AN EXVIVO COMPARATIVE STUDY TO EVALUATE THE EFFICACY OF THREE ROOT CANAL IRRIGATION SYSTEMS IN REDUCTION OF *ENTEROCOCCUS FAECALIS*

Dissertation submitted to

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CERTIFICATE

This is to certify that this dissertation titled **"AN EXVIVO COMPARATIVE STUDY TO EVALUATE THE EFFICACY OF THREE ROOT CANAL IRRIGATION SYSTEMS IN REDUCTION OF ENTEROCOCCUS FAECALIS"** is a bonafide record of work done by **DR. DIANA STANLY** under our guidance during her postgraduate study period between 2009-2012.

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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CONTENTS

LIST OF TABLES

LIST OF GRAPHS

INTRODUCTION

The main goal of root canal treatment in both vital and necrotic teeth is either prevention or elimination of a microbial infection in root canal system. Endodontic long-term success is not due to a single factor but relates to three aspects of treatment, which is called as 'endodontic triad'. This is composed of instrumentation, disinfection and obturation. These three components of the triad are interwoven.[57] Instrumentation alone does not prepare the canal system for obturation and disinfection is key to augmenting the process and optimizing the obturation.

Disinfection comprises removal of the residual tissue in the canal system and the associated bacteria through flushing the canal system with irrigating solution.[57] However the intricacies of the canal anatomy with its fins, lateral canals and apical deltas make it impossible for the instrumentation of the canals to reach all of the fine aspects of the anatomy. The key is to remove as much residual tissue as possible and the more thorough the irrigation process, lower the remaining bacterial level. Irrigation of the canal system permits removal of residual tissue in the canal anatomy that cannot be reached by instrumentation of the main canals.

It has been demonstrated that bacteria and their products play an essential role in the development and perpetuation of pulpal and periradicular diseases [35]. Although the root canal flora is dominated by obligate anaerobic bacteria, some facultative strains, e.g. *Enterococcus faecalis*, have been involved in persistent infections, influencing the prognosis of the root canal treatment[45]. *E. faecalis* is probably the only species that can adapt to and tolerate the ecologically demanding conditions in the filled root canal. It has the ability to penetrate dentinal tubules, sometimes to a deep extent, which also enable them to escape from the action of instruments and substances used during treatment.[27] Hence Eradication of *E. faecalis* from the root canal with the chemo-mechanical preparation and using disinfecting irrigants and antibacterial dressing is difficult[38].

Various irrigating solutions have been used during and immediately after root canal preparation to remove debris, necrotic pulp tissue and to eliminate microorganisms that cannot be reached by mechanical instrumentation. Ideal root canal irrigants should have requirements such as a broad antimicrobial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms, dissolve necrotic pulp tissue remnants, inactivate endotoxin, prevent the

formation of a smear layer during instrumentation or dissolve the latter once it has formed.[21]

Numerous irrigants have been recommended for use in the treatment of root canal infections. Sodium hypochlorite (NaOCl) has been widely used as an irrigant since its introduction in endodontics by Walker in 1936. In addition to bleaching, deodorizing and tissue-dissolving properties, NaOCl has been demonstrated to be an effective disinfectant agent.[45] NaOCl is effective against *E. faecalis* both in buffered and unbuffered solutions. However, there is no one unique irrigant that can meet all these requirements, even with the use of methods such as lowering the pH, increasing the temperature, as well as addition of surfactants to increase the wetting efficacy of the irrigant. Thus contemporary endodontics, dual irrigations such as sodium hypochlorite with Ethylenediamine tetraacetic acid (EDTA) or chlorhexidine (CHX) are often used as initial and final rinses to complement the shortcoming that are associated with the use of a single irrigant.[33]

More importantly, these irrigants must be brought into direct contact with the entire canal wall surfaces for effective action, particularly for the apical portions of small root canals. Throughout the history of endodontics, endeavors have continuously been made to

develop more effective irrigant delivery and agitation systems for root canal irrigation. These systems might be divided into two broad categories, ie manual agitation techniques and machine-assisted agitation devices.[21]

In conventional needle irrigation, replenishment and exchange of irrigant in the apical third and the effectiveness of chemical debridement are dependent on the depth of penetration. Boutsioukis et al[5] showed in a computational fluid dynamic model that the exchange of irrigant only occurs 1–1.5 mm past a side-vented needle, and the irrigant beyond that point remains stagnant. Chow et al [13] found that the exchange of irrigant does not extend much beyond the tip of the irrigating needle. Vapor lock that results in trapped air in the apical third of root canals might also hinder the exchange of irrigants and affect the debridement efficacy of irrigants.[50]

Machine - assisted agitation devices such as Endovac creates negative pressure by placing a suction needle (cannula) into the root canal. It is designed to deliver irrigating solution to the apical end of the canal system and into the root canal irregularities and suck out debris. Recent in vitro studies have demonstrated that the Endovac system can provide better cleaning at the most apical part of the prepared canal,

presents reduced risk of apical extrusion of irrigants, and promotes a better intracanal disinfection than conventional irrigation.[21]

The purpose of this in vitro study was to compare the effectiveness of three irrigation systems: the Endovac system, Max I probe and Navitip FX in reduction of *Enterococcus faecalis* population.

The objectives of this study were:

1. To evaluate the effectiveness of the negative pressure technique and positive pressure technique in the reduction of *E. faecalis* population from the root canal.
2. To compare the antimicrobial efficacy of new irrigation systems in reduction the *E. faecalis* population from the root canal.

