Comparative Evaluation of Anti Bacterial Activity of Four Endodontic Sealers on Enterococcus Faecalis - An in Vitro Study

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Abstract

Aim and Objectives: To evaluate the antibacterial activity of four endodontic sealers on Enterococcus faecalis - an in vitro study.

Materials and Methods: Enterococcus faecalis was used as a test organism and a direct contact test was performed. The sealers to be tested were grouped as Group I- Zinc oxide eugenol based sealer (Tubliseal EWT), Group II- Calcium hydroxide based sealer (Acroseal), Group III- Epoxy resin based sealer (AH Plus), Group IV- Polydimethyl Siloxane based sealer (Roekoseal) and Group V- Control (Absence of sealer).

The antibacterial activity of the sealers were tested under three different conditions. Samples were used within 20 min. after mixing (designated as fresh samples),Samples were prepared and allowed to set for 24 hrs and 7 days in a humid atmosphere at 37^0C temp. For each set of samples, the reading were taken at duration of 2,5,20 and 60 mins. after placement of bacterial inoculum. For the test, the sealers were mixed and placed on the side wall of microtitre plate wells. Direct contact test was performed.

Results: Data was collected by recording the colony forming unit/ml with digital colony counter. The results obtained were subjected to statistical analysis by one way ANOVA test. Group comparison showed significant difference between the groups. Inter group comparison between the groups using Tukey Post Hoc Test showed that in comparison to Group V (Control group), the Group I (Tubliseal EWT) in fresh and 24 hrs samples showed significant difference, Group II (Acroseal) and Group III (AH Plus) showed a significant difference in fresh samples. and IV (Roekoseal) did not show any

significant difference with control group.

Conclusion: The sealers evaluated in this study showed different inhibitory effects. Zinc oxide Eugenol based sealer (Tubliseal EWT) was the most effective and Polydimethyl Siloxane based sealer (Roekoseal) was the least effective against Enterococcus faecalis, where as calcium hydroxide based sealer (Acroseal) is effective only in fresh sample and epoxy resin based sealer (AH Plus) was effective only for a short period. Inhibition of the bacterial growth is related to the direct contact of the microorganism with the sealer. Hence the incorporation of antimicrobial components into root canal sealers may become an essential factor in preventing the regrowth of residual bacteria and control of bacterial re-entry into the root canal space.

Keywords: antibacterial properties, colony forming unit, direct contact test, endodontic sealers, enterococcus faecalis.

INTRODUCTION

The main objective of endodontic therapy is to eliminate bacteria from the infected root canal and to prevent root canal infection.[1] The majority of the bacteria found in the root canal system may be eliminated by the biomechanical cleaning and shaping of the root canal space. However, microorganisms might still survive these challenges due to the anatomical complexities of many root canals, such as dentinal tubules, ramifications, deltas and fins which cannot be sufficiently cleaned, even after meticulous mechanical procedures. Enterococcus faecalis is a recalcitrant candidate among the causative agents of failed endodontic treatment. According to Sundquist et al. 38% of the failed root canal systems were contaminated with Enterococcus faecalis.[2]

E. faecalis is not favored by the conditions in the untreated canal, and when present, they are a small percentage of the initial flora in the root canal. However, once they enter the canal system and become established, they can resist antimicrobial treatment, including interim medications, and will persist after obturation.[3]

Antibacterial activity of sealer to entomb and kill the surviving microorganisms and to attain a fluid tight seal by serving as filler for canal irregularities and minor discrepancies between the root canal and core materials, thus preventing re-entry and colonization of bacteria. The use of sealers with antibacterial properties may be advantageous especially in clinical situations of persistent or recurrent infection.The endodontic sealers have been shown to give the greatest antimicrobial effects immediately after spatulation, following which there is a gradual loss of antimicrobial effects over time.[4]

