antimicrobial effects of root canal sealers - AH plus, Apexit Plus, Epiphany SE, and RoekoSeal when in contact with Enterococcus faecalis. The direct contact test was used to assess the anti microbial properties og the materials. The materials were examined immediately after setting and 1,2, 7 and 14 days after aging in phosphate buffered saline. The authors suggested that Apexit Plus had a short-term antibacterial effect of 1 day on E. faecalis, whereas Epiphany SE enhanced bacterial growth for at least 7 days. AH plus and RoekoSeal were ineffective.

Alfredo et al (2008)[3] evaluated the bond strength of AH Plus and Epiphany sealers to human root canal dentine irradiated with a 980 nm diode laser at different power and frequency parameters, using the push-out in 60 canine roots the specimens were prepared with a tapered bur and irrigated with sodium hypochlorite, ethylene-di-aminetetraacetic acid and distilled water and divided into five groups- one control and four experimental groups were submitted to 980 nm diode laser irradiation at different power (1.5 and 3.0 W) and frequency (continous wave and 100 Hz) parameters. Half of specimens in each group had their canals filled with AH Plus sealer and half with Epiphany. The push-out test was performed. The specimens were split longitudinally and examined under SEM to assess the failure modes after sealer displacement. The conclusion include that the 980 nm diode laser irradiation of root canal dentine increased the bond strength of AH Plus sealer, but did not affect the adhesion of Epiphany sealer.

Bouillaguet et al (2008)[4] evaluated the long-term sealing ability of four contemporary endodontic sealers [Pulp Canal Sealer (PCS), AH-Plus, GuttaFlow and Epiphany] using a fluid filtration technique in palatal roots of 40 human maxillary molar teeth. The root canals were prepared using a crown-down technique. 24 hours after filling the roots were connected to an automatic flow recording device filled with double distilled water under pressure to measure leakage flow rates were assessed at 6, 12 or 24 hr and after 1- year of storage. They concluded that GuttaFlow and Epiphany allowed less fluid movement along filled straight roots.

Resende et al (2009)[26] assessed the physicochemical properties and the surface morphology of AH Plus, Epiphany, and Epiphany SE root canal sealers. Five samples of each material were employed for each test according to ANSI/ADA specification 57. The results suggest that the setting time, flow and radiopacity tests conformed to ANSI/ADA standardization. The dimensional change in all groups and the solubility of Epiphany were greater than values considered acceptable, with higher amounts of calcium ion release. Epiphany SE revealed more organized, compacted, and homogeneous polymers in a reduced resin matrix when compared with the other groups.

Shanahan et al (2011)[29] provided a review on Root canal filling using Resilon stating that within the limit of the in-vitro studies Resilon appears to perform adequately in comparison to gutta-percha, however, as a result of the questionable merit of such studies, it cannot presently be considered an evidence-based alternative to the current gold standard gutta-percha.

Nawal et al (2011)[20] evaluated the antimicrobial efficacy and flow properties for Epiphany, Guttaflow and AH-Plus sealer with the use of Enterococcus faecalis ATCC 29212 as a test organism. They concluded that antimicrobial activity of the sealers was greatest for Epiphany followed by AH-Plus sealer and Guttaflow. Epiphany sealer had the maximum flow followed by AH-Plus sealer and Guttaflow.

L. Kqiku et al (2011)[17] evaluated the active versus passive dye microleakage and apical sealing ability of laterally condensed gutta-percha/AH Plus versus Resilon/Epiphany in their *in vitro* study. One hundred and twenty teeth were instrumented and divided into experimental, positive and negative control groups. In group 1, the teeth were obturated with gutta-percha/AH Plus and in group 2 the teeth were obturated with Resilon/Epiphany. The apical seal was evaluated with a passive and active dye penetration test. Absorbance of the extracted dye was determined with a spectrophotometer. They concluded that canals obturated with Resilon/Epiphany showed less apical leakage than those obturated with gutta-percha/AH Plus, regardless of the type of dye penetration test used.

Assmann et al (2012)[2] evaluated the bond strength to root dentin of 2 mineral trioxide aggregate (MTA)–based sealers (Endo-CPM sealer and MTA

Fillapex) and of 1 epoxy resin–based sealer (AH Plus sealer). Forty-five extracted human teeth with single roots were prepared by using the step-back technique. Irrigation with 2.5% NaOCl and a final rinse with 17%

Ethylene-di-aminetetraacetic acid and distilled water were performed. Canals were filled by using Endo- CPM sealer, MTA Fillapex, or AH Plus sealer by means of the gutta-percha lateral condensation technique. After 7 days, the roots were sectioned perpendicularly to its long axis, and the push-out test was carried out. From the results it can be concluded that Endo-CPM sealer presented advantages when a post preparation was required. MTA Fillapex presented acceptable resistance to dislodgement, which was similar to that observed in samples filled with AH Plus sealer.

Economides et al (2012)[9] evaluated ex vivo, the push-out bond strength of a new filling material (Smart seal) compared with gutta-percha/AH-26. A total of 40 extracted single-rooted human teeth were used. After instrumentation using the ProTaper rotary system, the root canals were filled as follows: Group 1, Smartseal sealer and a 0.06 taper Smartpoint calibrated to apical tip size 30; Group 2, Smartseal sealer and an F3 SmartpointPT; Group 3, AH-26 sealer and a single F3 ProTaper gutta-percha cone and Group 4, AH-26 sealer and gutta-percha using the cold lateral condensation technique. Two successive disk shaped slices were cut from each root sample and the bond strength was measured using the push-out test. The author concluded that there was no difference in adhesion to dentine between the Smart seal system and gutta-percha/AH-26 applied using either the single cone or lateral condensation technique.

Reddy et al (2013)[25] carried out a study to determine the sealing ability of four root end filling materials- Intermediate Restorative Material (IRM), Mineral Trioxide Aggregate, Geristore and Retroplast using a glucose leakage model. 100 extracted teeth were used for this study. The teeth were divided into 6 groups – 4 experimental groups of 20 teeth each and 2 control groups of 10 teeth each. In the positive control, no root end filling was done and in the negative control, the teeth were completely coated with nail varnish. All the teeth were instrumented, their apices were resected. 3mm deep root end preparations were prepared with retro tips. The root end cavities of the experimental groups were filled with the retrograde filling materials. The materials were manipulated according to the manufacturers' instructions. Each tooth was mounted in a glucose leakage device as described by Xu and coworkers. The amount of glucose was determined by a UV-VIS recording spectrophotometer at 500-nm wavelength. According to the results of their study, MTA showed the least leakage at both 7th and 14th days and hence can be considered as the material of choice for root end filling.

Lumbini et al (2013)[19] provided an overview about Smart seal- New Age obturation stating that Smartseal is a recently introduced root canal obturating system based on polymer technology. Its principle is based on the hydrophilic nature of the obturating points which can absorb surrounding moisture and expand resulting in filling of voids and spaces. According to the author since, its introduction, Smartseal has been widely reported to be successfully used in endodontic therapy.

Didato et al (2013)[6] evaluated the time-based lateral hygroscopic expansion of a water-expandable endodontic obturation point. They compared the time-based lateral expansion of two sizes and two batches of water-expandable obturation points (CPoint, EndoTechnologies, LLC) and a similar-sized gutta-percha point (control) at various distances from the point apex: 5, 10, and 15 mm. They concluded that when exposed to water, the lateral expansion of a new hydrophilic endodontic obturation point significantly increases in dimension within 20 min, whereas a conventional gutta-percha point does not.

Eid et al (2013)[8] conducted a study to evaluate the effects of C-Point on the viability and mineralization potential of odontoblast-like cells. The biocompatiability of CPoint and commercially available gutta-percha points evaluated using rat odontoblast-like cell line. They concluded that the in vitro biocompatibility of C-Point is comparable to gutta-percha with minimal adverse effects on osteogenesis after elution of potentially toxic components.

Ruiz et al (2013)[27] in their study evaluated the physical properties of AH Plus alone and mixed with 1% or 2% chlorhexidine (CHX); 0.1%, 0.2%, 0.3%, and 0.5% of cetrimide (CTR); and combinations of both. Setting time, flow, solubility, and radiopacity of AH Plus were evaluated following the ANSI/ADA Specification No. 57/2000. Five samples of each material were tested for each property. They concluded that the addition of CHX, CPR, and combinations of both to AH Plus did not alter the physical properties specified by ANSI/ADA requirements.

Arora et al (2014)[1] evaluated and compared a novel polyamide polymer based obturating system and Gutta-percha and sealer in filling simulated lateral canals and their homogeneity when used for obturating the root canals using cone beam computed. A total of 60 freshly extracted human single rooted teeth with fully formed apices were selected for this study. Teeth were de-coronated, and roots were standardized to a working length of 15 mm. Root canal preparation was carried out with rotary Protaper file system in all groups. The specimens were then randomly divided into three groups A, B, and C (n = 20). Ten samples from each group were decalcified and simulated lateral canals were made at 2, 4, and 6 mm from the root apex. Remaining ten samples from each group were maintained calcified. Group A was obturated with SmartSeal system. Group B was obturated with sectional backfill method. Group C was obutrated with cold lateral compaction method (control). Decalcified samples from the respective groups were analyzed with digital radiography and photography and the measurement of the linear extension and area of lateral canal filling was done using UTHSCSA software. Calcified samples were subjected to cone beam computed tomography image analysis sectioned axially.They concluded that polyamide polymer obturation proved to have greater efficiency when compared with Gutta-percha system, when used for obturation with regards to adaptation of the sealer and penetration into the simulated lateral canals.

