"COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF THREE ENDODONTIC SEALERS AGAINST *ENTEROCOCCUS FAECALIS* USING DIRECT CONTACT TEST: AN IN-VITRO STUDY"

By

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This dissertation is

dedicated to

Almighty God,

My Parents,

&

My Brother

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Date: ***Dr. ABHIJIT SAJO SEBASTIAN***

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LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	*E. faecalis*	*Enterococcus faecalis*
2	CFU	Colony Forming Units
3	ANSI/ADA	American National Standard/ American Dental Association
4	mm	Millimetre
5	MPa	Mega Pascals
6	ADT	Agar Diffusion Test
7	DCT	Direct Contact Test
8	µl	Microlitre
9	BHI	Brain Heart Infusion broth
10	°C	Degrees centigrade
11	nm	Nanometre
12	CLSM	Confocal Laser Microscopy
13	h	hours
14	MTA	Mineral Trioxide Aggregate
15	MRCT	Membrane Restricted Contact Test
16	ANOVA	Analysis of Variance
17	SD	Standard Deviation
18	SE	Standard Error

19	CI	Confidence Interval
20	n	Number of specimens
21	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed
22	<	Less than
23	>	Greater than

ABSTRACT

Aim:

Comparative evaluation of antimicrobial efficacy of three endodontic sealers against *Enterococcus faecalis* using Direct Contact Test : An In-vitro study

Study design:

PREPARATION OF THE INOCULUM

The facultative strain of *E. faecalis* (MTCC 439) was grown aerobically from frozen stock cultures in Brain Heart Infusion broth at 37°C. Microorganisms were then subcultured in BHI broth under laminar air flow to ensure their purity. The suspension was then adjusted to 0.5 McFarland scale= 1.5×10^8 CFU/ml

PROCEDURE

Endodontic sealers were mixed or dispensed on to a mixing pad according to the manufacturer's instructions.

The 96- well microtiter plate was held vertically, i.e., the plate's surface was maintained perpendicular to the floor, and half of the side wall of the 22 wells (per sealer) **(n=22)** was coated using a cavity liner applicator.

GROUP 1: AH Plus

GROUP 2: Guttaflow Bioseal

GROUP 3: Ceraseal

8 hours later, corresponding to the recommended setting time of sealers, a 100 µl of bacterial suspension was placed on the test material. The plate was then held in a

vertical position and the walls were then inspected for evaporation which occurred within 1 hour at 37 °C. This ensures direct contact between bacteria and test material.

Then, keeping the plate horizontal, BHI broth (200µl) was added to each of these wells and gently mixed. Following this, the plate was incubated at 37 °C.

Optical Density i.e. the absorbance readings were obtained by analyzing the microtiter plates with a spectrophotometer on the 1^{st}, 3^{rd}, 5^{th} and 7^{th} days at 630nm.

Results:

One Way ANOVA was done to check the statistical association between the three sealers Independent t test was done to check the statistical association between two individual sealers to compare their mean difference.

Intra group comparison between each sealer individually was done using Tukeys multiple posthoc procedures

A statistically significant difference were seen between all the groups (<0.05). Guttaflow Bioseal performed better than AH Plus and Ceraseal.

Conclusion:

Within the limitations of the present study, it can be concluded that none of the sealers could completely eradicate the *E. faecalis* but Guttaflow Bioseal showed the best result.

Key words: endodontic sealers, *Enterococcus faecalis*, direct contact test, antibacterial efficacy, optical density

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INTRODUCTION

Bacteria are the primary etiological agents for pulpal necrosis and periapical lesions .[1] After initiation of infection coronally, the infection advances apically until bacterial products or the bacteria themselves are able to irritate the periapical tissues, resulting in apical periodontitis.[2]

Primary endodontic infections are polymicrobial, with obligatory anaerobic bacteria dominating the microbiota. In cases of unsuccessful endodontic therapy and canals with chronic infections, *Enterococcus faecalis*, yeast and *Candida albicans*, have been identified as the most typically recovered species.[2]

Gram-positive cocci, *E. faecalis* can be found individually, in pairs, or in short chains. They are facultative anaerobes, meaning they can grow with or without oxygen. In the human intestinal lumen, Enterococcus species survive in large numbers [10^5-10^8 Colony-Forming Units (CFU) per gramme of faeces]. They do not harm their hosts in the majority of cases.[3] It is, nevertheless, a stubborn candidate among the many causes of unsuccessful endodontic therapy. *E. faecalis* was found in 38 percent of the failed root canal systems. The capacity of *E. faecalis* to adhere to collagen and remain viable within tubules is the main cause of endodontically treated teeth failing frequently. These microbes can live in the root canals as a monoinfection and can flourish even in low-nutrient environments. It is difficult to eradicate *E. faecalis* from the root canal utilising only chemomechanical preparation with disinfecting irrigants and antibacterial dressings.

Most endodontic sealers in use today, do not result in a perfect and long-lasting seal with the root canal wall. Microleakage is therefore a serious clinical problem and a major cause of failure of endodontic therapy. [1] Hence, using a biocompatible endodontic sealer that hermetically seals the root canal and possesses long-term antimicrobial and antibiofilm properties will aid in reduction of residual infection and create an environment that discourages bacterial colonization.[4]

An ideal root canal sealer should have good adherence to the canal walls as well as the filling material, establish an excellent seal, be radiopaque, and non-staining. It should be dimensionally stable as well. The clinician should be able to easily mix and introduce the sealer into the canals, and if required it should also be easy to remove from the canals. It must be bactericidal or bacteriostatic in nature and insoluble in tissue fluids. The sealer should preferably cause no irritation to the periapical tissues, have a sufficient working period, and be free of mutagenic or carcinogenic properties.[5]

Schroeder pioneered the use of epoxy resin-based sealers in endodontics, and modern variations of the original formulation are commonly used today. Because of their lower solubility, improved apical seal, and micro-retention to root canal dentin, epoxy resin sealers have been utilised.[6]

AH Plus (Dentsply) is an epoxy-resin based sealer that comes in a two-paste system that comes in two tubes and a double barrel syringe. The resin paste is made up of epoxy resin, calcium tungstate, zirconium oxide, and aerosol iron oxide. The amine paste consists of adamenthas amine, N, N didenyl – 5 – oxanone diamine, zirconium oxide, aerosil, and silicone oil. It has a 4 hour working time, an 8 hour setting time, and a 26mm film thickness. It is thixotropic, radio opaque, and has a

flow of 36 mm, which meets the ANSI/ADA (2000) requirements. It has a very low shrinkage rate and high dimensional stability. The adhesive bond strength of AH Plus sealer to dentin was measured at 4 MPa by Pecora et al. The AH Plus sealer's bond strength to dentin is improved by using the continuous chelation irrigation protocol. AH Plus has been examined in multiple studies for possible interactions with living tissue and has been determined to be innocuous and safe. AH Plus is regarded as the "Gold Standard" sealer because of its exceptional qualities, such as low solubility, modest expansion, adherence to dentin, and superior sealing ability.[6] It is, however, non-bioactive and does not have any osteogenic potential.[4]

GuttaFlow Bioseal (Coltène/Whaledent, Altstatten, Switzerland) is a silicone-based sealer that claims to outperform GuttaFlow and GuttaFlow 2 in terms of biological properties. The mixture consists of gutta-percha powder, polydimethylsiloxane, platinum catalyzer, and zirconium dioxide. It also contains calcium silicate particles, making it acceptable for use in fluid-contaminated environments and allowing for the release of calcium ions needed for in situ apatite nucleation. This is an intriguing way to get a bioactive gutta-percha sealer that could be useful in endodontic and regenerative therapy. The new material outperforms AH Plus in terms of physicochemical properties, such as dentin penetrability and cytocompatibility.[4]

Cements based on a calcium and silicate composition, such as mineral trioxide aggregate, have recently been introduced to modern dentistry. These cements are utilised for a variety of clinical applications, including pulp capping in primary and permanent teeth, root-end filling, perforation repair, and apical plug for teeth with open apices, because of their superior sealing ability and biocompatibility.[7] They are

also known to have bioactive properties. Bioactive materials like glass and calcium phosphate interact with the surrounding tissue to stimulate the formation of more durable tissues with greater strength and acid resistance.[8]

There are two significant benefits to using these materials as root canal sealers. The first is their biocompatibility, which prevents rejection by the surrounding tissues, and the second is the presence of calcium phosphate in these bioceramic materials, which enhances the material's properties by resulting in a chemical composition and crystalline structure that is similar to teeth and bone apatite materials resulting in improved bonding between the sealer and the root dentin.[9]