REVIEW OF LITERATURE

Chow et al in (1983)[13] evaluated the effectiveness of root canal irrigation using hypodermic needle & syringe and he stated that there was little flushing and displacement of particles much beyond the tip of the needle. He concluded that the clinical extent of effectiveness of irrigation is a function of the depth of insertion of the needle and small bore needles were more effective than larger ones.

Bystrom et al (1983)[10] conducted an invivo study to find the antibacterial effect of 0.5% sodium hypochlorite solution as root canal irrigant was studied in fifteen single-rooted teeth. Each tooth was treated at five appointments, and the presence of bacteria in the root canal was studied on each occasion. No antibacterial intracanal dressings were used between the appointments. When 0.5 percent hypochlorite was used no bacteria could be recovered from twelve of fifteen root canals at the fifth appointment.These results suggest that 0.5 percent sodium hypochlorite solution is more effective than saline solution as a root canal irrigant.

Bystrom et al (1985)[11] evaluated the antibacterial effect of irrigating infected root canals with 0.5 and 5 per cent sodium hypochlorite solutions clinically. The results indicated that there was no

difference between the antibacterial effect of these two solutions. The combined use of EDTA and 5% sodium hypochiorite solution was more efficient than the use of sodium hypochiorite solutions alone. An important observation was that bacteria surviving instrumentation and irrigation rapidly increased in number in the period between appointments when no intracanal medicament was used.

Kahn et al (1995)[26] evaluated the efficacy of a variety of endodontic irrigating devices. His study utilized plastic blocks with artificial canals to simulate the clinical setting. The canals were instrumented, and red food dye was introduced into each canal. The blocks were placed in a jig to simulate maxillary and mandibular arch orientation. Irrigation was performed with: (a) B-D 22-gauge needle; (b) Monoject Endodontic Needle 23 and 27 gauge; (c) Max-i-Probe 25-, 28,and 30-gauge probes; (d) Cavi-Endo ultrasonic handpiece; and (e) Micromega 1500 subsonic handpiece. He concluded that the Max-i-Probe probes were the most effective instrument used to clear dye from the simulated canals in both the mandibular and maxillary positions.

Siqueira et al (1997)[45] evaluated the effectiveness of 4.0% sodium hypochlorite (NaOCl) used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal was tested

invitro. And he concluded that there were no statistically significant differences between the experimental groups. However, NaOCl applied by the three methods tested, was significantly more effective than the saline solution (control group) in disinfecting the root canal.

Siqueira et al (1999)[42] evaluated in vitro reduction of the bacterial population in the root canal by the mechanical action of instrumentation and irrigation. Root canals inoculated with a *Enterococcus faecalis* suspension were instrumented using hand NiTi flex files, Greater Taper (GT) files, and Profile 0.06 taper Series 29 rotary instruments. Irrigation was performed using sterile saline solution. It was concluded that the instrumentation and irrigation can mechanically remove more than 90% of bacterial cells from the root canal.

Siqueira et al (2000)[43] evaluated the in vitro intracanal bacterial reduction produced by instrumentation and irrigation with 1%, 2.5% and 5.25% sodium hypochlorite (NaOCl) or saline solution .The three NaOCl concentrations showed large zones of inhibition against *E. faecalis*. He concluded that regular exchange and the use of large amounts of irrigant should maintain the antibacterial effectiveness of the NaOCl solution, compensating for the effects of concentration.

Gomes et al (2001)[19] assessed in vitro, the effectiveness of several concentrations of NaOCl (0.5%, 1%, 2.5%, 4% and 5.25%) and two forms of Chlorhexidine gluconate (gel and liquid) in three concentrations (0.2%, 1% and 2%) in the elimination of *E. faecalis*. He concluded that even though all tested irrigants possessed antibacterial activity, the time required to eliminate *E. faecalis* depended on the concentration and type of irrigant used.

Love et al (2001)[27] evaluated a study to identify a possible mechanism that would explain how *E. faecalis* could survive and grow within dentinal tubules and reinfect an obturated root canal. He concluded that the virulence factor of *E. faecalis* in failed endodontically treated teeth may be related to the ability of *E. faecalis* cells to maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum.

Calt et al (2002)[12] evaluated the effects of EDTA on smear layer removal and on the structure of dentin, after 1 and 10 min of application. He concluded that 1 min EDTA irrigation is effective in removing the smear layer. However a 10-min application of EDTA caused excessive peritubular and intertubular dentinal erosion. Therefore they suggested that this procedure should not be prolonged >1 min during endodontic treatment**.**

Niu et al (2002)[33] examined dentinal erosion caused by final irrigation with EDTA and NaOCl. When the root canal was irrigated with 15% EDTA alone, the dentine had a smooth and plane appearance, and dentinal tubule orifices were regular and separated. When the root canal was irrigated with EDTA followed by NaOCl the dentine was eroded and the dentinal tubule orifices were irregular and rough. However, more debris was removed by irrigation with EDTA followed by NaOCl than with EDTA alone .He concluded that final irrigation with 6% NaOCl accelerates dentinal erosion following treatment with 15% EDTA.

Bardford et al (2002)[6] He observed apical pressures from different needles inserted deeply into small round and ovoid canals as instrumentation progressed. Low-pressure (5 psi) air was injected through the needles, and apical pressures were recorded after each instrument. Pressures varied greatly within each test group. Generalities that can be drawn are that binding the needle within the canal gives higher pressures than with the needle slightly short of binding and that pressures were higher with apexes instrumented to size 30 and higher. With the needle tightly bound, neither needle size, needle design, nor canal shape resulted in statistically significant mean pressure differences. With the needle slightly withdrawn, larger bore needles

gave higher pressures than small diameter needles. Caution is advised with the clinical use of pressurized air in the drying of root canals

Usman et al (2004)[51] compared in an in situ model the efficacy of root canal debridement in the apical 3 mm when instrumenting to a GT size 20 or a GT size 40 at working length and he concluded that the apical third cleanliness could be predicted mainly by instrument size and to a lesser extent by the canal length. Irrigant volume, number of instrument changes, and depth of penetration of irrigation needle were not likely to explain differences in debridement.