Many studies have been performed to assess the anti microbial efficacy of different root canal sealers. The agar diffusion test was the most commonly used technique but had many limitations as it was dependent on diffusion and physical properties of tested materials. With the introduction of direct contact test by Weiss et al. anti bacterial activity of the endodontic sealers is tested based on measuring the effect of close contact between test bacteria and tested material on the kinetics of bacterial growth. Moreover it is a quantitative assay which allows insoluble materials to be tested.[5]

The Direct Contact Test has been used to evaluate the in vitro antibacterial activities of numerous endodontic sealers. In the present in vitro study antimicrobial activity of four endodontic sealers Zinc oxide eugenol based sealer (TubliSeal EWT), calcium hydroxide based sealer (Acroseal), Epoxy resin based sealer (AH Plus), Polydimethyl siloxane based (Roekoseal) is assessed and compared by direct contact test on Enterococcus faecalis.

Aim & objective

The aim of this in vitro study is to evaluate the antibacterial activity of various endodontic sealers on Enterococcus faecalis.

The objective of this study is to evaluate and compare the antibacterial activity of four endodontic sealers (Tubliseal EWT, Acroseal, AH Plus and Roekoseal) on Enterococcus faecalis by a direct contact test over a period of time.

Methodology

A comparative, in vitro study to evaluate the antibacterial activity of four endodontic sealers on Enterococcus faecalis by a direct contact test was undertaken in the Department of Microbiology, Dr. B. Lal's Institute of Biotechnology, Jaipur, in association with the Department of Conservative Dentistry and Endodontics, Government Dental College, Jaipur.

STUDY MATERIALS

Endodontic Sealers

1. Zinc Oxide Eugenol based sealer (Tubliseal EWT-SybronEndo, Glendora, CA)
2. Calcium hydroxide based sealer (Acroseal-Septodont, France)
3. Epoxy resin based sealer (AH Plus- Dentsply DeTrey GmbH, Konstanz, Germany)
4. Polydimethyl Siloxane based Sealer (Roekoseal-Coltene Whaledent)

Test microorganism

Enterococcus faecalis employed for testing antimicrobial activity of endodontic materials was obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh. (MTCC No - 439).

METHODOLOGY

Growth of microorganism

E. faecalis (MTCC No. 439) obtain from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh were grown aerobically from frozen stock cultures in Tryptone Soya Broth (TSB) at 37^0C. Inoculum was prepared by the resuspension of cells to predetermined optical densities related to known concentration of 0.5 Mac Farland Standard.

Grouping of the specimen:

Endodontic Sealers were divided into 5 groups.

GROUP	SPECIMEN
GROUP I	Zin oxide eugenol based (Tubliseal EWT)
GROUP II	Calcium hydroxide based (Acroseal)
GROUP III	Epoxy-diamine based (AH Plus)
GROUP IV	Polydimethyl siloxane based (Roekoseal)
GROUP V	The growth of the micro organism in the absence of the sealer. (Negative control)

The sealers were prepared in strict compliance with the manufacturers' recommendation and they were subjected to Direct Contact Test.

The antibacterial activities of the sealer were tested under three different conditions. Samples were used within 20 min. after mixing (designated as fresh samples), Samples were prepared and allowed to set for 24 hrs in a humid atmosphere at 37^0C temp, Samples were prepared and allowed to set for 7 days in a humid atmosphere at 37^0C temp. For each set of samples, the readings were taken at duration of 2,5,20 and 60 mins. after placement of bacterial inoculum.

The 96 wells of a microtitre plate, 24 wells were utilized per sealer of each 6 were designated for 2,5,20 and 60 min. respectively. The wells were held vertically, i.e., the plate's surface was maintained perpendicular to the floor plane and the side wall were coated with freshly mixed tested material. Even and thin coating was achieved by using a small size round ended dental instrument.

After 20 min, a 10 μL bacterial suspension (10^8 bacteria) was placed on the test material. The plate was held in a vertical position and wells were inspected for evaporation of the suspension's liquid, which occurred within 1 hr at 37°C. This ensured direct contact between bacteria and tested material. Tryptone Soya Broth (TSB) 300 μL was added to each of these wells and gently mixed for 2 min.