Endodontic sealers containing calcium silicates have been introduced in recent years as a result of these advantageous properties. Ceraseal (Meta Biomed Co., Cheongju, Korea), a novel bioactive calcium silicate-based, premixed endodontic sealer that contains calcium silicates, zirconium oxide, and a thickening agent, is known to eliminate the pathogens in the root canal due to its high pH.[10]

The antibacterial activity of endodontic sealers has been studied in numerous studies. The antibacterial effect was seen to be strongest immediately after spatulation, followed by a gradual reduction of antimicrobial effects over time.[1] Several studies have been conducted to evaluate the antibacterial effectiveness of various root-canal sealers. The Agar-Diffusion Test (ADT) was once the most extensively used method for determining the suitability of dental materials. The Agar-Diffusion Test (ADT) was once the most widely used method for evaluating dental materials. However, it is better suited for soluble materials or vapours due to its relative insensitivity and reliance on diffusion and physical features of the examined materials.[11] Weiss et al. described a Direct Contact Test (DCT) assay that examines

the antibacterial efficacy of endodontic sealers to address some of the drawbacks of ADT. DCT is a quantitative assay that can be used to test materials that are insoluble in water. It can also be utilised in standardised ageing studies.[12]

Although literature supports the antimicrobial properties of GuttaFlow Bioseal and Calcium silicate-based sealers, there is no literature documented on the antimicrobial efficacy comparing these three sealers, i.e., AH-Plus, GuttaFlow Bioseal and Ceraseal. Hence, the aim of the study was to evaluate and compare the antimicrobial efficacy of AH-Plus, GuttaFlow Bioseal and Ceraseal through the direct contact test.

OBJECTIVES OF THE STUDY

AIM

To evaluate and compare the antimicrobial efficacy of three endodontic sealers- Epoxy resin-based sealer (AH-Plus), Silicone based sealer (GuttaFlow Bioseal) and Calcium silicate-based sealer (Ceraseal) against *Enterococcus faecalis* using DCT.

OBJECTIVES

1. To evaluate the antimicrobial efficacy of Epoxy resin-based sealer (AH-Plus), Silicone based sealer (GuttaFlow Bioseal) and Calcium silicate-based sealer (Ceraseal) against *E. faecalis* using DCT.

2. To compare the antimicrobial efficacy of Epoxy resin-based sealer (AH-Plus), Silicone based sealer (GuttaFlow Bioseal) and Calcium silicate-based sealer (Ceraseal) against *E. faecalis* using DCT.

HYPOTHESIS

NULL HYPOTHESIS: -

There is no difference in the antimicrobial efficacy of AH Plus sealer, Guttaflow Bioseal sealer and Ceraseal sealer against *E. faecalis* evaluated using Direct Contact Test.

ALTERNATE HYPOTHESIS: -

There is a difference in the antimicrobial efficacy of AH Plus sealer, Guttaflow Bioseal sealer and Ceraseal sealer against *E. faecalis* evaluated using Direct Contact Test.

REVIEW OF LITERATURE

In a study done by **Anumula et al.,** Direct Contact Test was used to evaluate the bactericidal activity of four endodontic sealers: zinc oxide eugenol sealer (DPI), glass ionomer sealer (Ketac Endo Applicap), polydimethylsiloxane based sealer (Gutta Flow), and resin based sealer (Endo Rez). The most effective sealer against Enterococcus faecalis was zinc oxide eugenol, and the least effective sealer was urethane dimethacrylate resin, whilst glass-ionomer-based and polydimethyl-siloxane-based sealers were barely effective for a short time. The antibacterial properties of endodontic sealers were found to deteriorate over time in this investigation. These findings show that inclusion of antimicrobial components in root canal sealers may become an important factor in preventing bacterial regrowth in the root canal system.[1]

A study was done by ***Ruiz-Linarez et al.*** to investigate the in vitro antibacterial and antibiofilm activity of GuttaFlow Bioseal and AH Plus, after 1 day, 1 week and 4 weeks of aging which concluded that GuttaFlow Bioseal showed increased antibacterial and antibiofilm activity at 1 and 4 weeks as determined by DCT and CLSM, while AH Plus indicated an opposite property in which its antimicrobial activity decreased over time.[4]

In a study done by **Cobankara et al.** both ADT and DCT were used to examine the bactericidal activity of five different endodontic sealers (RoekoSeal, Ketac-Endo, AH Plus, Sealapex, Sultan). RoekoSeal had no antibacterial action in ADT. The bactericidal activity of AH Plus, Sealapex, and Sultan was not significantly different (p 0.05), while that of Ketac-Endo was lower than these sealers (p 0.05). The results, however, were altered in the DCT. Bacterial growth was completely inhibited

by AH Plus and Sultan. In the first 19 hours, KetacEndo had an antibacterial effect similar to AH Plus and Sultan. Bacterial growth was seen in RoekoSeal and Sealapex. It was found that the results of microbiological studies are influenced by the procedure, timing, or contents of the investigated materials. As a result, it is suggested that when testing the antibacterial capabilities of dental materials, more than one assaying method be utilised.[11]

A study was done by ***Weiss et al.*** to investigate the antibacterial characteristics of two endodontic sealers (AH 26 and Endoflas). According to the ADT, AH26 outperformed Endoflas in terms of antibacterial activity. When the DCT was used, the opposite outcome was produced. Endoflas may have more effective antibacterial components that are either less soluble or diffusible in the surrounding agar media and thus showed inferior results in the ADT. It was determined that inclusion of antimicrobial components in root canal sealers could play a critical role in limiting bacterial regrowth and controlling bacterial re-entry into the root canal space.[12]

A study was done by **Zhang et al.** to test the antibacterial activity of seven different endodontic sealers (AH Plus, Apexit Plus, iRoot SP, Tubli Seal, Sealapex, Epiphany SE, and EndoRez) against *E. faecalis* 20 minutes after mixing (fresh samples), 1, 3, and 7 days later. Fresh iRoot SP, AH Plus, and EndoRez were found to efficiently eliminate *E. faecalis*. iRoot SP and EndoRez remained effective for 3 and 7 days, respectively. Sealapex was moderately effective throughout the investigation and, together with EndoRez, was the only sealer that could completely remove E. faecalis.[13]

A study was done by ***Mickel et al.*** to assess the antibacterial activity of four root canal sealers (Sealapex, Roth 811, Kerr EWT, and AHPlus) against *E. faecalis*. With a mean of 1.1 mm, Roth 811 had the greatest zone of inhibition, followed by Sealapex with 0.8 mm and Kerr EWT with 0.5 mm. There was no zone of inhibition in AH-Plus. The inhibition zone in the positive control was 10.0 mm on average.[14]

A study was done by ***Abduljabbar et al.*** to investigate the antibacterial effect of three calcium silicate-based root canal sealers (Endosequence/BC Sealer, CeraSeal, and BioRoot RCS) against Enterococcus faecalis at various incubation times (24 h, 48 h, 72 h, and 7 days). According to the conclusions of this experiment, all of the sealers tested inhibited bacterial growth, with BioRoot RCS having the most antimicrobial activity and CeraSeal having the lowest. The antibacterial capabilities improved when the incubation time was increased.[15]

A study was done by ***Pizzo et al.*** to evaluate the antibacterial activity of four endodontic sealers (AH Plus, Endomethasone, Pulp Canal Sealer and Vcanalare) by using DCT. It concluded that the antibacterial activity of the sealers is dependent on the time period between mixing and testing. When freshly mixed, all sealers had a bactericidal effect, however only Vcanalere's bactericidal effect lasted for 7 days after setting.[16]

Chakravorthy et al. used the ADT to assess the bactericidal activity of four bioceramic sealers (Guttaflow Bioseal, BioRoot RCS, MTA fillapex, and AH plus) after 24, 48, and 72 hours. BioRoot RCS had the best antibacterial properties, followed by MTA fillapex and Guttaflow Bioseal, both of which had equal inhibitory effects. The antibacterial impact of AH Plus was the least.[17]

A study was done by ***Nirupama et al.*** to evaluate the antibacterial effectiveness of four endodontic sealers (AH Plus, Tubliseal EWT, EndoRez, and iRoot SP) against three microorganisms: *E. faecalis, Candida albicans*, and *Staphylococcus aureus*. The study found that the antimicrobial activity of AH Plus was the highest while that of EndoRez was the lowest.[18]

A study was conducted by ***Roshdy et al.*** to assess the antibacterial impact of AH Plus and Ceraseal sealers with and without the addition of silver nanoparticles against Enterococcus faecalis. The antibacterial activity of AH Plus sealer was higher than CeraSeal sealer against Enterococcus faecalis. The bactericidal activity of both sealers was boosted by adding silver nanoparticles gel.[19]