Veltri et al (2004)[52] studied the abilities of ProTaper and GT Rotary files to shape the curved canals of extracted mandibular molars. He concluded that dentin removal was same for the files but working time was shorter for ProTaper files.

Berutti et al (2004)[4] evaluated the influence of manual preflaring and torque on the failure rate of rotary nickel-titanium ProTaper instruments Shaping 1 (S1), Shaping 2 (S2), Finishing 1 (F1), and Finishing 2 (F2). These factors were evaluated using an in vitro method by calculating the mean number of Endo-Training-Blocks shaped before file breakage under different conditions. He concluded that manual preflaring creates a glide path for the instrument tip and is a major

determinant in reducing the failure rate of these rotary nickel-titanium files. All instruments worked better at high torque.

Fukumoto et al (2004)[17] evaluated the effectiveness of a new root canal irrigation technique with intracanal aspiration in removing the smear layer and to assess irrigant extrusion ex vivo. He concluded that irrigation using the intracanal aspiration technique allowed more effective removal of the smear layer than that performed by the conventional method in an apically resected canine tooth. The intracanal aspiration technique produced limited extrusion of the irrigant beyond the apical foramen.

Sedgley et al (2005)[41] evaluated the mechanical efficacy of irrigation in reducing bacteria in the root canal which is dependent on depth of placement of the irrigation needle. He concluded that the mechanical efficacy of 6 ml of irrigant in reducing intracanal bacteria was significantly greater when delivered 1 mm compared with 5 mm from working length.

Dunavant et al (2006)[15] compared the efficacy of root canal irrigants against *E. faecalis* biofilms using a novel in vitro testing system. Biofilms grown in a flow cell system were submerged in test irrigants for either 1 or 5 minutes. He proved that both 1% NaOCl and

6% NaOCl were more efficient in eliminating *E. faecalis* biofilm than the other solutions tested.

Al-Hadlaq et al (2006)[1] conducted in an invitro study to evaluate the cleaning efficacy of new brush-covered irrigation needle, the NaviTip FX and concluded that using the NaviTip FX produced cleaner coronal thirds of instrumented root canals compared to the control group. On the other hand, the middle and apical thirds were not statistically significantly different between the two groups.

Beber et al (2006)[2] evaluated the efficacy of 0.5%, 2.5% and 5.25% sodium hypochlorite (NaOCl) as intracanal irrigants associated with hand and rotary instrumentation techniques against *Enterococcus faecalis* within root canals and dentinal tubules and he concluded that higher concentrations, NaOCl, was able to disinfect the dentinal tubules, independent of the canal preparation technique used.

Bulacio et al (2006)[9] evaluated the minimum inhibitory concentration (MIC) and the antibacterial effect (AE) of 2.5% NaOCl, 0.2% chlorhexidine gluconate (CHX) and 17% EDTA on *Enterococcus faecalis.* The antibacterial capacity was assessed by diffusion in agar.He concluded that with NaOCl 2.5%: *Enterococcus faecalis* was totally inhibited for 24 hours in the apical area and for 8 hours in the middle area. CHX 0.2% elicited a reduction of more than 5 log CFU and EDTA

17% induced a reduction of more than 3 log CFU at all the time points examined in the apical and middle areas.

Vinothkumar et al (2007)[54] tested the mechanical efficacy of various irrigating needle tip designs on bacteria inoculated into instrumented root canals. He concluded that irrigation using safety needles with single side port was significantly effective.

Nielsen et al (2007)[32] compared the efficacy of the Endovac irrigation system and needle irrigation to debride root canals at 1 and 3 mm from working length. One tooth of each matched pair was instrumented and irrigated by using the Endovac and the other tooth of the matched pair was instrumented and irrigated with a 30-gauge ProRinse irrigating needle. All teeth were irrigated with NaOCl and EDTA for a predetermined amount of time, and total volume of irrigant used was recorded. After instrumentation and irrigation, the teeth were fixed, decalcified, and sectioned at 1 mm and 3 mm from working length. The amount of remaining debris was determined as percentage of the area of the canal lumen. This study concluded that there was better debridement at 1 mm from working length by using the Endovac compared with needle irrigation.

Hockett et al (2008)[24] determined whether irrigation with apical negative pressure was more effective than traditional positive-pressure

irrigation in eradicating *Enterococcus faecalis* from preshaped root canals. He concluded that apical negative-pressure irrigation has the potential to achieve better microbial control than traditional irrigation delivery systems.

Estrela et al (2008)[16] evaluated efficacy of the sodium hypochlorite (NaOCl) and chlorhexidine (CHX) on *Enterococcus faecalis* was evaluated by systematic review and meta-analysis. He concluded NaOCl or CHX showed low ability to eliminate *E. faecalis* when evaluated by either PCR or culture techniques.

Goel et al (2009)[18] compared the effect of continuous, intermittent passive ultrasonic irrigation (PUI) and active scrubbing of irrigants with NaviTip FX (Ultradent, South Jordan, UT) in removing smear layer and he concluded that NaviTip FX and intermittent PUI showed significantly lower smear score than other groups at the 3 mm level. Both brush and intermittent ultrasonic activation were effective in the removal of smear layer from the apical third.