Cotti et al (2014)[5] evaluated the cytotoxicity of the new experimental self-adhesive, methacrylate-based hybrid root canal sealer XT and compared it with the epoxy resin-based AH Plus Jet in their in vitro study published. The cytotoxicity of the tested materials was evaluated after 1, 24, 48, and 72 hours by using growing and confluent mouse fibroblast cell line L929. L929 fibroblasts were maintained in Dulbecco modified medium containing 10% fetal calf serum at 37̊ C and 5% CO2. At confluence, cells were seeded in 24-well plates at concentration of 1.5×10^5 cells (growing cells) or 2.5×10^5 (confluent cells) for each well. An amount of 5 mL of each root sealer was placed into individual wells containing a monolayer of L929 cells to mimic the in vivo condition of the possible extrusion of sealer in the periapical tissues. Neutral Red and [3-(4,5-dimethylthiazol-2-yl)- 2,5 diphenyl tetrazolium bromide] were used for the cytotoxicity evaluation. Untreated cells were used as control. Results were confirmed by examination with optical microscope. They concluded that XT was less cytotoxic than AH Plus Jet as indicated by viability and morphologic analyses, and its initial cytotoxicity decreased progressively over time.

E. Iriboz et al (2014)[16] evaluated the effectiveness of the ProTaper and Mtwo retreatment systems for removal of resin-based obturation techniques during retreatment. A total of 160 maxillary anterior teeth were enlarged to size 30 using ProTaper and Mtwo rotary instruments. Teeth were randomly divided into eight groups. Resilon + Epiphany, gutta-percha + Epiphany, gutta-percha + AH Plus and gutta-percha + Kerr Pulp Canal Sealer (PCS) combinations were used for obturation. ProTaper and Mtwo retreatment files were used for removal of root canal treatments. After clearing the roots, the teeth were split vertically into halves, and the cleanliness of the canal walls was determined by scanning electron microscopy. Specimens obturated with gutta-percha and Kerr PCS displayed significantly more remnant obturation material than did specimens filled with resin-based obturation materials. Teeth prepared with Mtwo instruments contained significantly more remnant filling material than did teeth prepared with ProTaper. ProTaper files were significantly faster than Mtwo instruments in terms of the mean time of retreatment and time required to reach working length. The Resilon + Epiphany and AH Plus + gutta-percha obturation materials were removed more easily than were the Epiphany + gutta-percha and Kerr PCS + gutta-percha obturation materials. Thus, they concluded that although ProTaper retreatment files worked faster than did Mtwo retreatment files in terms of removing root canal obturation materials, both retreatment systems are effective, reliable and fast.

Pawar et al (2014)[21] in their in-vitro study evaluated and compared the micro-leakage of three sealers; Endosequence bioceramic (BC) sealer, AH Plus and Epiphany. Study was done on 75 extracted human single rooted permanent teeth, which were decoronated and the root canals were instrumented. The specimens were randomly divided into three groups (n = 25) and obturated by continuous wave condensation technique. Group A: using Endosequence BC, Group B: using AH Plus sealer, Group C: using Resilon Epiphany system. Micro-leakage was evaluated using dye penetration method. Teeth were split longitudinally and then horizontally markings were made at 2, 4 and 6 mm from the apex. Dye penetration evaluation was done under stereomicroscope (30X magnification).The results suggested that newly introduced BC sealer and Epiphany sealer sealed the root canal better compared to AH Plus Sealer.

Souza et al (2014)[28] evaluated and compared, by means of bacterial infiltration, the quality of sealing obtained by Tagger's hybrid (TH) and Single Cone (SC) techniques, in association with AH Plus/Gutta-percha (AH) and Epiphany/Resilon (ER). Palatal roots of 70 maxillary molars were instrumented and divided randomly into six groups: G1, TH/AH; G2, SC/AH; G3, TH/ER; G4, SC/ER; G5, negative control; G6, positive control. The roots were sterilized and monitored for 56 days to detect bacterial leakage using Enterococcus faecalis. From the results it can be concluded that none of the groups were able to prevent bacterial leakage and the lowest ability to prevent infiltration was obtained when applied SC/ER to filling the canal.

Elbatouty et al (2015)[7] evaluated the push-out bond strength of bioceramic root canal sealer (Endo Sequence BC) in comparison to a resin-based (AH Plus) sealer and a zinc oxide-eugenol-based (Kerr EWT) sealer. Sixty-three roots were randomly divided into three groups (n = 21) according to the root canal sealer: group 1, EndoSequence BC; group 2, AH Plus; and group 3, Kerr EWT. 2mm thick horizontal sections from the coronal, middle, and apical thirds of each root were sliced for the push-out bond strength measurement using a universal testing machine after 7, 14 and 30 days. Modes of failure were evaluated using a scanning electron microscope They concluded that the EndoSequence BC samples showed the highest mean push-out bond strength values after 1 and 4 weeks, followed by AH Plus and Kerr EWT. After 2 weeks, the AH Plus samples showed the highest mean push-out bond strength values followed by EndoSequence BC. The time after obturation and the basic composition of the sealer are important factors in determining the bond strength of the sealer to the root canal wall.

Hegde et al (2015)[15] conducted a comparative assessment of apical sealing ability of a novel hydrophilic vs. conventional hydrophobic obturation systems- Smart-Seal System, Resilon, and conventional Gutta-Percha system using a bacterial leakage. Seventy freshly extracted human single rooted teeth with fully formed apices were randomly divided into three groups (20 each) and two control groups (5 positive and 5 negative). Teeth were de-coronated, and roots were standardized to a working length of 16 mm. Root canal preparation was done with rotary pro-taper file system in all groups. Group A was obturated using Smart-Seal system (Hydrophilic), Group B using

Resilon/Epiphany system (Hydrophilic), and Group C using Gutta-Percha (GP)/AH plus system (Hydrophobic) in a single cone technique. Using Enterococcus faecalis, a split chamber bacterial leakage model was developed to evaluate the sealing ability of three obturation systems. Samples will be monitored every 24 hours for 60 days. They concluded that hydrophilic obturations of the root canal shows a better resistance to bacterial leakage as compared to hydrophobic obturations.

Hedge et al (2015)[14] conducted a scanning electron microscopic push-out bond strength study to evaluate the effect of different final irrigation activation techniques affect the bond strength of self-expanding Smart-Seal obturation at the different thirds of root canal space- manual dynamic activation (MDA), Canal Brush activation, ultrasonic activation (UA) and Endo-Activator. One hundred single-rooted human teeth were prepared using the Pro-Taper system to size F3, and a final irrigation regimen using 3% sodium hypochlorite and 17% EDTA was performed. The specimens were randomly divided into five groups (*n* = 20) according to the final irrigation activation technique used as follows: No activation (control), manual dynamic activation (MDA), CanalBrush activation, ultrasonic activation (UA) and EndoActivator. Five specimens from each group were subjected to scanning electron microscopic observation for assessment of the smear layer removal after the final irrigation procedures. All remaining roots were then obturated with Smart-Seal obturation system. A push-out test was used to measure the bond strength between the root canal dentin and Smart-Seal paste. From the study it was concluded that UA improved the bond strength of Smart-Seal obturation in the coronal and middle third and MDA/Endo-Activator in the apical third of the root canal space.

Hegde et al (2015)[13] evaluated the Sealing ability of three hydrophilic single cone obturation systems – single cone C-Points/smartpaste biosealer; single cone bioceramic (BC) impregnated gutta-percha/endosequence BC sealer; single cone Resilon/RealSeal SE using a glucose leakage. A total of 90 freshly extracted human maxillary single‑rooted teeth was selected, and their crowns were cut. The root canal of each sample was instrumented using a rotary crown down technique and then divided into four experimental (n = 20 each) and two control groups (n = 5 each). Samples in the experimental groups were filled as follows: Group 1, cold lateral condensation using gutta‑percha/AH Plus; group 2, single‑cone C‑points/smart‑paste bio‑sealer; group 3, single‑cone bio‑ceramic (BC) impregnated gutta‑percha/ endo‑sequence BC sealer; group 4, single‑cone Resilon/ RealSeal SE after 7 days, the sealing ability of root canal fillings was tested at different time intervals using glucose leakage model. Glucose leakage values were measured using a spectrophotometer and statistically analyzed. They concluded that CPoints/ smartpaste Bio and BC impregnated gutta-percha/endosequence BC sealer combinations provided the superior sealing ability over the lateral condensation technique.

Hedge et al (2015)[12] did a comparative assessment of fracture resistance of roots obturated with three hydrophilic obturation systems- novel C-Point system, Resilon/Epiphany system, and EndoSequence BC sealer; and one hydrophobic gold standard gutta-percha/AH Plus system in ninety freshly extracted, human, single rooted mandibular premolars. The specimens were de-coronated and randomly divided into 6 groups. Specimens were prepared and obturated with respective materials. Each group was then subjected to fracture testing by using a universal testing machine. The force required to fracture each material was recorded. They concluded that, in contrast to hydrophobic systems, hydrophilic systems showed higher fracture resistance in a single-rooted premolar.

Hegde et al (2015)[11] evaluated the effects of calcium hydroxide (CH), triple and double antibiotic pastes (DAPs) on the bond strength of Smart-Seal obturation, C-points with Endosequence Bio-ceramic (BC) sealer to the root canal dentin in sixty-four freshly extracted single-rooted human mandibular premolars that were de-coronated and prepared using rotary Pro-taper system with full sequence till F3. The specimens were randomly divided into a control group and three experimental groups that received an intracanal dressing with the materials. The dressing was removed after 3 weeks and then obturated with C-points and Endosequence BC sealer. A push-out test was used to measure the bond strength between the root canal dentine and the obturating system. They concluded that the DAP and CH did not affect the bond strength of the novel hydrophilic obutrating system. TAP improved the bond strength of Smart-Seal system in the middle and apical thirds.