A study was conducted by ***Kharouf et al.*** to compare the physicochemical qualities, filling ability, and antibacterial activity of a premixed calcium silicate-based sealer (Ceraseal) to a powder–liquid bioceramic sealer in a study (BioRoot). After 72 hours, both sealers were found to have killed 95 percent of E. faecalis. After 72 hours, there was no discernible difference in the bactericidal effect of sealers. As a result, bioceramic sealers may play a significant role in bacterial growth control. Furthermore, CS exhibited better filling ability and lower solubility than BioRoot sealer possibly because of its chemical composition and mixing procedure.[20]

A study was done by ***Eldeniz et al.*** to compare the antibacterial activity of EndoREZ, a resin-based sealer, to five other sealers: AH 26, Diaket, Sultan, Apexit, and RoekoSeal, against three bacteria (*Enterococcus faecalis, Staphylococcus aureus, and Pseudomonas aeruginosa*). EndoREZ, Apexit, and RoekoSeal did not show any antibacterial action according to the ADT. AH 26 and Sultan were found to be strong

bacterial growth inhibitors according to the DCT results. The study concluded that EndoREZ is not as effective a bacterial growth inhibitor as Sultan and AH 26.[21]

A study was done by ***Farmakis et al.*** to test the antibacterial activity of six endodontic sealers (Roth 601, AH-26, TopSeal, AH-Plus, GuttaFlow, and EndoREZ) in both unset and set states against *Enterococcus faecalis* and *Proteus vulgaris*. It was discovered that the antibacterial activity of AH-26, AH-Plus, TopSeal, and Roth 601 against *Enterococcus faecalis* and *Proteus vulgaris* varied based on the test used. In the DCT, only Roth 601 was effective against Proteus vulgaris after setting. The findings also suggested that minute changes in the composition of the similarly manufactured sealers, TopSeal and AH-Plus, could explain the varied levels of activity against *Enterococcus faecalis* expressed through the zones of inhibition seen in the ADT.[22]

A study was conducted by ***Heyder et al.*** to compare the antibacterial efficiency of various sealers (AH Plus, Hermetic, RoekoSeal, Sealapex, Apexit Plus, 2Seal, EndoREZ, and ProRoot MTA). It was concluded that Hermetic caused considerable suppression of *E. faecalis*, *F. nucleatum*, and *P. gingivalis*, as evidenced by ADT and DCT results. In the DCT, AH Plus had a suppressive effect against *E. faecalis* and *F. nucleatum*. *E. faecalis* was not suppressible with any of the other sealers tested.[23]

A study was done by ***Kapralos et al.*** in which a modified direct contact test and a membrane restricted test was used to investigate the antibacterial activity of the sealers, AH Plus, TotalFill BC sealer, RoekoSeal, and Guttaflow 2 for planktonic grown and 24-hour-old biofilms of Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, and Streptococcus mutans It was discovered that

AH Plus is highly bactericidal against a variety of bacteria, both planktonic and in biofilms. After 24 hours, however, the antibacterial activity is lost. Up to 7 days after setting, TotalFill BC sealer had a strong antibacterial impact on planktonic bacteria. Incase of the DCT, TotalFill BC sealer demonstrated lower antibacterial activity than AH Plus against biofilms of S. aureus and E. faecalis. When a membrane was utilised to separate the biofilm and sealer, it showed similar results for all the species tested. Guttaflow 2 and RoekoSeal demonstrated no antibacterial effect against bacteria in biofilms or planktonic microorganisms.[24]

A study was done by ***Kayaoglu et al.*** in which they used the DCT and the MRCT to assess the antibacterial activity of root canal sealers (MCS, AH Plus, Grossman's sealer, Sealapex, Apexit) against *E. faecalis*. MCS, AH Plus, and Grossman's sealer were found to be effective in lowering the CFUs incase of DCT as well as MRCT. Sealapex and Apexit (calcium hydroxide-based sealers) proved ineffective.[25]

A study was done by ***Morgental et al.*** to determine the pH and bactericidal activity of Endo CPM Sealer and MTA Fillapex using ADT and DCT, when compared to white MTA and Endofill. They found that MTA Fillapex and Endofill had an antibacterial effect against E. faecalis before setting, but it was lost after 7 days of mixing. White MTA and Endo CPM Sealer had no antibacterial activity before or after setting, despite their alkaline pH.[26]

A study was conducted by ***Poggio et al.*** in which ADT and DCT was used to assess the antimicrobial activity of several root canal sealers (BioRoot RCS, MTA Fillapex, and Pulp Canal Sealer) against *Enterococcus faecalis*. According to the ADT results, BioRoot RCS, MTA Fillapex, and Sealapex Root Canal Sealer had the

lowest antibacterial activity, while Pulp Canal Sealer and AH + sealers had a considerable rise in antibacterial effect. Both EasySeal and N2 sealers had significantly larger mean diameters of the inhibition zone. After 6 minutes of contact, the DCT results showed that AH + and Sealapex Root Canal Sealer had no bactericidal effect. The antibacterial activity of AH plus and Sealapex Root Canal Sealer increased significantly after 15 and 60 minutes of contact. When compared to AH plus, the antibacterial impact of Sealapex Root Canal Sealer was significantly stronger. After 6 minutes of contact, BioRoot RCS, MTA Fillapex, Pulp Canal Sealer, and N2 demonstrated the least number of colonies generated per millilitre. Except for N2, the other examined sealers showed a considerable increase in antibacterial activity after 15 and 60 minutes (BioRoot RCS, MTA Fillapex and Pulp Canal Sealer).[27]

A study was done by ***Shakya et al.*** to investigate the antibacterial effect and flow characteristics of various endodontic sealers (AH Plus, MTA Fillapex, CRCS, and GuttaFlow 2) on *Enterococcus faecalis* using ADT and DCT. Except for Gutta Flow 2, all sealers were found to have antibacterial activity against E. faecalis. Calcibiotic Root Canal Sealer (CRCS) had the greatest zone of inhibition at 24 hours, while AH plus had the lowest. In AH plus, CRCS, and MTA Fillapex, the zone of inhibition reduced after 7 days. At both time periods, DCT revealed a significantly lower number of organisms in AH Plus, CRCS, and MTA than controls. Gutta Flow 2 did not show any significant antimicrobial action. Maximum and minimum flow was shown by AH Plus and CRCS respectively.[28]

MATERIALS AND METHODS

SOURCE OF DATA:

The study was conducted in the Department of Conservative Dentistry and Endodontics, , KLE Academy of Higher Education & Research (KLE University), KLE VK Institute of Dental Sciences, Belagavi (Karnataka)

The Direct Contact Test was carried out in KLE's Dr Prabhakar Kore Basic Sciences Research Centre, KLE University, Belagavi.

MATERIALS USED FOR THE STUDY:

- AH-Plus (Epoxy-resin based) (Dentsply DeTrey)
- GuttaFlow Bioseal (Silicone based) (Coltene/ Whaledent)
- Ceraseal (Calcium silicate based) (Meta Biomed)
- Brain Heart Infusion broth
- Saline
- *Enterococcus faecalis* suspension (MTCC 439)

ARMAMENTARIUM USED FOR THE STUDY

- 96-well microtiter plate (TARSONS)
- Finn pipette - 100µl calibration (eppendorf Research plus)
- Incubator (KEMI)
- Mixing spatula
- Mixing pad
- Cavity liner applicator tips (Denmax)
- Microplate spectrophotometer (LISA Plus; 630nm)

SAMPLE SIZE ESTIMATION:

$S = \frac{S_1 + S_2}{2}$

Z_α= 1.96 at 5% α error.

Z_β= 1.032 at 15% β error.

S1=0.08

S2=0.07

d=0.068

$$n = \frac{2\sigma^2 (Z_\alpha + Z_\beta)^2}{d^2} = 22 \text{ (in each group)}$$

METHODOLOGY

Preparation of the bacterial inoculum:

The facultative strain of *E. faecalis* (MTCC 439) was grown aerobically from frozen stock cultures in Brain Heart Infusion broth at 37°C. Microorganisms were then subcultured in BHI broth under laminar air flow to ensure their purity.[1] The suspension was then adjusted to 0.5 McFarland scale= 1.5×10^8 CFU/ml.[29]

Direct Contact Test

Endodontic sealers were mixed or dispensed on to a mixing pad according to the manufacturer's instructions.

The 96- well microtiter plate was held vertically, i.e., the plate's surface was maintained perpendicular to the floor, and half of the side wall of the 22 wells (per sealer) **(n=22)** was coated using a cavity liner applicator.