The kinetics of the outgrowth in each well is monitored by- Measurement of colony forming unit/ml.

Measurement of colony forming unit/ml.

100 µL of broth was then transferred from well to an eppendrof containing 900 µL of TSB. 100 µl of the suspension from each eppendrofs was placed on TSA Petri plate. Petri plates were incubated at 37°C for 24 hrs. Colony forming units were counted by the digital colony counted and CFU/ml was calculated.

Colony forming unit (CFU) and calculating the CFU/ml-

CFU is used to determine the number of viable bacterial cells in a sample per ml. For each dilution, count the number of colony forming units. Typically numbers between 30 and 300 are considered to be in the range where one's data is statistically accurate.

CFU count with the help of Digital Colony Counter. Digital Colony Counters count bacterial and mold colonies in Petri dishes. This equipment have ensures quick and accurate measurements; and register the count on the digital display screen.

Calculating the number of bacteria per mL of serially diluted bacteria:

To calculate the number of bacteria per ml of diluted sample was:

$$\frac{\text{Number of CFU}}{\text{Volume plated (mL) x total dilution used}} \rightarrow \frac{\text{Number of CFU}}{\text{mL}}$$

Data were recorded, then plotted and statistically analyzed using One Way ANOVA and Tukey Post Hoc Test. The whole experiment was carried out under aseptic conditions and was repeated six times to ensure reproducibility.

DISCUSSION

It has been known for more than a century that bacteria colonize the root canal. The role of these bacteria and their by-products in the initiation and perpetuation of pulp and periapical diseases has been well established [6].

The golden rule in the practice of Endodontology is to debride and obturate the canals as efficiently and three dimensionally as possible and to prevent subsequent reinfection. Cleaning and shaping procedure, followed by the three-dimensional obturation of the root canal space, are common procedures used to achieve this goal. However, studies by Lin et al. and Siqueira et al. have demonstrated that part of the root canal space often remains untouched during chemomechanical preparation regardless of the technique and instruments employed.[7] Love, Molander et al., Sundquist et al. reported the presence of microorganisms in areas such as isthmuses, ramifications, deltas, irregularities and dentinal tubules even after thorough chemomechanical preparation of the root canal system [8,9] and it has also been postulated by Bystrom and Sjogren et al. 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 that the treatment will fail [10].

To survive in the obturated canal, microorganisms must withstand intracanal disinfecting measures and adapt to an environment in which there are few available nutrients. Therefore failure of endodontic treatment attributed to persistent microorganisms will only occur if they possess pathogenicity, reach sufficient numbers, and gain access to the periradicular tissues to induce or maintain periradicular disease.

The microbiota associated with failed cases differs markedly from untreated teeth (Primary root canal infection). In untreated canals, it is a polymicrobial infection of gram –ve and gram +ve bacteria and dominated by obligate anaerobes. Whereas failed cases (previously filled teeth) have a monoinfection of predominantly gram +ve micro organisms with equal proportions of facultative and obligate anaerobes and the most common isolated microorganism being Enterococcus faecalis. It has been recovered in 30–70% of canals of root filled teeth with persistent periapical lesions. Enterococcus

faecalis is highly resistant to intracanal dressings and is known to resist the antibacterial effect of calcium hydroxide[8,9].

Enterococcus faecalis is a gram positive, group D streptococci and a facultative anaerobe and is a micro-organism that can survive extreme challenges. Enterococci can grow at 10°C and 45°C, at pH 9.6, in 6.5% NaCl broth, and survive at 60°C for 30 minutes and in where nutrient are scarce. E. faecalis can adapt to adverse conditions, can enter the viable but non-cultivable (VBNC) state, a survival mechanism adopted by a group of bacteria when exposed to environmental stress, and resuscitate upon returning to favorable conditions and can invade the dentinal tubules.[11]

The aim of this study was to evaluate the antibacterial activity of four endodontic sealers on Enterococcus faecalis by a direct contact assay for Freshly mixed, after 24 hrs and after 7 days of incubation period . These included a recently introduced RoekoSeal (Polydimethyl siloxane) and AH Plus (Epoxy resin), Acroseal (Calcium hydroxide based) and TubliSeal EWT(Zinc oxide eugenol based).