Materials and Methods

MATERIALS AND METHODS

ARMAMENTARIUM

- Seventy extracted intact human mandibular first premolar teeth
- Diamond points (Mani burs)
- Endomotor (J.Morita, Japan)
- Hyflex CM rotary file system (Coltene USA)
    - Size 20/0.08%
    - Size 20/0.04%
    - Size 25/0.04%
    - Size 30/0.04%
    - Size 30/0.06%
- Distilled water
- 2% Sodium Hypochlorite
- 17% EDTA
- Syringe

- Endoactivator (Dentsply, Tusla)
- 0.5% Chloramine T
- C-Points size 30/ 0.06% taper (EndoTechnologies, LLC, Shrewsbury, MA, USA)
- Resilon cones – size 30/ 0.06% taper (SybronEndo, Orange, CA)
- Gutta-percha cones - size 30/ 0.06% taper (Dentsply, Tusla)
- Epiphany sealer (SybronEndo, Orange, CA)
- Smart seal Bio (EndoTechnologies, LLC, Shrewsbury, MA, USA)
- AH Plus sealer (Dentsply, Tusla)
- Glass beaker, Rubber stopper, rubber tube, glass pipette
- Micropipette
- 0.2% Sodium Azide (Chenchems, Chennai)
- 1 mol/L Glucose (Aspen laboratories, Delhi)

Equipment:

- Glucose leakage model, Glucose kit (Coral clinical systems, Goa)
- Spectrophotometer (Shimadzu, Japan)

METHODOLOGY

The study was approved by the dissertation and ethical committee of Ragas Dental College.

INCLUSION CRITERIA:

Seventy intact human mandibular first premolars with closed apices and single canals were included for the study.

EXCLUSION CRITERIA:

Teeth with dental caries, cervical abrasion, calcifications, previous restoration or endodontic manipulation, fractures or cracks, internal or external resorption and dilacerations were excluded.

Seventy freshly extracted human mandibular first premolars extracted for orthodontic purpose were selected according to the inclusion and exclusion criteria and stored in 0.5% Chloramine T at 4°c for one month. The teeth were de-coronated and root lengths were standardized to 15mm. A diamond bur was used to gain a straight-line access to the root canal. A size 10 K-File was inserted into the canal to verify the patency. The working length was determined by subtracting 1mm from the total length of the root. The chemo-mechanical preparation was done with Hyflex CM Ni-Ti files until size 30/0.06% taper using the J.Morita rotary system. Each canal was irrigated with 2% Sodium Hypochlorite with an Endoactivator after every instrument.

Copious irrigation of each root canal was carried out. The prepared teeth were divided into five groups. Three experimental groups of twenty teeth each and two groups of five teeth each, which will serve as positive and negative control.

Group I (n=20)

After preparation was completed, the canals were rinsed with an additional 5ml, 2% Sodium Hypochlorite solution followed by distilled water. The teeth were further irrigated with 17% EDTA followed by irrigation with distilled water. Each canal was dried using paper points.

AH Plus sealer was dispensed. It is a two paste system. A size 30/0.06% GP was taken and buttered with the AH Plus sealer and obturated.

Group II (n=20)

After preparation was completed, the canals were rinsed with an additional 5ml, 2% Sodium Hypochlorite solution followed by distilled water. The teeth were further irrigated with 17% EDTA followed by irrigation with distilled water. Each canal was dried using paper points.

Smart seal-Bio sealer was dispensed. It is a single paste system, which sets after coming in contact with water. A size 30/0.06% C-Point was taken and ‘buttered’ with Smart seal Bio sealer. A light pumping motion was used to fill the canal with sealer. Adequate fit of the C-Point was verified. Excess of the C-Point was sheared off.

Group III (n=20)

After preparation was completed, the canals were rinsed with an additional 5ml, 2% Sodium Hypochlorite solution followed by distilled water. The teeth were further irrigated with 17% EDTA followed by irrigation with distilled water. Each canal was dried using paper points.

Epiphany is a single bottle methacrylate based resin sealer. The sealer was placed into the canal. The size 30/0.06% Resilon cone was coated with the sealer and placed into the prepared root canal and Cured.

Group IV –Positive Control (n=5)

The irrigation protocol for the control group was the same as for the afore mentioned groups. The teeth that serve as positive control were obturated with Gutta-Percha WITHOUT any sealer.

Group V – Negative Control (n=5)

The teeth that were used as negative control were not obturated and coated completely with nail varnish.

The teeth in all the five groups were subjected to micro-leakage testing using the Glucose leakage Model and the leakage was assessed at different time intervals of 1,7,14,21 and 28 days.

DESCRIPTION OF GLUCOSE LEAKAGE MODEL:

All the teeth were coated with nail varnish except in the coronal and apical region. The coronal 4mm of the root specimens were then embedded in acrylic to form a cylinder around the root and enable intimate contact with the rubber tube used to connect the specimen to the glucose leakage apparatus. The apparatus is prepared by assembling a 5ml air tight glass glass jar fitted with a rubber stopper. Two holes are prepared on the rubber stopper to allow the 14 cm long glass tube which holds the tooth samples to pass into the glass beaker and the other hole is to withdraw the 0.2% NaN_3 present in the glass jar. The apical 2mm of the root are immersed in the 0.2% NaN_3 solution present in the glass jar. 1mol/L glucose solution is passed through the 14cm glass tube. In the Glucose leakage Model 10 µL of the sample was withdrawn after 24 hours, followed by 10 µL at regular intervals with the help of a micropipette. The sample withdrawn was then subjected to quantitative glucose testing by Glucose oxidase-Peroxidase test using a spectrophotometer at wavelength 505 ηm. The 10 µL of sample withdrawn was replenished with the same volume of 0.2% sodium azide.

METHODOLOGY

↓

Seventy single rooted human mandibular premolars with matured apex were selected. Root lengths were standardized to 15mm

↓

Instrumentation is completed with rotary Hyflex CM files using crown down technique.

↓

Irrigation with 2% sodium hypochloride and 17% EDTA

↓

Teeth were divided into three experimental groups of 20 each and two control groups of 5 each

↓

Obturation done by using three different 6% single cones obturating material with respective sealers for respective groups.

Experimental groups

Group – I **AH Plus / GP**	**Group – II** **SMART-SEAL**	**Group –III** **REAL-SEAL**

Stored in incubator at 37^{o}c for 1 week

↓

The teeth in all the groups were subjected to micro-leakage testing using the Glucose leakage Model. 10 μL of the sample solution is withdrawn after 24 hours

↓

Followed by 10 μL at regular intervals with the help of a micropipette.

↓

The 10 μL of sample withdrawn were replenished with the same volume of 0.2% sodium azide

↓

The samples withdrawn were subjected to quantitative glucose testing by Glucose oxidase-peroxidase test using a spectrophotometer

↓

Results recorded and subjected to statistical analysis

Figures

Fig 1: Tooth Samples (Mandibular Premolars)