GROUP 1: AH Plus

GROUP 2: Guttaflow Bioseal

GROUP 3: Ceraseal

8 hours later, corresponding to the recommended setting time of sealers, a 100 µl of bacterial suspension was placed on the test material. The plate was then held in a vertical position and the walls were then inspected for evaporation which occurred within 1 hour at 37 °C. This ensures direct contact between bacteria and test material.

Then, keeping the plate horizontal, BHI broth (200µl) was added to each of these wells and gently mixed. Following this, the plate was incubated at 37 °C.[11]

Optical Density i.e. the absorbance readings were obtained by analyzing the microtiter plates with a spectrophotometer on the 1^{st}, 3^{rd}, 5^{th} and 7^{th} days at 630nm.

Measurement of Optical Density:

Optical density is a measurement of turbidity based on the kinetics of bacterial growth, considering the fact that as the bacterial population increases, the absorbance reading, i.e. Optical Density given by the spectrophotometer increases. Optical density was measured using a spectrophotometer at 630 nm. [29]

The percentage of remaining bacterial cells were calculated using the following formula

$$\%f \text{ bacterial cells} = \frac{\text{Optical density of the positive control - Optical density of the test}}{\text{Optical Density of the positive control}} \times 100$$

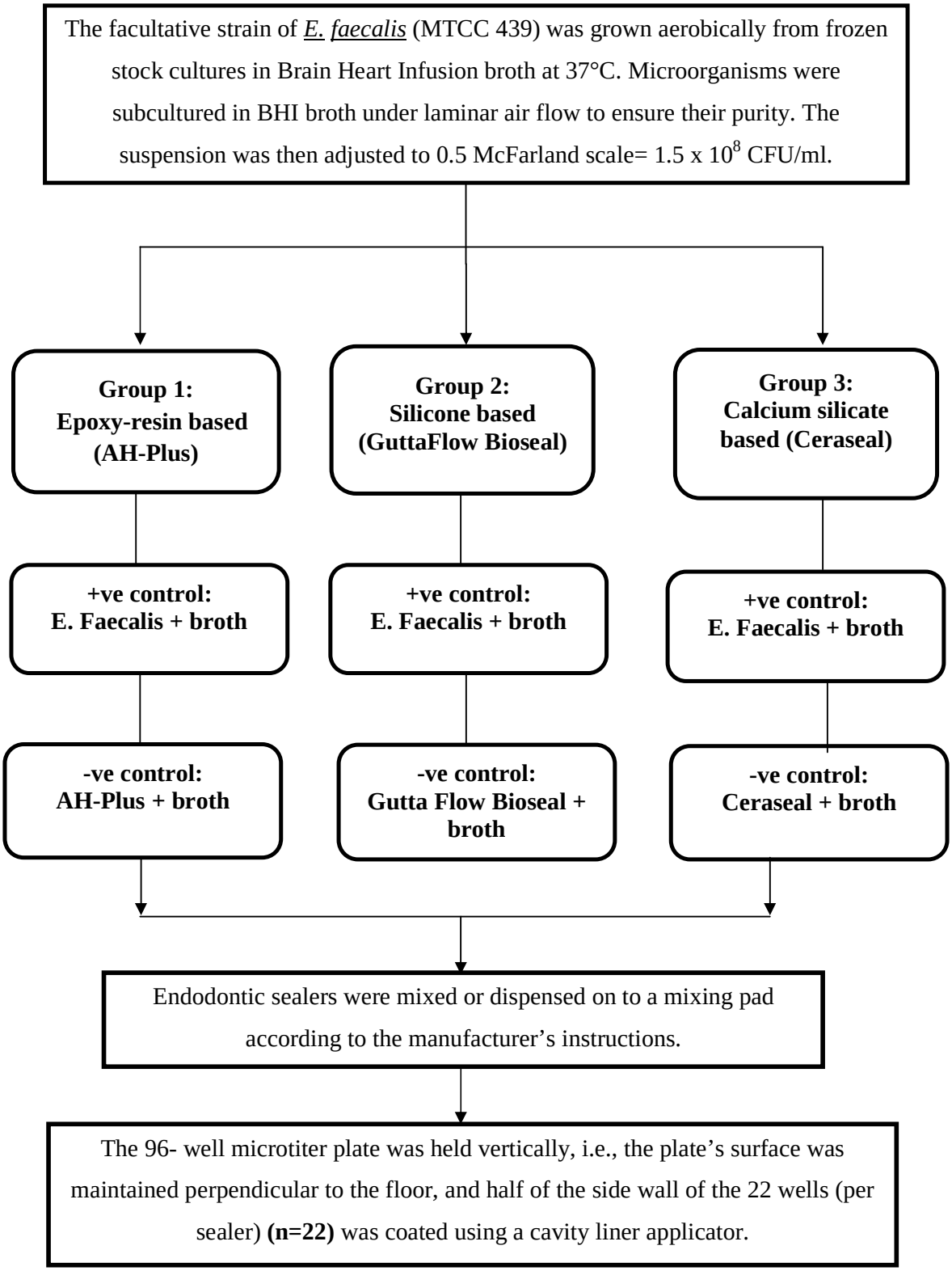
STATISTICAL ANALYSIS

One Way ANOVA was done to check the statistical association between the three sealers Independent t test was done to check the statistical association between two individual sealers to compare their mean difference.

Intra group comparison between each sealer individually was done using Tukeys multiple posthoc procedures

A statistically significant difference were seen between all the groups (<0.05). Guttaflow Bioseal performed better than AH Plus and Ceraseal.

FLOWCHART DEPICTING THE STUDY DESIGN



8 hours later, corresponding to the recommended setting time of sealers, a 100 µl of bacterial suspension was placed on the test material. The plate was then held in a vertical position and the walls were inspected for evaporation which occurred within 1 hour at 37 ℃. This ensures direct contact between bacteria and test material.

↓

Then, keeping the plate horizontal, BHI broth (200µl) was added to each of these wells and gently mixed. Following this, the plate was incubated at 37 ℃.

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Optical Density i.e. the absorbance readings were obtained by analyzing the microtiter plates with a spectrophotometer on the 1^{st}, 3^{rd}, 5^{th} and 7^{th} days at 630nm.