Endodontic literature reports the need to seal the root canal in a hermetic way. Leonardo and Leal affirmed that to seal a root canal means to fill it in all its extension with an inert, antiseptic material, obtaining the most hermetic seal possible. The endodontic sealers enhance the possible attainment of an impervious seal by serving as filler for root canal irregularities and minor discrepancies between the root canal and the core material. Most important requirements of sealers according to Grossman are biocompatibility, excellent seal, adequate adhesion and antimicrobial property [12].

Rappaport et al. stressed on the fact that “The ideal root canal cement should be bactericidal”. The need for an endodontic sealer with strong antimicrobial properties is questionable, especially since the antimicrobial effect of the various sealers is non-specific and can cause periapical tissue destruction. Al-Khatib demonstrated the need that it would be preferable to use a sealer that has relatively mild antibiotic activity and low toxicity [13].

ZnOE is utilized as a standard sealer and has a long time clinical record. Although dentin adhesive sealers are superior in ease of manipulation, radio opacity, setting time, excellent adaptation to canal walls and also strengthen roots compared to those roots which have been obturated with zinc oxide eugenol sealer, the anti bacterial activity of the newly introduced Polydimethyl siloxane based sealers (RoekoSeal) is questioned. Hence it is important to compare this newer generation sealers with the other sealers such as AH Plus (Epoxy resin), Acroseal (Calcium hydroxide based) and TubliSeal EWT (Zinc oxide eugenol based) sealers.

The endodontic sealers have shown to give the greatest antimicrobial effects immediately after spatulation, following which there is a gradual loss of antimicrobial effect over time. Because of various transitory and permanent products, it is essential to test the materials immediately after mixing.[14,15] In the present study, antibacterial activity of freshly mixed, 24 hr and 7 day sealers were examined at 2,5,20 and 60 min. after placement of bacterial inoculum.

The Direct Contact Test (DCT) has many advantages over Agar Diffusion Test (ADT).[4,5,14] It is a quantitative assay which allows water insoluble materials to be tested. It relies on direct and close contact between the test microorganism and the tested material and is virtually independent of the diffusion properties of both the tested material and the media. In addition to its reproducible and quantitative nature, the results of DCT unlike those of the agar diffusion test (ADT), were not affected by the size of the inoculum and were relatively insensitive to the size of the inoculum brought in contact with the tested material. It facilitates standardized measurements of a large number of specimens and their respective control simultaneously on the same microtitre plate and has the ability to monitor the bacterial growth, both in the

presence and in the absence of the tested materials. The present study has shown that direct contact test is an appropriate method of testing antimicrobial activity.[5,14]

Observations from this study showed that Tubliseal EWT showed maximum antibacterial activity in fresh and 24 hrs samples and no activity in 7 day samples. Acroseal showed antibacterial activity only in fresh samples after than activity reduced with time and no activity after 7 days. AH Plus showed only slight activity only in fresh samples after than no activity which is comparable to control group. Roekoseal did not showed any antibacterial activity, which showed no significant difference with the control group and significant difference with fresh & 24 hrs Tubliseal EWT and fresh Acroseal. This variation in the antibacterial activity of each tested sealer with time interval is in accordance with earlier studies.[4,5,14] This may be attributed to the diffusion of antimicrobial components present in these sealer.