Fig 2: Decoronation

Fig 3: Decoronated tooth samples

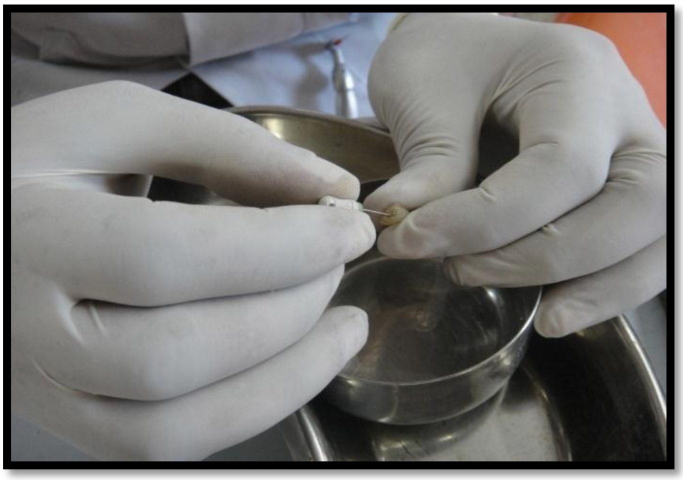


Fig 4: Initial cleaning and shaping

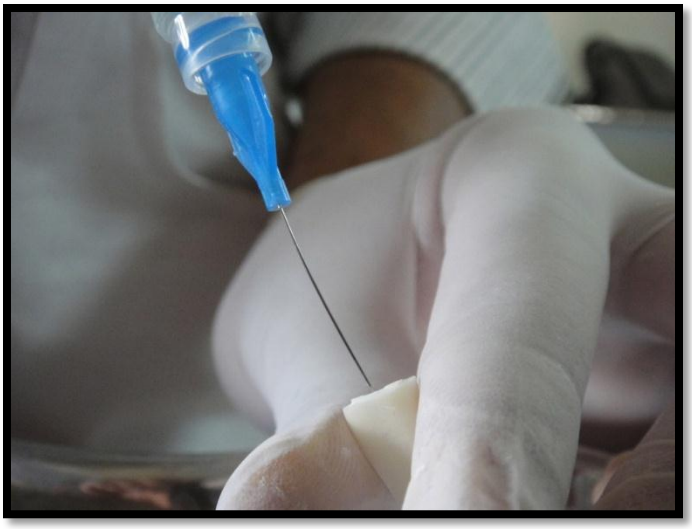


Fig 5: Irrigation of the canal

Fig 6: Endomotor and Endoactivator for cleaning and shaping

Fig 7: Experimental obturating systems

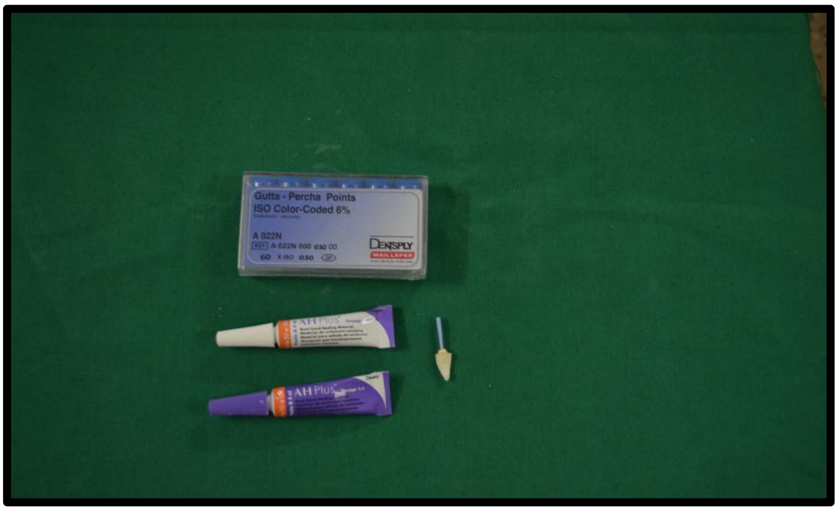


Fig 8: Gutta percha with AH Plus sealer

Fig 9: C-points with Bio ceramic sealer

Fig 10: Resilon with Epiphany

Fig 11: Samples stored at 37℃ in incubator

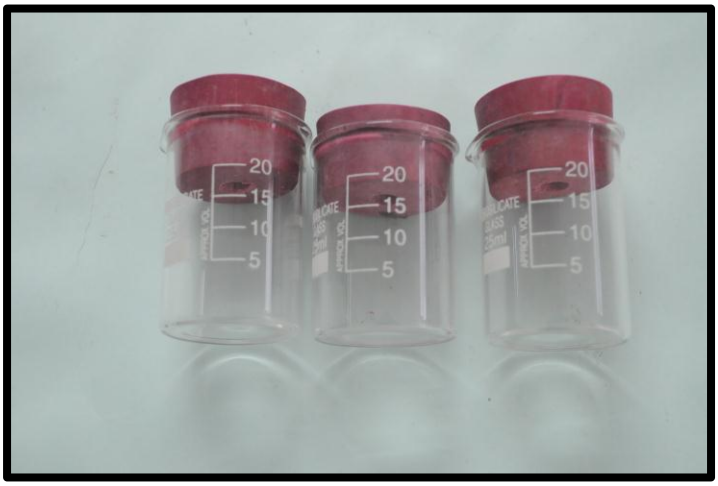


Fig 12: Air tight glass beaker with rubber stopper

Fig 13: Glass tube connecting the tooth sample

Fig 14: Apparatus to detect microleakage

Fig 15: 1 mol/L glucose solution

Fig 16: 0.2% NaN_3

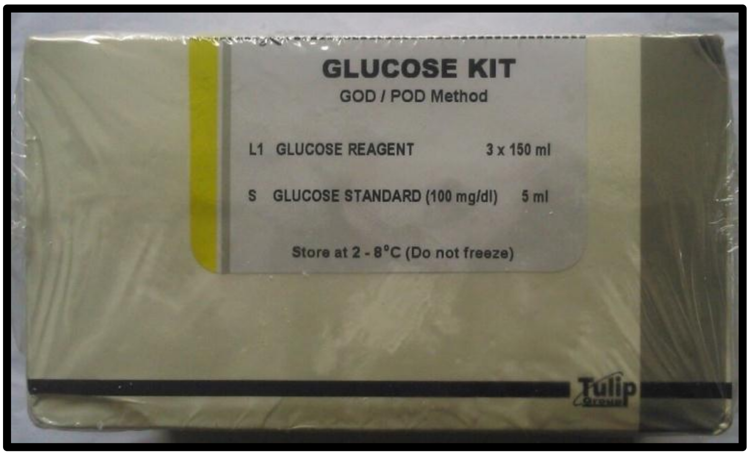


Fig 17: Glucose kit

Fig 18: Spectrophotometer

Fig 19: Spectrophotometer

Results

RESULTS

The results of the present study were subjected to statistical analysis with SPSS, Version 20 software to interpret the significant difference in the microleakage between experimental groups using Kruskal–Wallis and Mann–Whitney tests. To compare the leakage at different times within each group, Freidman and Wilcoxon signed ranks tests were used.

All level of statistical significance was set at a $P < 0.05$.

For each day tested, the positive controls had immediate substantial glucose leakage, which increased over time, where as the negative controls showed no detectable glucose leakage. This indicates that the seal of the glucose leakage system was effective and reliable.

The mean values and statistical comparisions between the experimental groups at each time interval were given in Table 1. The glucose leakage mean value of Group I Gutta-percha/AH plus on day 1 was 0.54 ± 0.07 mg/dl, at 7 days 4.93 ± 0.39, at 14 days 10.91 ± 0.59, at 21 days 13.11 ± 0.73, at 28 days 15.21 ± 0.52. The overall mean leakage value was 8.94 ± 5.48. There was a gradual increase of leakage from day 1 to day 28.

The mean values and statistical comparisions between the experimental groups at each time interval were given in Table 2. Group II Smart seal showed mean leakage value on day 1 0.30 ± 0.16, on the 7^{th} day 1.94 ± 0.46, on the 14th day 5.35 $\pm$ 0.77, on the 21 day 7.01 $\pm$ 0.57 and on the 28th day 7.81 $\pm$ 0.49. The overall mean was 4.48$\pm$ 2.96. There was a gradual increase of leakage from day 1 to day 28.

The mean values and statistical comparisions between the experimental groups at each time interval were given in Table 3. Group III Real seal showed mean leakage value on day 1 0.52 $\pm$ 0.10, on the 7th day 3.21 $\pm$ 0.48, on the 14th day 8.18 $\pm$ 1.32, on the 21 day 8.81 $\pm$ 1.10 and on the 28th day 10.01 $\pm$ 1.03. The overall mean was 6.14 $\pm$ 3.77. There was a gradual increase of leakage from day 1 to day 28.

INTERPRETAION OF RESULTS;

1. Glucose concentrations are seen more in Gutta-percha/AH Plus samples compared to C-points/bio ceramic sealer and Resilon/Epiphany systems
2. Sealing ability of C-Points with bio ceramic sealer was superior compared to Resilon cone with Epiphany sealer and Gutta-percha with AH-Plus.
3. Sealing ability of Resilon cone with epiphany sealer was better compared to Gutta-percha with AH plus.
4. Sealing ability of Gutta-percha with AH-Plus sealer was considerable.
5. Sealing ability of Positive Control group was least effective.
6. No leakage was seen in negative control group.

Tables and Graphs

Table 1: Microleakage in Group I(Guttapercha/AH Plus)					
S.NO	DAY 1	DAY 7	DAY 14	DAY21	DAY 28
1	0.52	5.02	11.02	13.32	15.66
2	0.54	5.08	12.07	14.42	15.45
3	0.56	5.05	10.9	13.3	15.2
4	0.43	5.07	11.2	13.6	15.6
5	0.45	5.01	11.4	12.3	14.7
6	0.65	4.33	10.34	13.88	15.61
7	0.66.	4.31	10.02	12.22	14.32
8	0.43	4.46	10.34	13.39	15.67
9	0.57	5.4	11.5	13.8	15.6
10	0.58	5.2	11.4	13.3	15.2
11	0.52	5.02	11.02	13.32	15.66
12	0.53	5.04	11.01	13.7	15.7
13	0.45	5.01	11.4	12.3	14.7
14	0.51	4.9	10.9	12.9	14.9
15	0.59	4.06	10.34	11.6	15.0
16	0.66	4.31	10.02	12.22	14.32
17	0.5	5.6	10.5	13.8	15.1
18	0.56	5.48	11.65	13.05	15.8
19	0.50	5.01	11.01	13.05	15.8
20	0.66	4.31	10.02	12.22	4.32
Mean±SD	0.54±0.07	4.93±0.39	10.91±0.59	13.11±0.73	15.21±0.52

Table 2: Microleakage in Group II(Smart Seal)					
S.NO	DAY 1	DAY 7	DAY 14	DAY21	DAY 28
1	0	1.34	5.62	7.72	7.98
2	0.01	2.23	4.67	5.89	7.76
3	0.24	2.56	6.89	7.78	7.89
4	0.26	2.22	4.76	6.66	8.99
5	0.23	2.33	4.45	6.65	8.87
6	0.22	1.34	3.33	5.89	6.62
7	0.3	2.28	5.07	7.01	7.7
8	0.21	1.32	5.44	6.78	7.56
9	0.4	2.22	5.2	7.3	7.8
10	0.41	1.35	5.33	4.64	7.43
11	0.24	2.56	6.89	7.78	7.89
12	0.25	1.5	5.5	6.5	7.5
13	0.3	2.28	5.07	7.01	7.7
14	0.35	2.3	5.4	7.0	7.5
15	0.38	2.29	5.67	7.74	7.9
16	0.4	2.22	5.2	7.3	7.8
17	0.7	1.46	5.72	7.1	7.89
18	0.45	1.87	5.62	7.71	7.99
19	0.46	1.9	5.73	7.01	7.85
20	0.21	1.32	5.44	6.78	7.54
Mean±SD	0.30±0.16	1.94±0.46	5.35±0.77	7.01±0.57	7.81±0.49