Zmener et al (2009)[56] evaluated the effectiveness of the NaviTipFX, a 30-gauge brush-covered irrigation needle, in removing debris and smear layer and concluded that in moderately curved root canals, a NaviTip FX used with 5.25% NaOCL and 17% EDTA solution with manual brushing was the most effective cleaning.

Desai et al (2009)[14] designed a study to evaluate the safety of various intracanal irrigation systems by measuring the apical extrusion of irrigant. It was designed to test worst case apical extrusion and was conducted using neutral atmospheric pressure and an open apex. The irrigation systems used were EndoVac Micro and Macro Cannula, Endo Activator, manual irrigation with Max-I-Probe needle, Ultrasonic Needle Irrigation, and Rinsendo. The results showed that Endovac Micro and Macro cannula groups did not extrude irrigant, and there was no statistically significant difference between these two groups and the Endo Activator group. Within the groups that produced extrusion, Endo Activator extruded statistically significantly less irrigant than Manual, Ultrasonic, and Rinsendo groups. There was no statistically significant difference among Manual, Ultrasonic, and Rinsendo groups. This study concluded that the Endovac did not extrude irrigant after deep intracanal delivery and suctioning the irrigant from the chamber to full working length.

Brito et al (2009)[7] compared the intracanal bacterial reduction promoted by chemo-mechanical preparation with 3 different irrigation techniques (Navitip needle Endo Activator, Endovac). The reduction in the bacterial populations was highly significant for all groups. The 3 experimental groups with NaOCl and EDTA as irrigants were

significantly more effective than the control group with saline in reducing CFU counts. There were no significant differences between the 3 techniques tested. He concluded that there was no evident antibacterial superiority of any of the irrigation techniques evaluated in the present in vitro model.

Parente et al (2010)[36] examined canal debridement efficacy by testing the null hypothesis that there is no difference between a 'Closed' and an 'Open' system design in smear layer and debris removal using either manual dynamic agitation or the Endovac for irrigant delivery. He concluded that Endovac is effective method to overcome the fluid dynamics challenges inherent in closed canal system.

Miller et al (2010)[29] compared the antimicrobial efficacy of root canal irrigation with the Endovac to endodontic needle irrigation in the apical 5 mm of root canals infected with *Enterococcus faecalis*. He concluded that although there were fewer cfu/mg when using the Endovac, there was not a statistically significant difference between the Endovac and needle groups.

Tay et al (2010)[50] compared effect of vapor lock on root canal debridement by using a side-vented needle for positive-pressure irrigant delivery and concluded that presence of an apical vapor lock effect adversely affects debridement efficacy.

Shin et al (2010)[48] evaluated the efficacy of Endovac system in comparison with that of a conventional needle irrigation method when the root canals were enlarged to various sizes and concluded that Endovac left significantly less debris behind than the conventional needle irrigation methods.

Mitchell et al (2010)[30] He conducted a study to compare extrusion of irrigants delivered with a 27-G needle or the Endovac system during instrumentation and final irrigation of teeth. He used a different method to determine the apical extrusion .Teeth were secured embedded in 0.2% agarose gel (ph =7.3-7.4) containing 1 mL 0.1% m-cresol purple, which changes color at a pH of 9.0. Teeth received NaOCl and EDTA irrigation with the 27-G slot needle or the Endovac system. The amount of irrigation was controlled for each sample. Photographs were taken and analyzed by using Adobe Photoshop to determine the amount of extrusion .The results revealed that 50% extrusion N40 with (6/12), 8.33% extrusion E40 with (1/12), 58.33% N60 with (7/12), and 8.33% E60 with (1/12). The overall extrusion frequency, regardless of apical preparation size, Endovac showed 8.33%. This study showed significantly less extrusion risk using the Endovac system compared with needle irrigation.

Boutsioukis et al (2010)[5] evaluated the effect of needle-insertion depth on the irrigant flow inside a prepared root canal during final irrigation with a syringe and two different needle types using a Computational Fluid Dynamics (CFD) model.He concluded that needle insertion depth was found to affect the extent of irrigant replacement, the shear stress on the canal wall, and the pressure at the apical foramen for both needle types.

Siu et al (2010)[46] compared the debridement efficacy of Endovac irrigation versus conventional needle irrigation in vivo. Seven adult patients with a total of 22 matched pairs of single-canaled vital teeth with fullyformed apices were recruited. Canals were instrumentedto a master apical file size #40/.04 taper and he concluded Endovac irrigation resulted in significantly less debris at 1 mm from WL compared with conventional needle irrigation. There was no significant difference at the 3-mm level.

Heilborn et al (2010)[23] did a histologic study to compare the Endovac system at two different exposure times to the traditional positive-pressure irrigation technique for root canal cleaning efficacy and to measure the volume of irrigation at the apical third and concluded that the apical negative-pressure irrigation system Endovac has the potential to achieve significantly better root canal cleaning at the apical

third of root canals and in less exposure time than required with traditional positive-pressure irrigation.

Ozdemir et al(2010)[34v]evaluated the effects of Ethylenediamine tetraaceticacid (EDTA) and sodium hypochlorite (NaOCl) on *Enterococcus faecalis* biofilm growth in root canal dentin of young and old individuals and concluded that combination of EDTA and NaOCl significantly reduced the amount of intracanal biofilm in both age groups (P< .01). However, the bacterial counts of *E. faecalis* in the old group were still higher (P < .05).He suggested that root canals from elderly population are more susceptible to canal infection. However, combined application of EDTA and NaOCl significantly reduces the amount of intracanal biofilm.