The present investigation showed Zinc oxide eugenol based sealer to have a maximum anti bacterial activity and complete inhibition of bacterial growth in fresh and 24 hrs samples and no activity after 7 days of incubation period, which is in accordance with the previous syudies. [15,16]

It has been established that eugenol is a potent antibacterial agent and is conceivable that it plays a major role within the activity of ZnOE based sealers.[15] ZnO has no antibacterial activity. Furthermore, if the ZnOE contacts wet tissue, the eugenol concentration increases. However, it can inhibit white cell chemotaxis, synthesis of prostaglandins and nerve activity. Several biochemical mechanisms have been proposed to explain the cytotoxicity of eugenol and its utilization in restorations to prevent bacterial penetration but at high concentrations, eugenol can also create an undesired cytotoxicity effect [5].

Calcium hydroxide was introduced to endodontics by Herman in 1920 for its pulp-repairing ability. In endodontics, it is mainly used for pulp- capping procedures, as an intracanal medicament, in some apexification techniques,and as a component of several root canal sealers. The two most important reasons for using calcium hydroxide as a root-filling material are stimulation of the periapical tissues in order to maintain health or promote healing and secondly for its antimicrobial effects. The exact mechanisms are unknown, but the following mechanisms of actions have been proposed:

1. Calcium hydroxide is antibacterial depending on the availability of free hydroxyl ions. It has a very high pH (hydroxyl group) that encourages repair and active calcification. There is an initial degenerative response in the immediate vicinity followed rapidly by a mineralization and ossification response.
2. The alkaline pH of calcium hydroxide neutralizes lactic acid from osteoclasts and prevents dissolution of mineralized components of teeth. This pH also activates alkaline phosphatase that plays an important role in hard tissue formation.
3. Calcium hydroxide denatures proteins found in the root canal and makes them less toxic.
4. Calcium hydroxide activates the calcium-dependent adenosine triphosphatase reaction associated with hard tissue formation.
5. Calcium hydroxide diffuses through dentinal tubules and may communicate with the periodontal ligament space to arrest external root resorption and accelerate healing.

In the present study, Acroseal (calcium hydroxide based sealer) demonstrated complete inhibition of bacterial of bacterial growth in fresh samples which is similar to Tubliseal EWT and there after loses its antibacterial property and shows slight activity after 24 hrs and no activity after 7 days. Pinheiro et al. reported zone of inhibition of E. faecalis by Acroseal sealer is similar to the Zinc oxide eugenol. Eldeniz et al. evaluated the pH and calcium ion release of three calcium hydroxide based sealers (Acroseal, Apexit and Sealapex)as well as time required to kill microorganism (E. faecalis), they concluded Acroseal

sealer presented less calcium ion release and pH than Apexit and Sealapex, and less lasting to kill the microorganism.[17]

In the present study, AH Plus (Epoxy resin based sealer) demonstrated slight antibacterial activity only in fresh samples there after no activity found in 24 hrs. and 7 day samples. Pizzo et al. reported that in DCT only fresh AH Plus possessed antibacterial activity, whereas 24- hrs and 7-day old samples did not show antibacterial effect against E. faecalis. Similar results were reported by Kayaoglu et al. The present study also showed that fresh AH plus had slight antibacterial effect, whereas set samples did not show antimicrobial activity. Gomes et al. e also showed the polymerization reaction in AH Plus result in antibacterial reaction for a short duration.[18] AH Plus a resin-based sealer, did not show any zone of inhibition. This could be because of the lack of release of formaldehyde.[3] Miyagak et al also demonstrated that AH Plus did not show any antibacterial activity against E. faecalis. AH 26 is also resin based sealer had a potent antibacterial property due to the presence of formaldehyde and of AH Plus was due to the presence of Bis-phenol diglycidyl ether. AH Plus lacked formaldehyde and had a lesser antibacterial activity when compared to AH 26.[5,14]

In present study Roekoseal, is a recently introduced Polydimethyl siloxane based sealer, showed no antibacterial activity in all samples at all the time intervals. There was no significant difference with the control group. In earlier study, showed absolutely no antimicrobial activity at all duration. In study by Cobankara et al. no antibacterial activity by ADT but shows some antibacterial activity by DCT.[15]

In this study control group showed the increased optcal density and CFU/ml at different time interval (2,5,20 and 60 min.) because of bacterial multiplication in every 26 min. and both the methods of determination of bacterial growth (by measurement of optical density and CFU/ml) showed similar results.