Table 3: Microleakage in Group III(Real seal)					
S.NO	DAY 1	DAY 7	DAY 14	DAY21	DAY 28
1	0.53	3.02	9.13	9.22	10.45
2	0.67	2.98	6.89	8.43	10.62
3	0.45	2.78	5.45	6.32	7.45
4	0.54	3.03	8.12	9.23	9.99
5	0.56	4.42	6.65	7.34	8.88
6	0.65	3.44	8.88	9.21	10.0
7	0.21	3.39	8.67	9.92	10.23
8	0.54	3.9	9.12	9.3	10.34
9	0.51	3.04	9.2	9.5	10.32
10	0.5	3.06	9.1	9.7	10.43
11	0.67	2.98	6.89	8.43	10.62
12	0.54	3.01	9.12	9.5	10.7
13	0.56	4.42	6.65	7.34	8.88
14	0.58	3.02	8.6	9.4	10.4
15	0.57	3.02	9.12	9.23	10.47
16	0.45	2.78	5.45	6.32	7.4
17	0.48	2.8	9.3	9.8	10.9
18	0.41	3.01	8.93	9.09	10.71
19	0.51	3.07	9.2	9.6	10.44
20	0.54	3.06	9.11	9.25	10.38
Mean±SD	0.52±0.10	3.21±0.48	8.18±1.32	8.81±1.10	10.01±1.03

Table 4: Kruskal-Wallis test Glucose leakage at various time intervals

	Group						P value Based on Kruskal-Wallis test
	Group I (AH Plus/G.P)		Group II (Smart Seal)		Group III (Real Seal)		
	Mean	SD	Mean	SD	Mean	SD	
1	.54	.07	.30	.16	.52	.10	<0.001**
7	4.93	.39	1.94	.46	3.21	.48	<0.001**
14	10.91	.59	5.35	.77	8.18	1.32	<0.001**
21	13.11	.73	7.01	.57	8.81	1.10	<0.001**
28	15.21	.52	7.81	.49	10.01	1.03	<0.001**

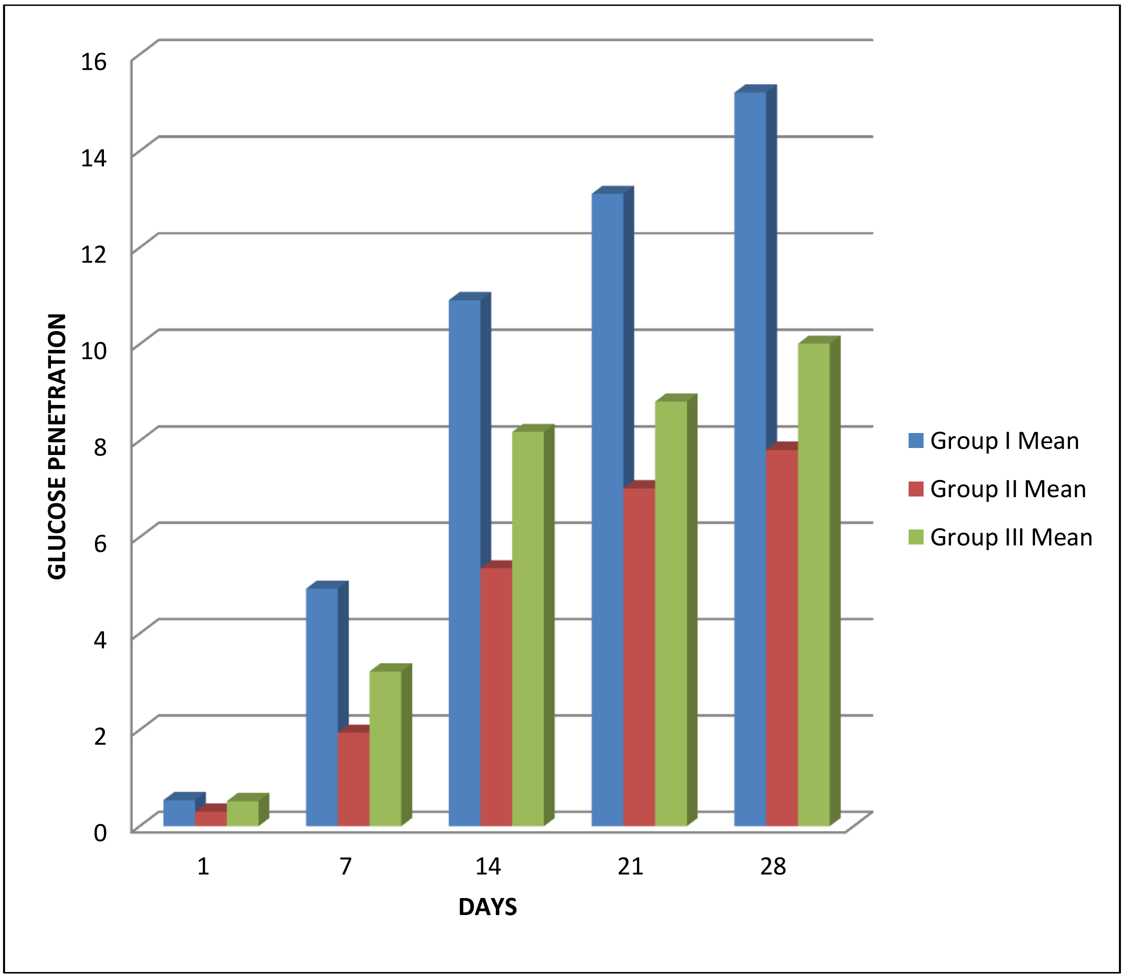
** Denotes significant at 1% confidence level

Table 5: Mann-Whitney test Glucose leakage at various time intervals between groups

	Group						P value based on Mann-Whitney test		
	Group I (AH Plus/G.P)		Group II (Smart Seal)		Group III (Real Seal)		Group I and II	Group I and III	Group II and III
Days	Mean	SD	Mean	SD	Mean	SD			
1	.54	.07	.30	.16	.52	.10	<0.001**	0.640	<0.001**
7	4.93	.39	1.94	.46	3.21	.48	<0.001**	<0.001**	<0.001**
14	10.91	.59	5.35	.77	8.18	1.32	<0.001**	<0.001**	<0.001**
21	13.11	.73	7.01	.57	8.81	1.10	<0.001**	<0.001**	<0.001**
28	15.21	.52	7.81	.49	10.01	1.03	<0.001**	<0.001**	<0.001**