Brunson et al (2010)[8] determined the effect that apical preparation size and preparation taper had on the volume of irrigant delivered to the working length of a root canal preparation in a clinically relevant amount of time. He concluded that an increase in apical preparation size and taper resulted in a statistically significant increase in the volume of irrigant. In addition, an apical enlargement to ISO #40 with a 0.04 taper will allow for tooth structure preservation and maximum volume of irrigation at the apical third when using the apical negative pressure irrigation system

Gondim et al (2010)[20] compared the postoperative level of pain after root canal therapy using either endodontic needle irrigation or a negative apical pressure device in vivo and concluded that use of a negative apical pressure irrigation device can result in a significant reduction of postoperative pain levels in comparison to conventional needle irrigation.

Vijaykumar et al (2010)[53] compared the reduction of *E. faecalis* counts in root canals produced by irrigation with distilled water,hydrogen peroxide, sodium hypochlorite, chlorhexidine, and combinations of solutions, in vitro. Reduction of colony counts in distilled water group was significantly lower than the mean reduction in all the other groups. However, no other contrasts are statistically significant. Combination of sodium hypochlorite and chlorhexidine showed the most effective antimicrobial activity followed by sodium hypochlorite and hydrogen peroxide together. Hydrogen peroxide was the least effective irrigant when used alone.

Retamoza et al (2010)[39] investigated the concentration of sodium hypochlorite and the irrigation time required to disinfect dentin cylinders infected with *Enterococcus faecalis* and concluded that High concentration and long exposure to NaOCl are needed for elimination of *E. faecalis* contaminated dentin.

Susin et al (2010)[49] compared canal and isthmus debris debridement efficacies of the manual dynamic irrigation (MDI) and apical negative pressure (ANP) techniques in the mesial root of mandibular first molars with narrow isthmi, using a closed canal design and he concluded that neither technique completely removed debris from the isthmus regions. However, the Endovac system, which encompasses the ANP concept, removed considerably more debris from narrow isthmi in mandibular mesial roots.

Paragliola et al (2010)[35] examined the effect of different root canal irrigant agitation protocols in the penetration of an endodontic irrigant into dentinal tubules and concluded that the use of an ultrasonic agitation to increase the effectiveness of the final rinse procedure in the apical third of the canal walls.

MATERIALS

- Fifty five intact human maxillary anterior teeth
- Normal saline
- Sodium hypochlorite 3 % (PRIMA DENTAL)
- 17% EDTA (PULP DENTAL)
- 20 ml syringe
- Irrigation devices : 1. NaviTip FX (Ultradent)
  2. Max I probe (Dentsply)
  3. Endovac (Discus Dental)
  4. Syringe needle
- Fifty five 20 ml test tubes
- 1-ml syringe
- Self cure acrylic resin (DPI)
- *Enterococcus faecalis* (ATCC 29212)
- Brain –Heart infusion agar
- Trypticase soy broth
- Fifty five disposable peptic plates
- Type II GIC (FUJI)
- Hand Gloves
- Face mask

ARMAMENTARIUM

- Airoter hand piece (NSK)
- #2 round bur (Mani)
- K files 10-25 (Mani)
- Rotary protaper files (Dentsply)
- Anthogyr (SybroEndo)
- Ultrasonic scaler (Satelec)
- Incubator
- Vortex machine

METHODOLOGY

Specimen Preparation

Fifty five extracted intact human permanent maxillary anteriors were selected for this study .The external surface of the tooth were debrided ultrasonic scaler tips. Conventional access cavities were done by using # 2 round burs. Patency with an #10 stainless steel K-file was achieved, and the working length was set at 1 mm back from the total root length.The working length was standardized to 20mm. Teeth exceeding 20mm in length were adjusted to 20 mm by incisal reduction. To standardize the apical constriction size, root canals were instrumented at the apical foramen up to a K-type file #25 in reaming action, under irrigation with saline. Apical foramen were sealed with Type II GIC. Then teeth were mounted vertically up to the cervical region in blocks made of a self cure resin. This makes handling and identification of the samples easier. The blocks containing the teeth were sterilized in autoclave for 20 minutes at 121^0c.

Contamination of the specimen

A suspension was prepared by adding 1 mL of a pure culture of *E. faecalis* (ATCC 29212), grown in trypticase soy broth (TSB) for 24

hours, to 5 mL of fresh TSB. Each root canal was filled with .1 ml of *E. faecalis* suspension by using sterile 1-ml syringes. Sterile K-type files #15 were used to carry the bacterial suspension to the entire root canal length. Blocks were then placed inside a rectangular surgical tray and incubated at 37^{o} C for 7 days in 100% humidity.

Testing Procedures

After 7 days of experimental contamination, teeth were randomly divided into 3 experimental groups of 15 teeth each according to the irrigation technique used and a control group consisting of 10 teeth. Groups were as follows: In group 1, root canals were irrigated by using brush covered 30-gauge NaviTip FX (Ultradent, South Jordan, UT). In Group 2, root canals were irrigated by using 30-gauge Max-i-Probe (Dentsply-Rinn, Elgin, IL). In group 3, root canals were irrigated by using the Endovac system (Discus Dental, Culver city, CA). All the experimental groups were irrigated with 3% NaOCl (sodium Hypochlorite) and 17% EDTA. Positive control group canals were irrigated with 27-gauge syringe, with saline solution as the irrigant with 20 ml syringe to deliver irrigation solution for all the groups.