The sealers evaluated in this study showed different inhibitory effects which may be related to their different composition. Over all ZnOE based sealers and calcium hydoxide based sealers proved to be effective against the microorganisms.

Thus, the incorporation of antimicrobial components into root canal sealers may become an essential factor in preventing the re growth of residual bacteria and control of bacterial re-entry into the root canal space and may also be of benefit in the treatment of persistent or recurrent infections. Additional studies in vitro and in vivo, however, are needed to evaluate the antimicrobial effects within dentinal tubules and biocompatibility of these sealers.

CONCLUSION

Anti microbial activity of root canal sealers may help to eliminate residual microorganisms unaffected by the effects of both chemo mechanical preparation and intra canal medication and in controlling infection.

Within the limitations of the study following conclusions were drawn:

1. Inhibition of the bacterial growth is related to the direct contact of the microorganism with the sealer
2. The anti bacterial activity of tested endodontic sealers on Enterococcus faecalis in an ascending order is as follows: Roekoseal, AH Plus, Acroseal and Tubliseal EWT sealer.
3. Study showed that antibacterial activity of all endodontic sealers were reduced with the time interval. As the sealers set it loses its antibacterial property.
4. Group I, Tubliseal EWT (Zinc oxide Eugenol based sealer) showed antibacterial activity upto the 24 hrs & no activity after 7 day.
5. Group II, Calcium hydroxide based sealer (Acroseal) had an initial antibacterial activity on fresh sample, which reduced with time after 24 hrs and no activity after 7 days.
6. Group III, Epoxy resin based sealer (AH Plus) showed slight antibacterial activity only in fresh

sample and there after was almost similar to the control group (Group V).

7. Group IV, Polydimethyl Siloxane based Sealer (Roekoseal) had no antimicrobial property and was almost similar to the control group.

Additional studies in vitro and in vivo, however, are needed to evaluate the antimicrobial effects within dentinal tubules and biocompatibility of these sealers.

Summary

Antibacterial activity of endodontic sealers can improve the success rate of endodontic treatment provided the physical properties are not compromised. The dentin adhesive sealers are superior in ease of manipulation, radio opacity, setting time, and excellent adaptation to canal walls, but the anti bacterial activity of the Epoxy resin based sealer and Polydimethyl siloxane based sealers is questioned. An in-vitro experimental study was formulated to evaluate the antibacterial activity of four endodontic sealers on Enterococcus faecalis by a Direct Contact Test.