** Denotes significant at 1% confidence level

GRAPH I: Glucose leakage at various time intervals for All the three groups

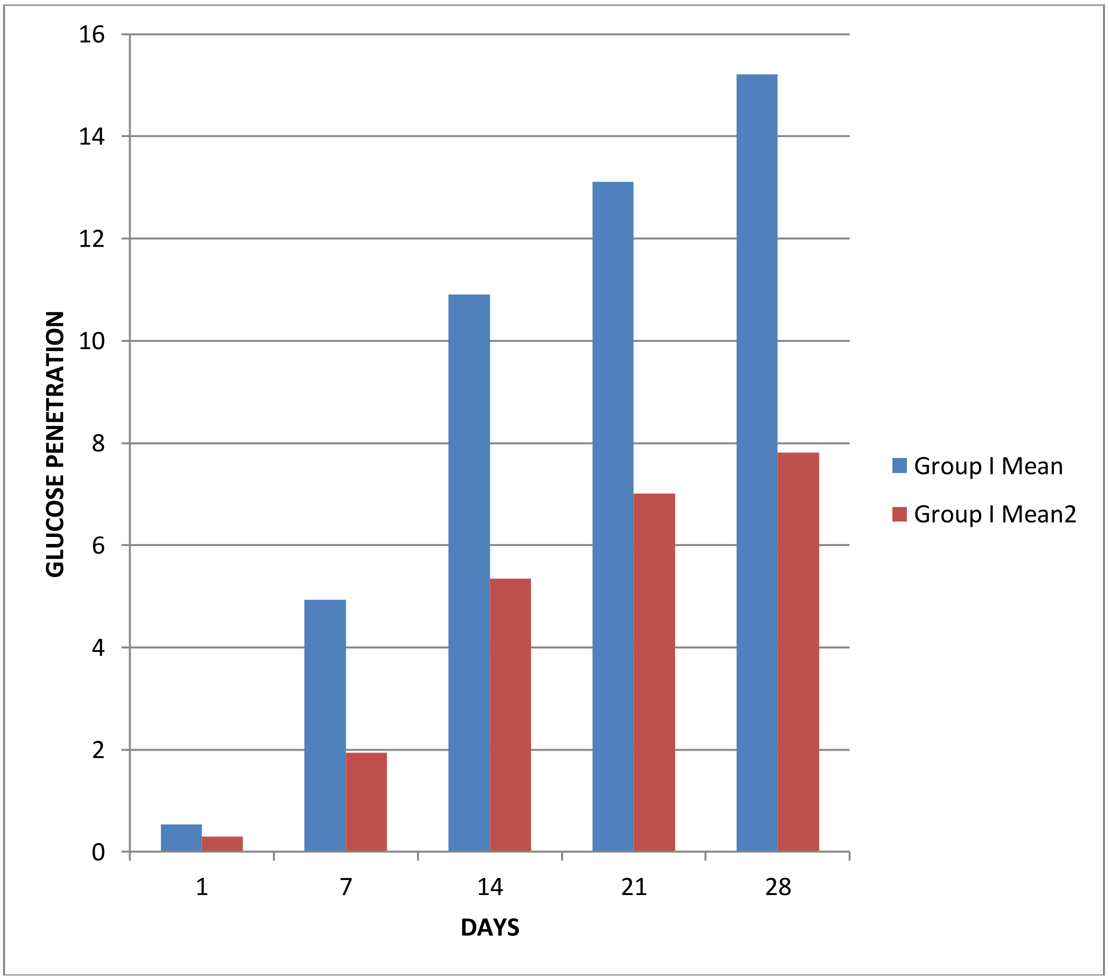


Group I - Gutta-percha with AH Plus

Group II – C- points/ Bioceramic sealer

Group III – Resilon/Epiphany

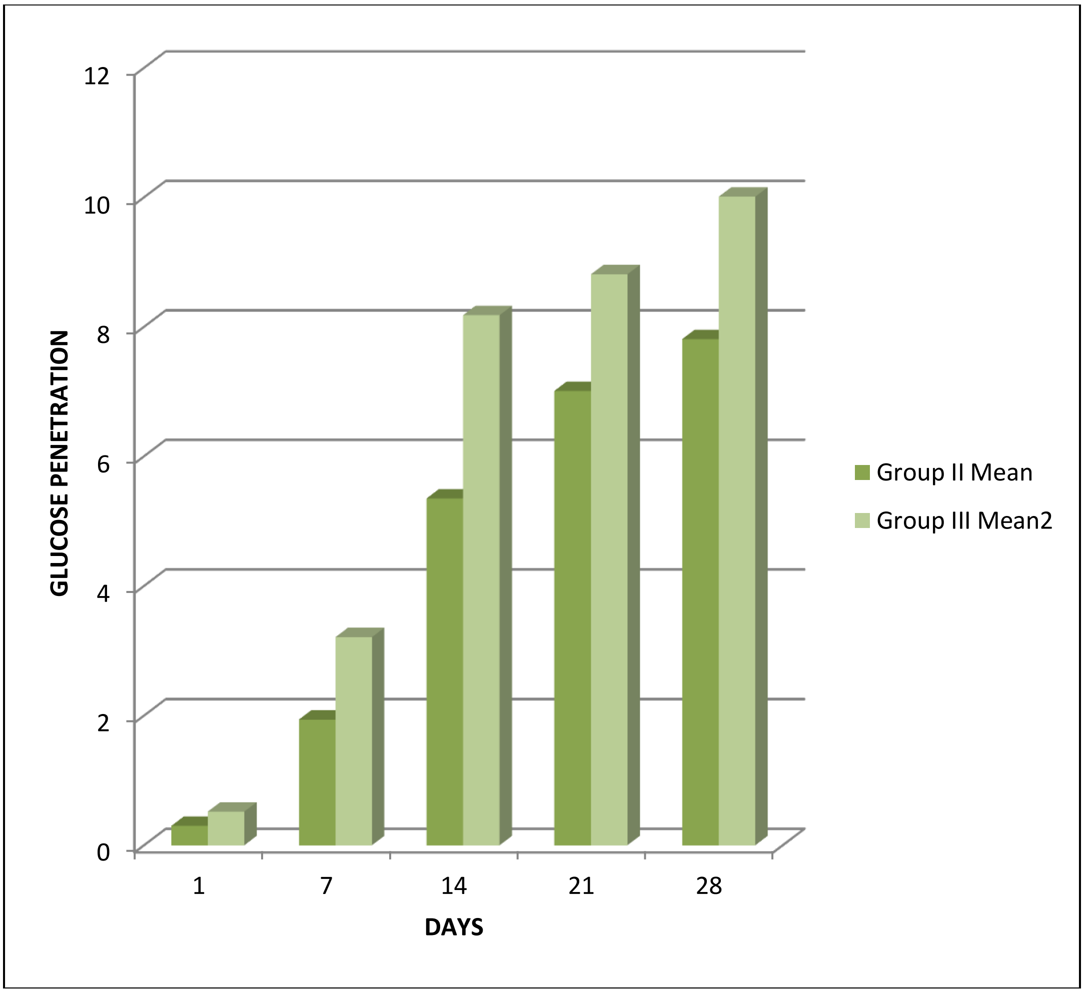
GRAPH II: Glucose leakage at various time intervals between Group I and Group II



Group I - Gutta-percha with AH Plus

Group II – C- points/ Bioceramic sealer

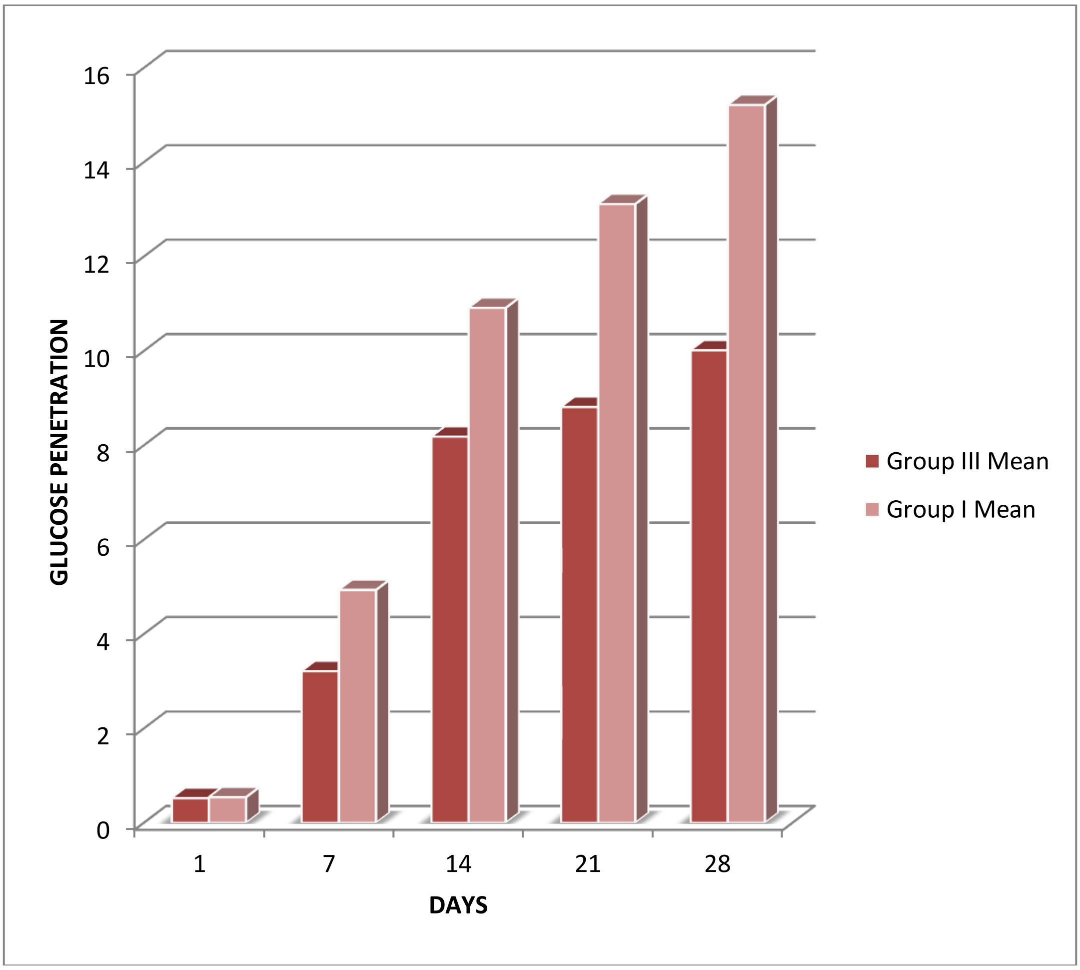
GRAPH III: Glucose leakage at various time intervals between Group II and Group III



Group II – C- points/ Bioceramic sealer

Group III – Resilon/Epiphany

GRAPH IV: Glucose leakage at various time intervals between Group I and Group III



Group I - Gutta-percha with AH Plus

Group III – Resilon/Epiphany

Discussion

DISCUSSION

The main objective of a root canal filling is to obturate the entire root canal system and produce an impervious apical seal. This prevents the penetration of micro-organisms and toxins from the oral cavity via the root canal into the peri-radicular tissues by sealing the root canal system at both the coronal and apical ends. Apical obturation prevents infection by anachoresis, and also blocks the portal of exit to the periapex for organisms which have survived, even after instrumentation and disinfection. To prevent the reinfection the whole pulp space is filled, thus blocking the dentinal tubules and accessory canals. A potential locus for multiplication of micro-organisms and all portals of exit to the body is sealed by these means.(10,18)

Obturation of a root canal is done by two materials one being the core and other is the sealer. Core material could either be cold or thermo plasticized. Warm condensation technique is considered as “golden” standard for endodontic treatment, that results in a friction fit, “cork-in-the-bottle” type sealing.(21)

Sealers play an important role in root canal filling. The ideal root canal sealer should be inert, dimensionally stable, and possess good antimicrobial activity and low toxicity towards the surrounding tissues. A good sealer adheres strongly to dentin and the core material.(32)

Various types of sealers are available, like the traditionally used Eugenol based, Non Eugenol, Calcium Silicate, Glass Ionomer sealers, Resin based sealers and to the most recent being Bio-Ceramic sealers. Each of them have their own inherent drawbacks. None of the traditionally available sealers fulfill all the ideal requirements of a sealer.