Group 1 and 2

In Group 1 after each instrument used, the canal was irrigated with 2 mL of NaOCl by using a 30-gauge NaviTip FX . In group II after

each instrument used, the canal was irrigated with 2 mL of NaOCl by using a 30-gauge Max-i-probe. For both the groups the needle was placed up to 2 mm short of the WL and while irrigating, the needle was moved in up and down motion to allow easy back flow of the irrigating solution and also to prevent the extrusion of the solution. After the F4 instrument was used, irrigation was with 3% NaOCl for 30 sec. The solution was left undisturbed in the canal for 60 seconds and then a final irrigation procedure was performed as follows: the canal was rinsed with 3% NaOCl for 30 sec, followed by 17% EDTA 30 sec, and again with 3% NaOCl for 30 sec.

Group 3

The canal and pulp chamber were kept full of irrigant throughout the procedures. After each instrument used, the canal was irrigated with NaOCl by using the **master delivery tip**. Specifically after apical preparation with the ProTaper F4 instrument, **macroirrigation** with 3% NaOCl was accomplished during a 30-second period while the irrigant was delivered coronally by the master delivery tip. For this step, the macrocannula was constantly moved up and down in the canal from a point just below the canal orifice to 4 mm short of the WL. NaOCl was then left undisturbed in the canal for 60 seconds. In sequence, 3 cycles of **microirrigation** were accomplished. During each cycle, the pulp

chamber was maintained full of irrigant, while the microcannula was placed at WL for 6 seconds. In sequence, the microcannula was positioned 2 mm from the WL for 6 seconds and then moved back to WL for 6 seconds. This up-down motion continued for 30 seconds, allowing 18 seconds of active irrigation directly at WL. After 30 seconds of irrigation, the microcannula was withdrawn from the canal in the presence of sufficient irrigant in the pulp chamber to ensure that the canal remained totally filled with irrigant and that no air was drawn into the canal space. This completed 1 microirrigation cycle. The first cycle used 3% NaOCl as the irrigant, the second cycle used 17% EDTA, and the third cycle used 3% NaOCl once again. At the end of the third cycle, the microcannula was left at WL to remove excess irrigant. The Endovac irrigation protocol was as per manufactures recommendation.

Positive control

In this group, instrumentation was performed as for Group 1. Irrigation was conducted with 27-gauge needle with 20ml syringe and saline was used as the irrigant.

Sampling Procedures

Before sample taking, the root canal was flushed with 1 ml of 10% sodium thiosulfate to neutralize the NaOCl. Each canal was then rinsed with saline, and a Hedstrom instrument #40 was used to file

vigorously the dentinal walls. Afterwards, the canal contents were aspirated with a 1-ml plastic syringe and then placed into tubes containing 1 ml of sterile saline. Two paper points #40 were also placed at the WL and also used to soak up the canal contents. Paper points were transferred to the same tubes containing 1 ml of saline. After agitation in vortex, 10-fold serial dilutions in saline, aliquots of 0.1 ml were plated onto Brain heart infusion agar plate (Difco) and incubated at 37^{o}C for 48 hours. The colony-forming units (CFUs) grown were counted and then transformed into actual counts based on the known dilution factors. The volumes of both sodium thiosulfate and saline before were all included in the total volume calculation for each group.

Materials and methodology

55 extracted intact human maxillary anterior teeth with single root and canal were collected, cleaned and stored in physiological saline at room temperature

↓

Conventional access cavities prepared using # 2 round burs. Working length was standardize to 20mmby incsial reduction

↓

Roots canals were enlarged till master apical file # 25 using K-file with saline irrigation.

↓

Apical Foramen were sealed with a type II GIC and mounted vertically up to the cervical region in self-cure resin blocks.

↓

A bacterial suspension was prepared by adding 1 ml of a pure culture of *E. faecalis* to 5 ml of fresh trypticase soy broth.

↓

Prepared specimen teeth were autoclaved for 20 minutes at 121^{o}c.

↓

Each root canal was inoculated with 0.1ml of *E. faecalis* suspension and teeth blocks were kept in metallic box and incubated at 37^{o}c for 7 days