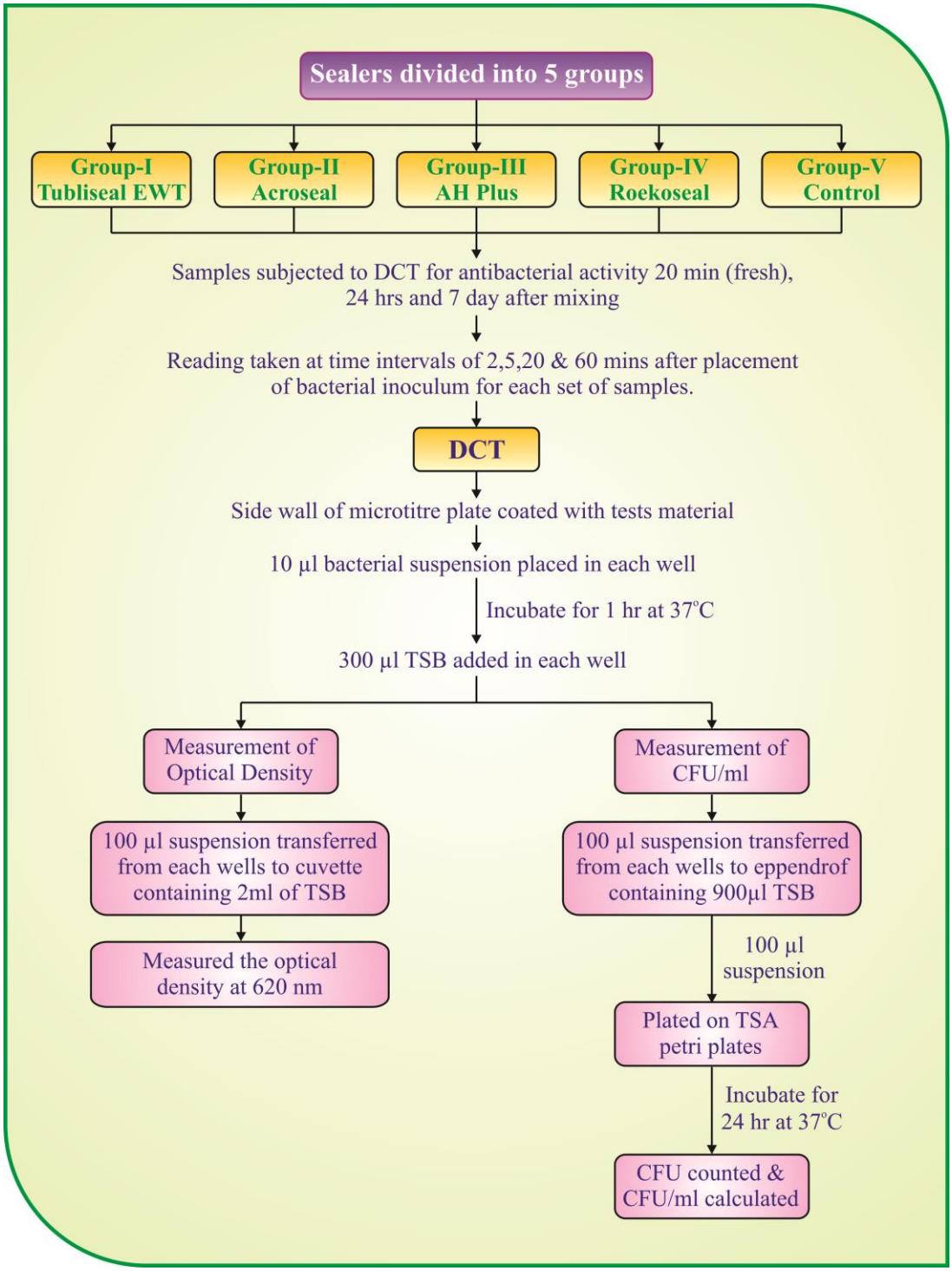
The study materials grouped and selected were Group I - Zinc Oxide Eugenol based sealer (Tubliseal EWT), Group II- Calcium hydroxide based sealer (Acroseal), Group III - Epoxy resin based sealer (AH Plus), Group IV- Polydimethyl Siloxane based Sealer (Roekoseal) and Group V (Control-absence of sealer). The sealers were mixed in strict compliance with the manufacturers' recommendations.

The direct contact test performed was based on turbidometric determination of bacterial growth in 96-well microtiter plates. The kinetics of the outgrowth in each well is monitored at 620 nm at 37^0C by the spectrophotometer and by plating on tryptone soya agar plate (TSA) and measuring the CFU/ml.