According to Schafer et al. (2003) the quality of the seal obtained with conventional Gutta-percha/ zinc oxide-eugenol is not perfect. Epoxy resin-based cements perform well as root canal sealers. AH Plus has been shown to have satisfactory physicochemical properties, low solubility and disintegration, good adhesion to dentine, antimicrobial action and good biological properties. Although AH-Plus has adequate long-term dimensional stability, its sealing ability remains controversial partly because AH-Plus does not bond to gutta-percha.[3,4]

According to Shipper et al. (2004) Epiphany is a dual-curing dimethacrylate resin that uses a primer. With this material, a thermoplastic core material is bonded to the resin-based sealer, root canals filled with Epiphany exhibit less microleakage than roots filled with gutta-percha and conventional sealers. Failures at the sealer–dentine interface may occur because of the polymerization of the methacrylate-based resin sealer immediately after its placement into the root canal. In addition, the coronal photo-activation of the sealer, following the manufacturers instructions, may reduce its flow and limit its contact with the primer and hence its penetration into the dentinal tubules. In a study Epiphany exhibited less antimicrobial activity than other sealers, except for AH 26 due to its hydrophilic resin form.

All these studies prove beyond doubt that there is a no ideal sealer that fulfills the requirements of a endodontic sealer henceforth we have selected a new obturating system Smart seal (C-points/ Bio-ceramic sealer) to check for its sealing ability, considering its unique property of self expanding in the root canals. Recent literature and studies have been limited on the properties of the C- points.

Didato et al (2013)[6] evaluated the time-based lateral hygroscopic expansion of a water-expandable endodontic obturation point. They compared the time-based lateral expansion of two sizes and two batches of water-expandable obturation points (CPoint, EndoTechnologies, LLC) and a similar-sized gutta-percha point (control) at various distances from the point apex: 5, 10, and 15 mm. They concluded that when exposed to water, the lateral expansion of a new hydrophilic endodontic obturation point significantly increases in dimension within 20 min, whereas a conventional gutta-percha point does not.

Eid et al (2013)[8] conducted a study to evaluate the effects of C-Point on the viability and mineralization potential of odontoblast-like cells. The biocompatibility of C-Point and commercially available gutta-percha points evaluated using rat odontoblast-like cell line. They concluded that the in vitro biocompatibility of C-Point is comparable to gutta-percha with minimal adverse effects on osteogenesis after elution of potentially toxic components. Literature evaluating the efficacy of C Point system as an obturation system is limited.

This brings in the need to find a new core/sealer which fulfills the ideal requirements. With this background the present study was contemplated with the aim to compare the sealing ability of three single cone obturating systems using a glucose leakage model.[24]

In the present study seventy human mandibular 1st premolars extracted for orthodontic purpose were selected according to the inclusion and exclusion criteria and were stored in 0.5% Chloramine T at 4°c for one month. Chloramine T controls infection and does not show any adverse effect on the organic phase of the dentin. The teeth were de-coronated and root lengths were standardized to 15mm. A diamond bur was used to gain a straight-line access to the root canal. A size 10 K-File was inserted into the canal to verify the patency.

Weines method was used to determine the working length. The working length was determined by subtracting 1mm from the total length of the root. The chemo-mechanical preparation was completed with Hyflex CM Ni-Ti files until size 30/0.06% taper using the J.Morita rotary system. After preparation is completed, the canals were rinsed with 5ml, 2% Sodium Hypochlorite solution using an endoactivator followed by distilled water. The teeth were further irrigated with 17% EDTA to remove the smear layer followed by irrigation with distilled water. Each canal was dried using paper points.

The teeth were coated with nail varnish except in the coronal and apical region. The coronal 4mm of the root specimens were then embedded in acrylic to form a cylinder around the root and enable intimate contact with the rubber tube used to connect the specimen to the Glucose leakage Apparatus.

After the initial instrumentation was done, the teeth were assigned into 5 groups. The groups were as follows: Three groups with 20 teeth in each, 2 groups with 5 teeth in each, which served as positive and negative control.

The groups were allocated with following intervention materials. Group I Gutta-percha with AH Plus sealer, Group II C Points with bioceramic sealer and Group III Resilon cone with Epiphany sealer, Group IV –Positive Control (n=5) obturated with Gutta-Percha WITHOUT any sealer and Group IV – Negative Control (n=5) were not obturated and were coated completely with nail varnish.

AH 26 is an epoxy resin recommended by Shroeder in 1957. This was later modified to AH Plus which is a paste-paste system. AH Plus is a sealer based on epoxy resin. According to the manufacturer, it has excellent sealing properties without the release of formaldehyde.

It consists of Epoxide paste Diepoxide, Calcium tungstate, Zirconium oxide, Aerosil, Iron oxide pigments and Amine paste 1-adamantane amine**,** N, N-dibenzyl-5-oxa nonandiamine, Calcium tungstate, Zirconium oxide, Silicone oil. AH Plus is able to flow into the orifices of the dentinal tubules, which is the reason for the comparatively good adhesion of AH Plus to dentin. It has less fracture resistance when used with gutta percha as compared to Resilon/Realseal. According to Almeida *et al.* leakage with AH Plus was significantly less than that with the ZnOE sealer.[18]

The resin core filling material, Resilon (Resilon Research LLC, Madison, CT), handles like gutta-percha. Obturation with Resilon cones were accomplished by use of Epiphany primer (Pentron Clinical Technologies, LLC, Wallingford, CT) and Epiphany resin-based sealer (Pentron Clinical Technologies). The RealSeal sealer is a dual-curing, resin-based composite sealer. The resin matrix is composed of bisphenol-A-glycidyl methacrylate (BisGMA), ethoxylated BisGMA, urethane dimethacrylate (UDMA), and hydrophilic difunctional methacrylate. The sealer with the aid of a primer adheres to the core material and dentin.[29]

According to cornelis, one of the factors that was instrumental in the development of resin-based sealers was the recognition that gutta-percha does not bond to dentin or to any conventionally used sealer, such as zinc oxide-eugenol (ZOE)-based cements and epoxy resins such as AH-26 or AH Plus.

This combination supposedly forms a mono-block in the root canal system. The Resilon material has been shown to be biocompatible, nonmutagenic, noncytotoxic, resolvable. It also has properties similar to those of gutta-percha, and is less irritating than epoxy resin or ZOE sealers. For retreatment purposes it may be softened with heat, or dissolved with solvents such as chloroform. The Epiphany Root Canal Sealant is a dual curable dental resin composite sealer. Studies recommend that EDTA or chlorhexidine (CHX) should be used as the final irrigant as sodium hypochlorite or hydrogen peroxide may weaken the seal.[26,39]

The most recent advancement in endodontic obturating materials is the evolution of Smart Seal system, a hydrophilic polymer. The system consists of obturation points (C-points) containing a polyamide core with an outer bonded hydrophilic polymer coating and an accompanying sealer which is further provided with polymer powder to be incorporated during the manipulation of the seal. The inner core of C-points is a mix of two proprietary nylon polymers: Trogamid T and Trogamid CX. The polymer coating is a cross-linked copolymer of acrylonitrile and vinylpyrrolidone which has been polymerized and cross-linked using allyl methacrylate and a thermal initiator. The lateral expansion of C-points is claimed to occur non-uniformly with the expandability depending on the extent to which the hydrophilic polymer is prestressed. Radioopacity of both the core and polymer coating is provided with the inclusion zirconia dioxide particles[15]

Various methods have been developed to assess the sealing ability of root canal filling materials. Methods such as dye leakage, fluid transport and bacterial penetration, had been frequently used for evaluation of micro-leakage. Other methods such as radio-labelled isotopes and electromechanical test have also been described. However, these methods often yielded large variations in the outcome and they are not considered to be reproducible and comparable.[33,42]

Assessment of bacterial leakage is considered to be more biologically relevant than that of dye or radioisotope penetration, but the conclusions might vary with the bacterial species used. Maintaining aseptic conditions throughout all steps of the experiment can be difficult. Radioisotope labeling and electrochemical technique were less frequently employed because they pose a radiation hazard and require sophisticated materials and apparatus.[43]

Several test methods have been described to evaluate the sealing quality of filled root canals. The most popular methods are fluid transport model (Wu et al. 1993) and the glucose leakage model (Xu et al. 2005). The latter can be seen as a further development of the fluid transportation concept, both measure passage of fluid along root filled teeth after subjecting them to constant pressure.[35.42.43]

The fluid filtration method, which was developed by Derkson et al for measuring dentin permeability, and later modified by Wu et al to evaluate endodontic leakage, is gaining popularity because it is sensitive and nondestructive and permits repeated observation of the same specimen over times. These techniques do not provide any information about the volume of tracer that penetrates which provides only semi-quantitative data with a high level of variation. However, the glucose model allows measurements of diffusion of the marker molecules as well. The glucose test might be more sensitive than the measurement of air-bubble movement, not only because the detected threshold measurement by eye is higher than that of the spectrophotometer, but also because the convective fluid transport was combined with glucose molecule diffusion.(35,42,43)

In the present study the glucose leakage model was used to completely evaluate the volume of tracer penetration. The advantages of this model are the relative ease of assembly and operation, the availability of the materials and equipment and the great sensitivity of the test. Glucose was selected as the tracer because of its small molecular size (MW = 180 Da) and is a nutrient for bacteria. The choice of tracer should be carefully chosen because its size and physicochemical properties may influence the result. The use of tracer of a small molecular size was favored by the previous studies conducted to obtain a relevant outcome.(31,35,43)

If the glucose could enter the canal from the oral cavity, bacteria that might survive root canal preparation and obturation could multiply and potentially lead to periapical inflammation. Glucose, therefore, was thought to be more clinically relevant than other tracers used in micro-leakage tests. Quantitative analysis of leakage was possible by determining the concentration of glucose in the apical reservoir that leaked through the filled root canal.[(43)]

The teeth in all the five groups were subjected to micro-leakage testing using the Glucose leakage Model. The samples were tested for leakage at 7 days intervals and the data were collected at baseline (day 1), 7^{th} day, 14^{th} day, 21th day and 28^{th} day.