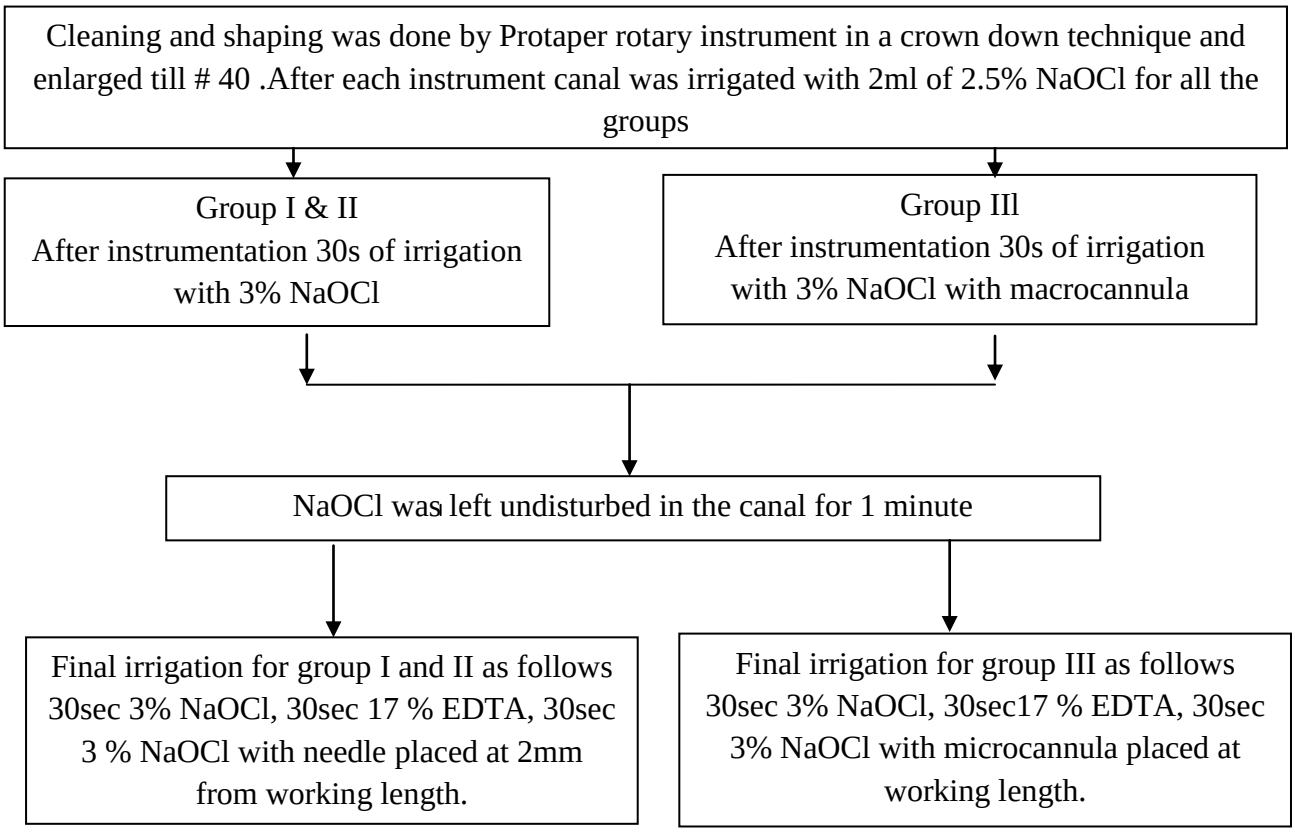
↓

After 7 days of experimental contamination teeth were randomly divided into 3 groups and a control group and irrigated with following irrigation protocol

↓

Group I(n=15)	Group II (n=15)	Group III (n=15)	Group IV (n=10)
30-gauge NaviTip FX	30-gauge Max-i-Probe	Endovac system	27-gauge needle with saline

Irrigation protocol



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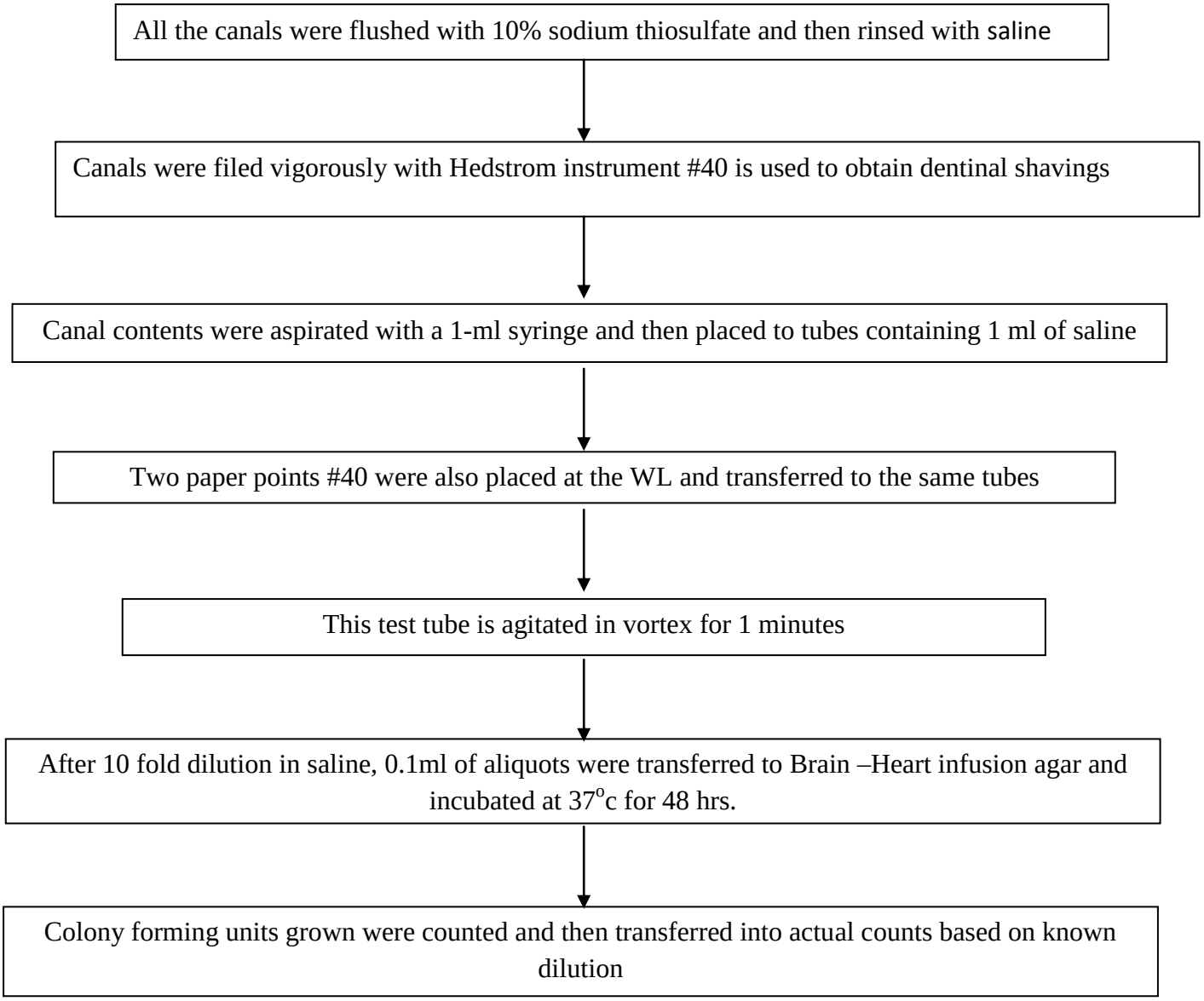
Group IV (Control group)

Instrumentation and irrigation was performed as similar to Groups I but irrigation was conducted with 27-gauge needle and saline was used as the irrigant.