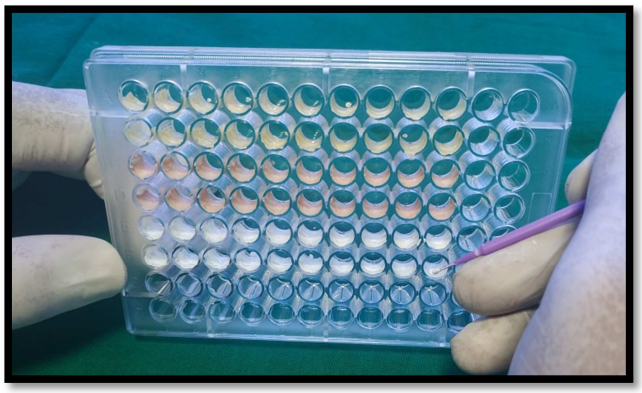


Fig 1:
Coating of the sealers on the side walls of the 96 well microtitre plate

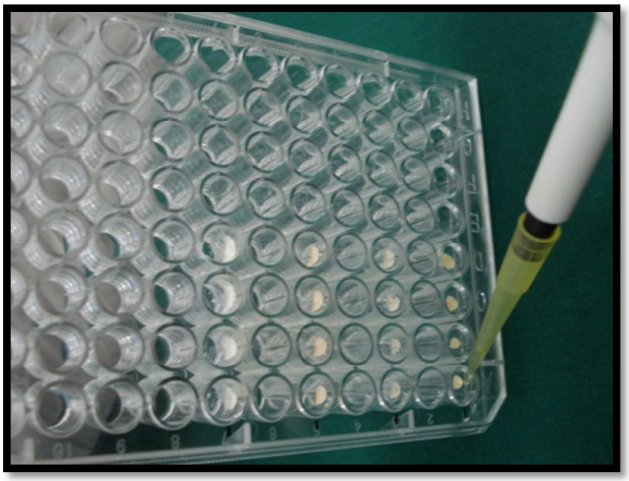


Fig 2:
Addition of the bacterial inoculum

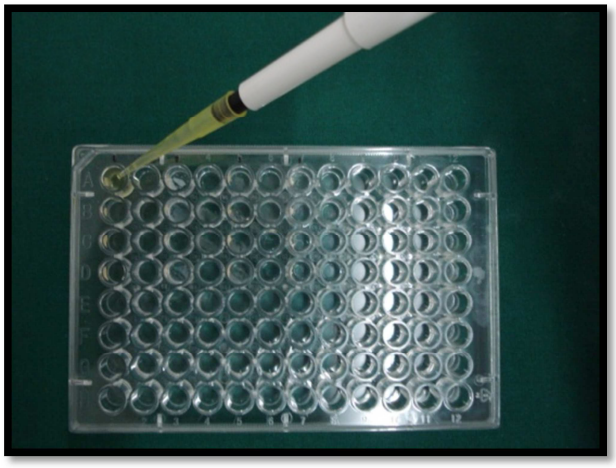


Fig 3: Addition of BHI broth to the wells

MATERIALS AND ARMAMENTARIUM

Fig 4:
AH Plus sealer

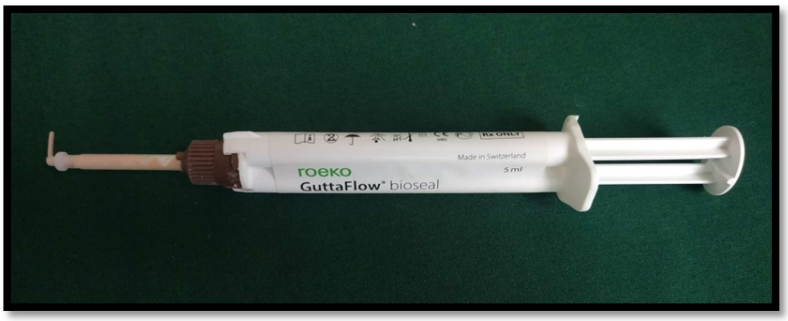


Fig 5:
Guttaflow Bioseal sealer

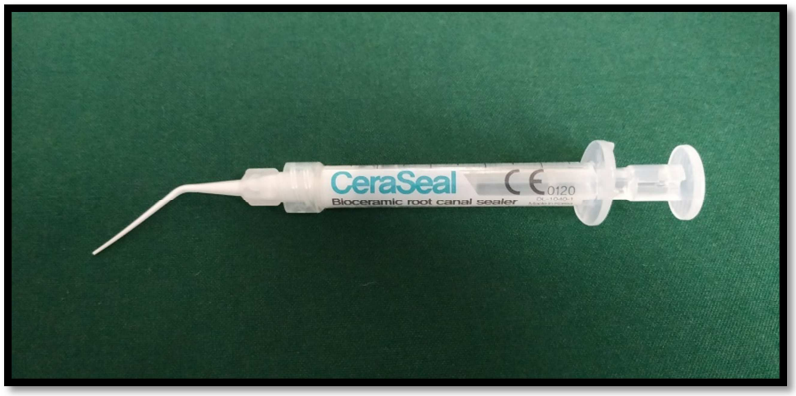


Fig 6:
Ceraseal sealer

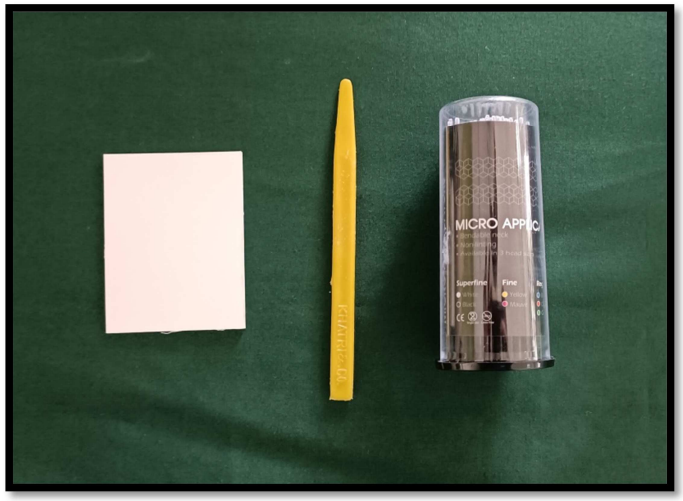


Fig 7:
Paper pad, agate spatula and applicator tips for manipulation and placement of sealers

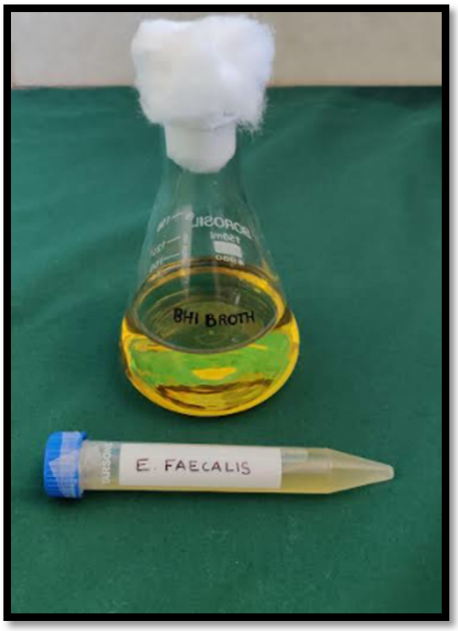


Fig 8:
E. faecalis inoculum

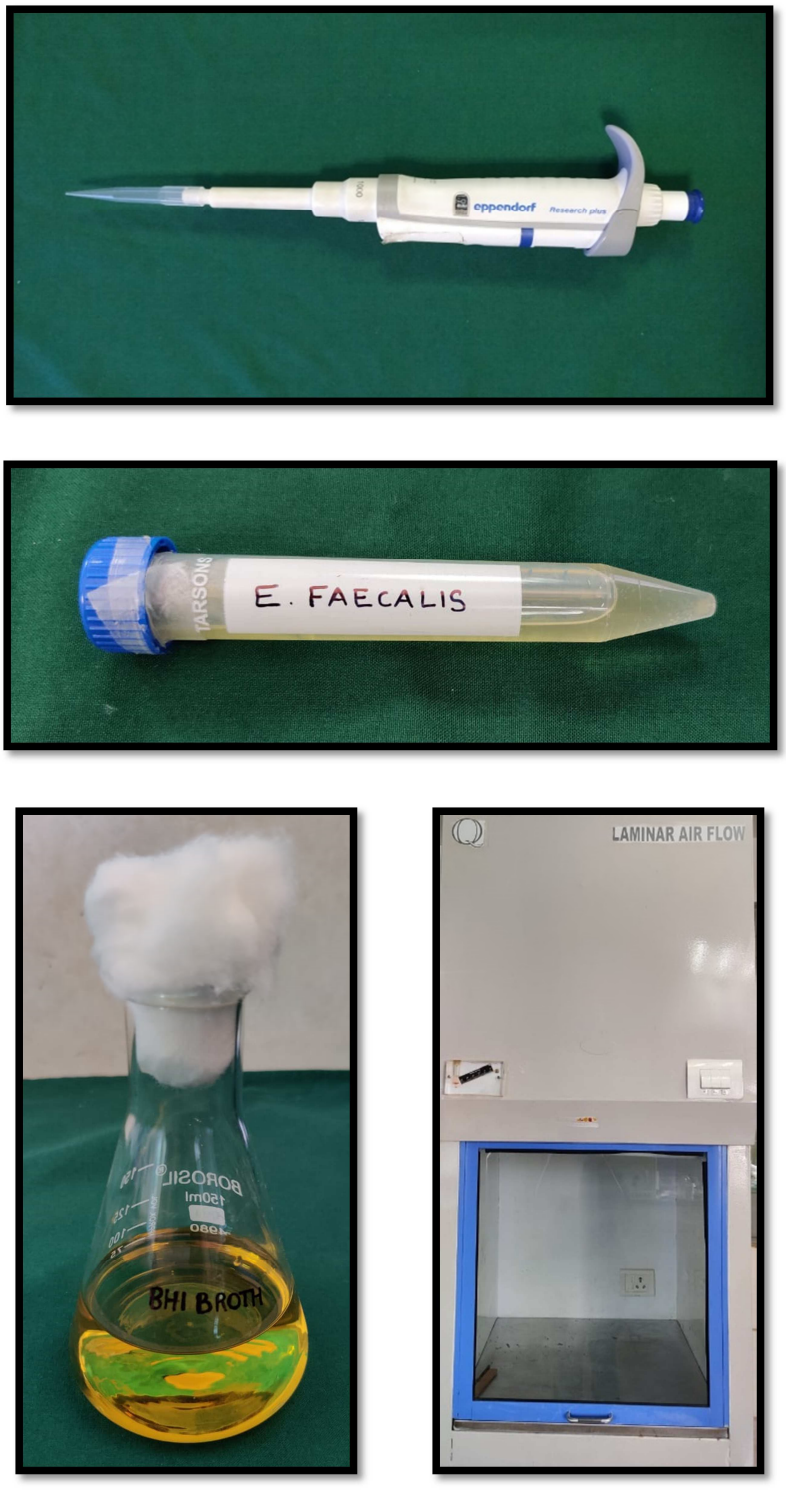


Fig 9:
Micropipette, E. faecalis inoculum, BHI broth and Laminar Air flow for microbiological assessment