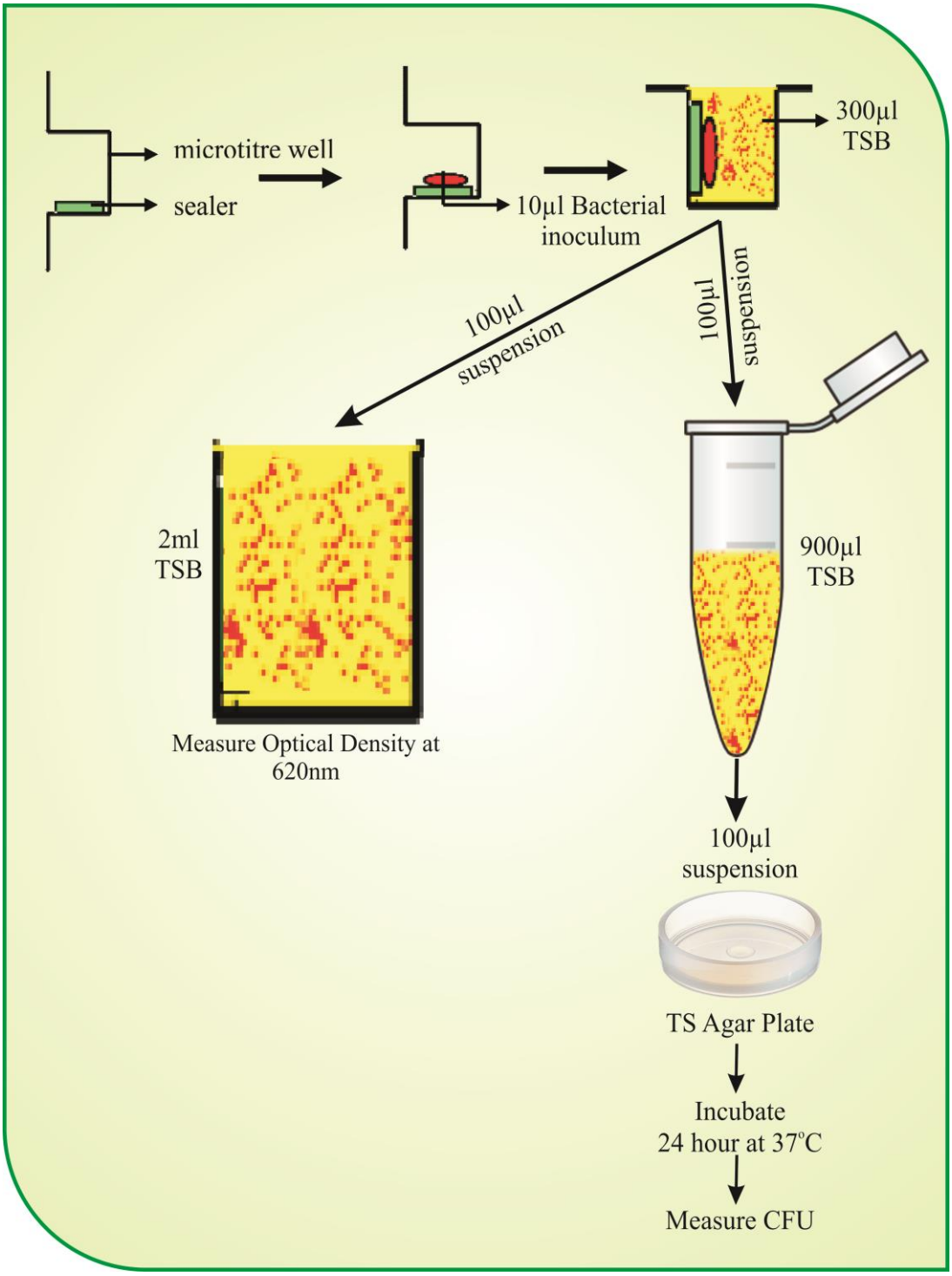
The results obtained were subjected to statistical analysis by one way ANOVA and Tukey Post Hoc Test. Zinc oxide Eugenol based sealer (Tubliseal EWT) was the most effective and Polydimethyl siloxane based sealer (Roekoseal) which showed similar growth as control was the least effective against Enterococcus faecalis. Fresh sample of Calcium hydroxide based sealers (Acroseal) showed significant anti bacterial property and slowly reduced with time. Epoxy resin based sealer (AH Plus) shows slight antibacterial activity only in fresh sample and there after no activity thereafter. Polydimethyl siloxane based sealer (Roekoseal) did not show antibacterial effect . Its antibacterial activity was similar to the control group.

I

NVESTIGATION DESIGN



DIAGRAMATIC VIEW OF DCT



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