In the Glucose leakage Model 10 µL of the sample was withdrawn after 24 hours, followed by 10 µL at regular intervals with the help of a micropipette. The sample withdrawn was then subjected to quantitative glucose testing by Glucose oxidase-Peroxidase test using a spectrophotometer at wavelength 505 ηm. The 10 µL of sample withdrawn was replenished with the same volume of 0.2% sodium azide. The study model used in the present study was similar to the technique used by Xu et al.[(43)]

To determine the concentration of glucose, the enzymatic glucose oxidase method was chosen because it provided the ultimate degree of specificity and high sensitivity when compared with other methods, such as copper or ferricyanide methods. With this method, glucose is oxidized by the enzyme glucose oxidase in the presence of oxygen to gluconic acid with the 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).[43]

The quantity of this oxidized chromogen is proportional to the glucose present initially in the first reaction, which quantity is determined by spectrophotometry. With this model, it was possible to quantify the endodontic micro-leakage continuously over time. The amount of microleakage was the cumulative value of leaked glucose. The reactivity of obturating materials with glucose could affect the results of the glucose leakage test. The results of Shemesh et al. indicated that all materials used in the current study did not show glucose reactivity.[31,43]

The results of the current study clearly demonstrate that none of the materials completely sealed the root apex in vitro (Table 1). Inadequate apical seals could result from the technique used to fill the canal system; for example, the use of a single-cone filling technique is often considered inferior to more sophisticated 3D compaction techniques. In the single-cone technique, the volume of sealer is high relative to the volume of the cone, and this ratio promotes void formation and reduces the quality of the seal. However, it must be noted that the concept of the single-cone technique has been recently re-visited, and that the volume of the sealer used in the present study was minimized because gutta-percha, C-point cones, Resilon cones were matched to the preparation. Use of the single-cone technique also allowed a comparison of the performance of all materials under relatively standardized conditions.[4]

For each day tested, the positive controls had immediate substantial glucose leakage, which increased over time, whereas the negative controls showed no detectable glucose leakage. This indicates that the seal of the glucose leakage system was effective and reliable.

The glucose leakage mean value of Group I gutta-percha/AH plus on day 1 was 0.54 $\pm$ 0.07 mg/dl, at 7 days 4.93 $\pm$ 0.39, at 14 days 10.91 $\pm$ 0.59, at 21 days 13.11 $\pm$ 0.73, at 28 days 15.21 $\pm$ 0.52. Overall mean leakage value was 8.94 $\pm$ 5.48. There was a gradual increase of leakage from day1 to day 28.

In the present study micro-leakage was found to be least in Group II and Group III. In Group II, Smart Seal showed mean leakage value on day 1 0.30 $\pm$ 0.16, on the 7th day 1.94 $\pm$ 0.46, on the 14th day 5.35 $\pm$ 0.77, on the 21 day 7.01 $\pm$ 0.57, on the 28th day 7.81 $\pm$ 0.49. The overall mean was 4.48$\pm$ 2.96. There was a gradual increase of leakage from day1 to day 28.

In Group III, Real Seal showed mean leakage value on day 1 0.52 $\pm$ 0.10, on the 7th day 3.21 $\pm$ 0.48, on the 14th day 8.18 $\pm$ 1.32, on the 21 day 8.81 $\pm$ 1.10, on the 28th day 10.01 $\pm$ 1.03. The overall mean was 6.14 $\pm$ 3.77. There was a gradual increase of leakage from day 1 to day 28.

On comparison of micro-leakage among the three groups, Group II which used Smart Seal showed the least mean glucose leakage.

In Group I the disparity in the finding could be explained by the fast setting and subsequent polymerization shrinkage of AH plus sealer, the lack of bonding between this sealer and gutta-percha, the low penetration ability of this sealer within the dentinal tubules and its hydrophobic property that prevents good adaptation of it in the incompletely dried canal.[(38)]

During root canal treatment, especially after rinsing the root canal system, it is obvious that fluid droplets are retained in the dentinal tubules and may not be completely removed through the use of paper points. Because the sealer may be exposed to tissue fluid and exudate, water sorption and solubility behaviour of the root canal sealers in the humid root canal system is of considerable importance.[(33)]

According to Tat and pashley, the currently marketed dentine adhesives, including the hydrophilic resin-based root canal sealer Epiphany, contain hydrophilic and ionic monomers, making them highly susceptible to water sorption and hydrolysis. This water sorption plasticizes polymers and lowers their physical/mechanical properties which decrease the life expectancy of the interfaces by hydrolysis and microcrack formation.[(23)]

According to Sano et al. one of the most important factors in the strength and stability of the resin/dentine bond is the incomplete resin infiltration into the demineralized dentine (hybrid layer). As a result, fluid movement occurs. This nanoleakage, or ingress of oral fluid through nanometer sized channels along collagen fibrils within the hybrid layer, is considered to be detrimental to bond integrity. C-factor can be more preponderant. Hence, any polymerizing endodontic sealer would be subjected to sizably voluminous polymerization stresses during the setting process, resulting in debonding and gap formation along the periphery of the root filling and thus can be a contributing factor for the increased leakage seen in this group. Therefore, in spite of the hydrophilic nature of Resilon, leakage was significantly more than other hydrophilic groups. According to Shipper et al., this material has been shown to be more resistant to leakage than gutta-percha for filling root canals.[38]

On comparison between the groups 1, 7, 14, 21 and 28 days we found that Group II (SMART SEAL) showed least leakage followed by Group III (REAL SEAL), Group I showed highest amount of glucose leakage. Statistical difference between the groups were found to be highly significant ($p<0.001$).

Leakage cannot be totally eliminated from the fate of a root canal treated teeth. Lateral canals, accessory canals and other anatomical variation play an important role in this, with periapical pressure being the leading factor.[21]

With the introduction of novel hydrophilic SmartSeal system (C-points) over the conventional hydrophobic Gutta-percha system, has widened our range of achieving a 3D seal.

The present study was carried out on mandibular premolars with straight canals. Hence, further studies has to be directed in teeth with complicated anatomy and curved root canals to evaluate the microleakage in the root canal system.

Summary

SUMMARY

This study was aimed to evaluate and compare the micro-leakage and to assess the sealing ability of three obturating materials. Seventy teeth with single canals (verified with radiograph) were selected for this study. Root length was standardized to 15mm and were randomly divided into 5 groups. Group I was Gutta-percha with AH-Plus sealer (n=20), Group II was C-Points with Bio ceramic sealer (n=20), Group III was Resilon with Epiphany sealer (n=20), Group IV (positive control)- Guttapercha without sealer (n=5) and Group V (negative control)- Teeth without obturation, coated with nail varnish (n=5)

Working length was obtained using 10 size stainless steel k-file. Chemo-mechanical preparation was performed by Ni-Ti rotary Hy-flex CM files(Size 20/0.08%,Size 20/0.04%,Size 25/0.04%,Size 30/0.04%,Size 30/0.06%) in crown down sequence with irrigation using 2% NaOCl and 17%EDTA with every change of each file. Irrigation was carried out passively with a Endoactivator with tip being placed 1mm short of working length and finally rinsed with distilled water.

The root canals were dried using paper points, obturated according to respective groups. After obturation samples were incubated at 37̊ c for 1 week. After incubation samples were subjected to glucose leakage test, later the solutions were subjected to spectrophotometer at 505 ηm at different time intervals (day 0, day 7, day 14, day 28).

The score values were recorded, tabulated and statistically analyzed by Mann-Whitney Test and Wilcoxon signed-rank tests.

Conclusion

CONCLUSION

With the introduction of novel hydrophilic Smart Seal system (C-points) over the conventional hydrophobic Gutta-percha system, has widened our range of achieving a three dimentional seal.

Within the limitations of this in vitro study, it can be concluded that:

1. C-Points with bio ceramic sealer showed least glucose leakage values hence proving superior sealing ability compared to other groups.
2. C-Points with bio-ceramic sealer showed superior sealing ability when compared with Resilon/Epiphany, Gutta-percha/AH-Plus and control groups.

From the above study it can be inferred that C-Points with bio ceramic sealer can be considered as a potent alternative for Resilon Epiphany system and Gutta-percha with AH-Plus sealer because of its superior sealing ability of the root canal system and negligible micro-leakage.

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Annexure

ANNEXURE

RAGAS DENTAL COLLEGE & HOSPITAL
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TO WHOM SO EVER IT MAY CONCERN

Date: 06-01-2016
Place: Chennai

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

The thesis topic **"EVALUATION OF MICROLEAKAGE OF THREE DIFFERENT SINGLE-CONE OBTURATION SYSTEMS BY QUANTITATIVE GLUCOSE LEAKAGE MODEL- AN *INVITRO* STUDY"** submitted by **Dr. Sreekanth kandepu** has been approved by the institutional review board of Ragas Dental College & Hospital on 5th May, 2014.

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