Fig 10:
Incubator

Fig 11:
Microplate spectrophotometer (LISA Plus; 630nm)

RESULTS

Table 1, 2, 3 and 4 : Score of % of remaining bacterial cells in AH Plus, Guttaflow Bioseal and Ceraseal and Control group

Table 1 : AH Plus

Name of the sealers	1st day	3rd day	5th day	7th day
AH Plus (22)	57	81	87	85
	58	84	95	84
	54	77	80	83
	55	95	81	86
	53	80	90	88
	50	87	81	80
	51	88	81	80
	54	75	82	85
	57	81	80	97
	55	77	88	84
	50	88	84	92
	57	81	88	92
	52	80	86	92
	58	87	81	95
	53	84	88	90
	56	83	85	91
	58	82	81	90
	55	80	87	91
	57	79	88	91
	53	77	91	92
	50	89	90	90
	59	90	97	98

Table 2 : Guttaflow Bioseal

Name of the sealers	1st day	3rd day	5th day	7th day
Guttaflow Bioseal	51	51	65	67
(22)	54	58	64	68
	57	54	60	64
	55	55	62	66
	58	53	68	65
	57	60	60	64
	51	58	61	63
	55	54	65	66
	51	57	60	67
	50	55	63	67
	58	50	64	65
	51	57	62	63
	55	50	60	60
	52	52	65	62
	51	55	60	65
	50	53	61	63
	56	56	64	66
	54	51	62	68
	55	57	66	67
	51	59	69	69
	51	58	60	68
	52	58	61	67

Table 3 : Cerseal

Name of the sealers	1st day	3rd day	5th day	7th day
Ceraseal	65	81	95	91
(22)	68	89	81	90
	64	78	88	90
	66	79	84	97
	68	75	95	90
	63	86	93	91
	60	85	84	90
	61	84	92	91
	68	84	91	91
	66	85	93	92
	66	83	91	90
	62	80	90	91
	60	85	98	98
	60	80	97	99
	61	88	99	90
	69	89	95	95
	62	89	92	99
	63	85	96	90
	60	84	89	90
	60	86	92	92
	69	85	93	91
	64	89	89	91

Table 4 : Control Group

-ve control	77	81	77	75
AH Plus	78	84	75	74
-ve Control Gutta flow	64	65	52	65
	57	61	50	70
-ve control Ceraseal	71	61	47	85
	68	64	45	74
+ve control	84	86	89	96
	82	83	86	94

Table 5: Summary of % of remaining bacterial cells in three sealers (AH plus, Gutta flow and Ceraseal) at different time intervals

Times	Compounds	Mean	SD	SE	95% CI for Mean	
					Lower Bound	Upper Bound
1st day	AH Plus	54.64	2.85	0.609	49.04	60.23
	Gutta flow	53.41	2.70	0.576	48.11	58.71
	Ceraseal	63.86	3.23	0.688	57.54	70.19
3rd day	AH Plus	82.95	5.08	1.082	73.01	92.90
	Gutta flow	55.05	3.02	0.643	49.14	60.96
	Ceraseal	84.05	3.86	0.823	76.48	91.61
5th day	AH Plus	85.95	4.85	1.035	76.44	95.47
	Gutta flow	62.82	2.70	0.576	57.52	68.11
	Ceraseal	91.68	4.59	0.979	82.68	100.68
7th day	AH Plus	88.91	4.98	1.063	79.14	98.68
	Gutta flow	65.45	2.28	0.487	60.98	69.93
	Ceraseal	92.23	3.13	0.668	86.09	98.36

Table 6: Comparison of three sealers (AH plus, Gutta flow and Ceraseal) with mean % of remaining bacterial cells at different treatment times by one way ANOVA

1. AH Plus

	Sum of squares	d.f.	Mean square	F	p-value
Between the groups	16558.045	3	5519.348	268.175	0.000
Within the groups	1728.818	84	20.581		
Total	18286.864	87			

2. Guttaflow Bioseal

	Sum of squares	d.f.	Mean square	F	p-value
Between the groups	2266.091	3	755.364	104.531	0.000
Within the groups	607.000	84	7.226		
Total	2873.091	87			

3. Ceraseal

	Sum of squares	d.f.	Mean square	F	p-value
Between the groups	11611.636	3	3870.545	275.488	0.000
Within the groups	1180.182	84	14.050		
Total	12791.818	87			

Table 7: Pair wise comparison of three sealers (AH plus, Gutta flow and Ceraseal) with mean % of remaining bacterial cells day wise

1. AH Plus vs Guttaflow Bioseal

Days	AH Plus		Guttaflow Bioseal		t-test	P value
	Mean	SD	Mean	SD		
1st day	54.64	2.85	53.41	2.70	1.46	0.150
3rd day	82.95	5.08	55.05	3.02	22.17	0.000
5th day	85.95	4.85	62.82	2.70	19.53	0.000
7th day	88.91	4.98	65.45	2.28	20.07	0.000

2. Guttaflow Bioseal vs Ceraseal

Days	AH Plus		Guttaflow Bioseal		t-test	P value
	Mean	SD	Mean	SD		
1st day	53.41	2.70	63.86	3.23	11.65	0.000
3rd day	55.05	3.02	84.05	3.86	27.77	0.000
5th day	62.82	2.70	91.68	4.59	25.41	0.000
7th day	65.45	2.28	92.23	3.13	32.41	0.000

3. Ceraseal vs AH Plus

Days	Ceraseal		AH Plus		t-test	P value
	Mean	SD	Mean	SD		
1st day	63.86	3.23	54.64	2.85	10.05	0.000
3rd day	84.05	3.86	82.95	5.08	0.80	0.427
5th day	91.68	4.59	85.95	4.85	4.02	0.000
7th day	92.23	3.13	88.91	4.98	2.64	0.011

Table 8: Comparison of three sealers (AH plus, Gutta flow and Ceraseal) with mean changes in % of remaining bacterial cells from 1st to 7th day by Tukey's multiple posthoc procedures

1. AH Plus

Times	Mean Difference	SE	p-value
1D-3D	-28.31818*	1.36785	0.000*
1D-5D	-31.31818*	1.36785	0.000*
1D-7D	-34.27273*	1.36785	0.000*
3D-5D	-3.00000*	1.36785	0.031*
3D-7D	-5.95455*	1.36785	0.000*
5D-7D	-2.95455*	1.36785	0.034*

*$p<0.05$

2. Guttaflow Bioseal

Times	Mean Difference	SE	p-value
1D-3D	1.63636*	0.81051	0.0470*
1D-5D	-10.40909*	0.81051	0.000*
1D-7D	-7.77273*	0.81051	0.000*
3D-5D	-12.04545*	0.81051	0.000*
3D-7D	-9.40909*	0.81051	0.000*
5D-7D	-2.63636*	0.81051	0.002*

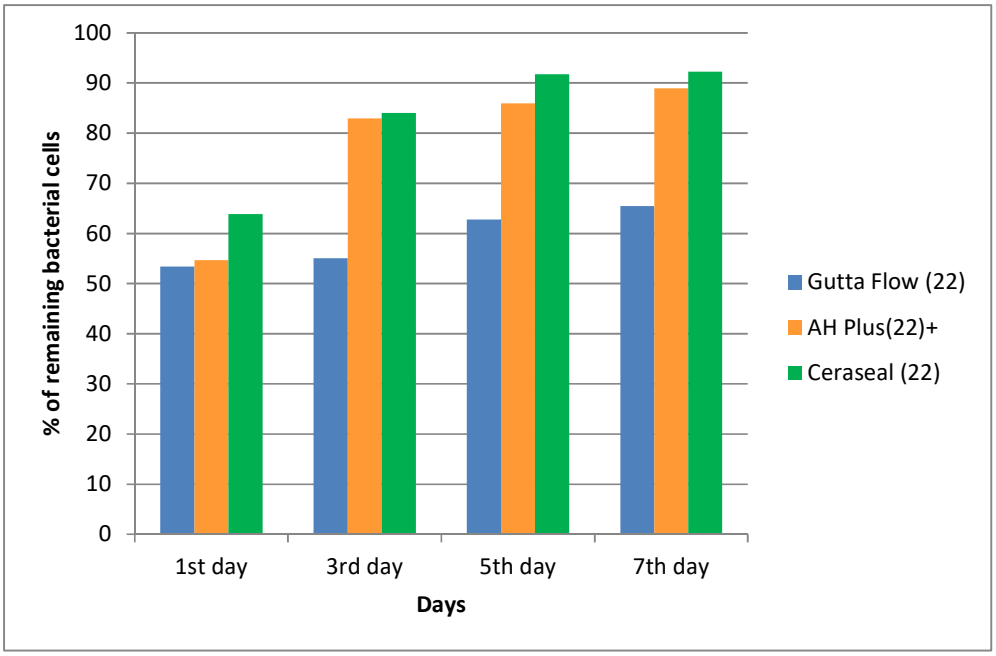
*p<0.05

3. Ceraseal

Times	Mean Difference	SE	p-value
1D-3D	-20.18182*	1.13016	0.000*
1D-5D	-27.81818*	1.13016	0.000*
1D-7D	-28.36364*	1.13016	0.000*
3D-5D	-7.63636*	1.13016	0.000*
3D-7D	-8.18182*	1.13016	0.000*
5D-7D	0.5455*	1.13016	0.631

*p<0.05

Figure 1: Comparison of three sealers (AH plus, Guttaflow Bioseal and Ceraseal) with mean changes in % of remaining bacterial cells from 1st day to 7th day



The results can be summarized as:

GROUP 1: AH Plus sealer showed intermediate inhibition of the bacteria on the 1st day compared to GROUP 2 : Guttaflow Bioseal sealer and GROUP 3 : Ceraseal sealer followed by a statistically significant decrease in efficacy till the 7thday . Intragroup comparison showed greatest antibacterial effect at 1st day, followed by a decrease in the effectiveness at 3rd day, 5th day and 7th day. The difference in the antibacterial effect was statistically significant on all the days.

GROUP 2 : Guttaflow Bioseal sealer showed the highest inhibition of the bacteria at 1st day compared to GROUP 1 : AH Plus sealer and GROUP 3 : Ceraseal sealer followed by a statistically significant decrease in efficacy till the 7thday . Intra group comparison depicted that the antibacterial efficacy was highest on 1st day followed by

a decline at 3 rd, 5th and 7th day . The difference in the antibacterial effect was statistically significant ($p < 0.05$) on all the days.

GROUP 3 : Ceraseal sealer showed the lowest antibacterial effect on the 1st day compared to GROUP 1 : AH Plus sealer and GROUP 2 : Guttaflow Bioseal sealer followed by a statistically significant decrease in the antibacterial efficacy on the 3rd and 5th day. Intragroup comparisons revealed a decrease in the antibacterial efficacy at 1st, 3rd ,5th and 7th day. The difference in the antibacterial effect was statistically significant ($p < 0.05$) on all the days except from 5th day to 7th day where the change was not significant ($p > 0.05$).

On comparing the groups, AH Plus sealer and Guttaflow Bioseal sealer, there was no statistical difference on the 1st day. Statistically significant difference (p value < 0.05) was seen at all the other time intervals tested i.e. 3rd day, 5th day and 7th day.

On comparing the groups, Guttaflow Bioseal sealer and Ceraseal sealer, there was a statistically significant difference (p value < 0.05) at all the time intervals tested i.e. 1st day, 3rd day, 5th day and 7th day.

On comparing the groups, Ceraseal sealer and AH Plus sealer, there was a statistically significant difference (p value < 0.05) on the 1st day, 5th day and 7th day. There was no statistically significant difference seen on the third day.

DISCUSSION

Endodontic infections are known to be caused primarily by bacteria and their byproducts. The incidence of anaerobes being found in root canals is high, particularly in cases where the infection has been present for a long time. Anaerobic bacteria may be particularly well adapted to live in the necrotic pulp and dentinal tubules, where blood and oxygen supply is limited or nonexistent.[11] Love et al. reported the presence of microorganisms in areas such as isthumuses, ramifications, deltas, irregularities and dentinal tubules even after thorough chemomechanical preparation of the root canal system Bystrom and Sjogren et al. hypothesised that if these microorganisms persist in the root canal at the time of root filling or if they penetrate into the canal after filling, there is a higher risk of treatment failure.[30,31]

In case of pulpal necrosis and apical periodontitis, using a sealer with high antibacterial activity aids in preventing or slowing the growth of any remaining microorganisms.[11] Endodontic sealers basically hold the cones of the filling material together and fill the space that the obturating material is unable to fill.[32]

An ideal endodontic sealer should seal properly, have a strong, long-lasting antibacterial activity and should have high biocompatibility and dimensional stability. The bactericidal activity of sealers may be able to eradicate any leftover pathogens and avoid recontamination in the event of coronal leakage, boosting the endodontic treatment's success rate.[12]

Enterococcus faecalis, a Gram-positive, facultative anaerobic microbe, was chosen for this study because it is the most common microorganism linked to post-treatment apical periodontitis and appears to be difficult to eradicate from the root

canal system due to several virulence factors found in *E. faecalis* that allow the organism to survive inside the root canal system even after root canal therapy. In the presence of human serum, it possesses the ability to penetrate dentinal tubules and bind to collagen.[33] Hence, from a therapeutic aspect, it is important to evaluate the antibacterial activity of endodontic sealers against this bacterium.[11]

Since it is a highly nutritive medium, Brain Heart Infusion medium is ideal for culturing a wide diversity of microorganisms. It is used to make inoculums used for antimicrobial susceptibility testing. It is also an ideal environment for anaerobic bacteria, yeasts, and moulds. This medium was chosen for this investigation because it is highly nutritious and well buffered, allowing a wide range of organisms, including E. faecalis, to flourish.[34]

One of the most commonly used procedures for determining antibacterial activity of dental materials was the Agar Diffusion Test (ADT). However, various restrictions should be highlighted, including inoculum density variability, insufficient culture media, agar viscosity, plate storage conditions, plate size and number of specimens per plate, and incubation duration and temperature. Other drawbacks of this method include its insensitivity and semiquantitative nature, as well as the fact that it does not distinguish between bacteriostatic and bactericidal capabilities of the compounds. Furthermore, the results of ADT are heavily influenced by the material's diffusibility, rather than only the material's toxicity for the specific microbe. Only water-soluble compounds can be tested as a result. Larger zones of inhibition are likely to result from a substance that diffuses more freely. As a result, in addition to direct cytotoxicity, the results may be influenced by the varied diffusion rates of the various sealers.[11]

Weiss as al.[14] and Fuss et al.[35] have previously investigated the Direct Contact Test (DCT), which has numerous advantages over the ADT. It is a quantitative assay that can be used to test the compounds that are not soluble in water. It is based on direct and close contact between the test microorganism and the tested material, and it is practically unaffected by the diffusibility of the tested material and the media. DCT offers a number of benefits, including repeatability, a quantitative assay, simultaneous testing of 50 samples, and continuous bacterial outgrowth measures with over 2400 readings per plate.[11] Hence, the DCT was chosen to assess the antimicrobial efficacy of the endodontic sealers used in this study.

The null hypothesis that there is no difference in the antimicrobial efficacy of the three endodontic sealers against *E. faecalis* evaluated using Modified Direct Contact Test was rejected.

Our results showed that the highest antibacterial effect was shown by Group 2: GuttaFlow Bioseal sealer followed by Group 1: AH-Plus sealer followed by Group 3: Ceraseal sealer.

AH Plus sealer was selected as a control because it is a widely used endodontic sealer used in endodontics.[4] AH Plus sealer showed intermediate inhibition of the bacteria on the 1st day compared to Group 2 : Guttaflow Bioseal sealer and Group 3 :Ceraseal sealer followed by a statistically significant decrease in efficacy till the 7thday . Intragroup comparison showed greatest antibacterial effect on the 1st day, followed by a decrease in the effectiveness on the 3rd day, 5th day and 7th day. The difference in the antibacterial effect was statistically significant on all the days. The bactericidal action of formaldehyde which is released during the setting process[4] or the toxicity of non-polymerized components, such as amines or epoxy

resins, could explain AH Plus's short-term antimicrobial potential. This fact has also been credited for explaining the lower antibacterial activity of AH Plus sealer in other studies.[4,11,13,16,22] and it is consistent with the results of the Modified Direct Contact test obtained in this study.

Guttaflow Bioseal is a silicone based sealer. It proved to be the best among the three sealers tested in this study. Guttaflow Bioseal sealer showed the highest inhibition of the bacteria on the 1st day compared to Group 1 : AH Plus sealer and Group 3 : Ceraseal sealer followed by a statistically significant decrease in efficacy till the 7thday . Intra group comparison depicted that the antibacterial efficacy was highest on the 1st day followed by a decline on 3 rd, 5th and 7th day . The difference in the antibacterial effect was statistically significant ($p < 0.05$) on all the days. The enhanced antibacterial efficacy of the sealer is attributed to the inclusion of calcium silicate particles, which provide an alkalizing activity by releasing calcium ions continuously after setting. The alkaline environment within the root canal has a pronounced bactericidal effect and can aid in the healing process by raising the pH of the periapical region, which aids in the activation of alkaline phosphatase leading to the formation of hard tissue.[4]

Ceraseal showed the least antibacterial efficacy when compared to Group 1 (AH Plus) and Group 2 (Guttaflow Bioseal). There was a statistically significant decrease in the antibacterial efficacy on the 3rd and 5th day. Intragroup comparisons revealed a decrease in the antibacterial efficacy at 1st, 3rd ,5th and 7th day. The difference in the antibacterial effect was statistically significant ($p < 0.05$) on all the days except from 5th day to 7th day where the change was not significant ($p > 0.05$). A calcium silicate-based bioceramic sealer consists of calcium phosphate, calcium

silicate cement, and calcium oxide. Calcium silicates undergo a hydration reaction when they come into contact with dentinal moisture, resulting in the creation of calcium silicate hydrogel and calcium hydroxide. This calcium hydroxide interacts partially with calcium phosphate to produce hydroxyapatite and water. As a result of the water generated, the cycle is restarted, resulting in the production of more calcium silicate hydrogel and calcium hydroxide, as well as an increase in pH (> 12.5). By the time the sealer hardens, its pH decreases to around 9.14, reducing its antibacterial effectiveness. These sealers are also hydrophilic, and their antibacterial effect is thought to be a result of a combination of their high pH, active calcium hydroxide diffusion, and hydrophilicity. Their antibacterial effect however, is considerably decreased after 7 days of mixing.[15]

Our results are in accordance with Ruiz-Linarez et al. and Chakravorthy et al., who demonstrated superior antibacterial efficacy of Guttaflow Bioseal against E. faecalis when compared to AH Plus.[4,17]

Furthermore, a study has demonstrated higher antibacterial efficacy of AH-Plus compared to Ceraseal.[19] This was consistent with the results of our study as well.

GuttaFlow Bioseal sealer seems to be a promising material in root canal therapy, given its capacity to kill E. faecalis and inhibit the formation of biofilms, as well as its adequate physicochemical and biological qualities.[4] However, further research is needed to determine how long the sealer's antibacterial and antibiofilm action lasts and how its qualities change over time based on the oral cavity environment of each patient.

CONCLUSION

The following conclusions were drawn from the present study:

- All the three sealers had different inhibitory effects on *Enterococcus faecalis* during the incubation period.

- All the test materials exhibited antibacterial activity against *E. faecalis*, but to a varying degree. Guttaflow Bioseal was the most effective as compared to AH Plus and Ceraseal at all the time intervals tested ie. at 1^{st}, 3^{rd}, 5^{th} and 7^{th} day. The antibacterial efficacy decreased over time till the 7^{th} day.

- The antibacterial activity of the tested endodontic sealers against *E. faecalis* in an ascending order is as follows: Ceraseal, AH Plus and Guttaflow Bioseal

SUMMARY

The study was conducted in the Department of Conservative Dentistry and Endodontics, , KLE Academy of Higher Education & Research (KLE University), KLE VK Institute of Dental Sciences, Belagavi (Karnataka)

The Modified Direct Contact Test was carried out in KLE's Dr Prabhakar Kore Basic Sciences Research Centre, KLE University, Belagavi.

Endodontic infections are polymicrobial in nature, with obligatory anaerobic bacteria dominating the microbiota in primary infections. In cases of unsuccessful endodontic therapy, *Enterococcus faecalis* and yeast, primarily *Candida albicans*, have been frequently identified as the species most commonly recovered from root canals undergoing retreatment. It is difficult to eradicate *E. faecalis* from the root canal using chemomechanical preparation techniques and antibacterial dressings alone. A majority of the root canal obturating materials on the market now do not provide a long-lasting flawless seal with the root canal wall. Microleakage is still a clinical issue and a possible cause of endodontic therapy failure. 1 As a result, using a biocompatible root canal sealer that not only hermetically seals the root canal but also has long-term antibacterial and antibiofilm qualities would aid in reducing residual infection and creating an environment that discourages bacterial colonisation.[4]

Thus, present investigation was undertaken to evaluate and compare the antimicrobial efficacy of three endodontic sealers- Epoxy resin-based sealer (AH-Plus), Silicone based sealer (GuttaFlow Bioseal) and Calcium silicate-based sealer (Ceraseal) against *Enterococcus faecalis* using DCT.

The facultative strain of *E. faecalis* (MTCC 439) was grown aerobically from frozen stock cultures in Brain Heart Infusion broth at 37°C. Microorganisms were then subcultured in BHI broth under laminar air flow to ensure their purity.[1] The suspension was then adjusted to 0.5 McFarland scale= 1.5 x 10^8 CFU/ml.[29]

Endodontic sealers were mixed or dispensed on to a mixing pad according to the manufacturer's instructions.

The 96- well microtiter plate was held vertically, i.e., the plate's surface was maintained perpendicular to the floor, and half of the side wall of the 22 wells (per sealer) **(n=22)** was coated using a cavity liner applicator.

GROUP 1: AH Plus

GROUP 2: Guttaflow Bioseal

GROUP 3: Ceraseal

8 hours later, corresponding to the recommended setting time of sealers, a 100 µl of bacterial suspension was placed on the test material. The plate was then held in a vertical position and the walls were then inspected for evaporation which occurred within 1 hour at 37 °C. This ensures direct contact between bacteria and test material.

Then, keeping the plate horizontal, BHI broth (200µl) was added to each of these wells and gently mixed. Following this, the plate was incubated at 37 °C.[11]

Optical Density i.e. the absorbance readings were obtained by analyzing the microtiter plates with a spectrophotometer on the 1^{st}, 3^{rd}, 5^{th} and 7^{th} days at 630nm.

The percentage of remaining bacterial cells were calculated using the following formula

$$\%f \text{ bacterial cells} = \frac{\text{Optical density of the positive control - Optical density of the test x100}}{\text{Optical Density of the positive control}}$$

The results obtained indicated that there is significant difference between all the three groups (p value <0.05). Among all the three groups, GROUP 2: Guttaflow Bioseal sealer showed the highest inhibition of the bacteria at 1st day compared to GROUP 1 : AH Plus sealer and GROUP 3 : Ceraseal sealer. There was a significant decrease in antibacterial efficacy from 1st day to 7th day for all the sealers tested except for GROUP 3: Ceraseal sealer where a significant decrease in antibacterial efficacy was seen from 1st day to 5th day. However, the decrease in efficacy was not significant from 5th day to 7th day.

Therefore, the 'null hypothesis' that there is no difference in the antimicrobial efficacy of AH Plus sealer, Guttaflow Bioseal sealer and Ceraseal sealer against *E. faecalis* evaluated using Direct Contact Test was rejected.

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ANNEXURE – I

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CERTIFICATE

This is to Certify that the synopsis titled

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF THREE ENDODONTIC SEALERS AGAINST ENTEROCOCCUS FAECALIS BY MODIFIED DIRECT CONTACT TEST: IN VITRO STUDY *Submitted by*

Dr. ABHIJIT SAJO SEBASTIAN *P. G. Student / Staff, Guided by* DR. SUNITA SHIVANAND *from Department of* CONSERVATIVE DENTISTRY & ENDODONTICS *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

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This is to certify that the Biostatistics aspect of the Dissertation / Research work of **Dr Abhijit Sajo Sebastian, Post Graduate Student**, under the guidance of **Dr. Sunita Shivanand** M.D.S, **Reader, Department of Conservative Dentistry and Endodontics**, entitled **"Comparative Evaluation of Antimicrobial Efficacy of Three Endodontic Sealers against *Enterococcus Faecalis* using Modified Direct Contact Test: An invitro study"** has been done under my guidance and considered satisfactory.

Place: Belagavi
Date: 14/12/2021

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ANNEXURE – III

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PLAGIARISM CHECK REPORT

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The soft copy of Research Work / Manuscript by DR. ABHIJIT SAJO SEBASTIAN entitled "COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF THREE ENDODONTIC SEALERS AGAINST ENTEROCOCCUS FAECALIS USING DIRECT CONTACT TEST: AN INVITRO STUDY"

under the guidance of DR. SUNITA SHIVANAND has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

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