Sampling procedure



RESULTS

The results of the present study were subjected to statistical analysis to interpret the significant differences among various irrigation systems. One way ANOVA, Post hoc Tukey tests were used for statistical analysis in the present study.

One way analysis of variance (ANOVA) is used to study the overall variance within groups. It is the extension of the between groups t-test to the situation in which more than two groups are compared simultaneously. However, it is not possible to identify the difference between the various groups with the help of the P values obtained from ANOVA. Therefore a specific statistical test was used for intra-group comparison. Hence, the Post hoc Tukey is done in order to determine which groups differ from each other. The Post hoc Tukey Test Honestly significant difference or HSD test is a Post hoc test designed to perform a pair wise comparison of the means to identify the specific groups in which significant difference expression occurs.

Unpaired t-test is applied to unpaired data of independent observation made on individuals of two different or separate groups or samples drawn from two populations.

In this study One way ANOVA followed by Tukey HSD test showed statistically significant difference among Experimental and control groups concerning *E faecalis* reduction in each group.

Table 1: demonstrates the mean and standard deviation values of for all the groups.

Table 2: demonstrates the Post hoc Tukey comparison between the groups.

To summarize the result:

- Mean rank score for *E.faecalis* reduction was highest in control group. (Fig 29)
- Mean rank score for *E.faecalis* reduction was lowest in Endovac group. (Fig 28)
- Experimental groups showed statistically significant difference when compared with control group.
- Among the experimental groups, Group III (Endovac group) showed statistically significant difference in reduction of bacteria.
- When group I & group II compared, there was no statistical differences between them in reduction of *E.faecalis.*

DISCUSSION

Apical periodontitis is an infectious disease caused by microorganisms colonizing the root canal system.[31] The endodontic treatment of teeth containing irreversibly inflamed pulp is essentially a prophylactic treatment because the radicular vital pulp is usually free of infection. The rationale is to treat the tooth in order to prevent further infection of the root canal system and the subsequent emergence of apical periodontitis. On the other hand, in cases of infected necrotic pulps or in root canal–treated teeth associated with apical periodontitis, an intraradicular infection is established. As a consequence, endodontic procedures should focus not only on prevention of the entry of new microorganisms into the root canal system but also on the elimination of those already located therein.[44]

The microbes grow in sessile biofilms, aggregates, coaggregate and also as planktonic cells suspended in the fluid phase of the canal. A biofilm is a community of microorganisms embedded in an Exopolysaccharide (EPS) matrix that adheres onto a moist surface whereas planktonic organisms are free-floating single microbial cells in an aqueous environment.[31] Four mechanisms that confer antimicrobial tolerance to cells living in a biofilm have been elucidated. The first is

the barrier properties of the EPS matrix. Extracellular enzymes such as β-lactamase may become trapped and concentrated in the matrix, thereby inactivating β-lactam antibiotics. The second mechanism involves the physiological state of biofilm microorganisms. Bacterial cells residing within a biofilm grow more slowly than planktonic cells; as a result, biofilm cells take up antimicrobial agents more slowly. The third suggested mechanism is that microorganisms within the biofilm experience metabolic heterogeneity. Microorganisms protected in biofilms are greater than one thousand times more resistant to biocides as the same organisms in planktonic form. There is consensus that apical periodontitis persisting after root canal treatment presents a more complex aetiological and therapeutic situation than apical periodontitis affecting teeth that have not undergone endodontic treatment.[15]

The influence of bacterial persistence in the root canals on treatment outcome is an important issue in endodontics, because bacteria have been shown to play a major role in persistence or emergence of apical periodontitis after root canal treatment. Indeed, studies have revealed that the outcome of the endodontic treatment is significantly influenced by the presence of bacteria in the root canals at the time of filling. This indicates that persisting bacteria can survive in treated canals and are able to induce or sustain periradicular tissue

inflammation, highlighting the concept that the eradication of bacteria from the root canal system should be the ultimate goal of the endodontic treatment of teeth with apical periodontitis. The predominant bacteria from the secondary infection include *Lactobacilli, Staphylococci, Enterococcus faecalis, and Propionibacterium.*[44]

Enterococcus faecalis is a facultative gram-positive anaerobe part of the human normal flora and an important pathogen in opportunistic infection in humans. It is a persistent organism that, despite making up a small proportion of the flora in untreated canals, plays a major role in the etiology of persistent periradicular lesions after root canal treatment. It is the most consistently reported organism from root canal failures, with a prevalence ranging from 22% to 77% of cases analysed. The organism is resistant to most of the intracanal medicaments, and can tolerate a pH up to 11.5, which may be one reason why this organism survives antimicrobial treatment with calciumhydroxide dressings. This resistance occurs probably by virtue of its ability to regulate internal pH with an efficient proton pump *E faecalis* can survive prolonged starvation and can grow as monoinfection in treated canals in the absence of synergistic support from other bacteria. Therefore, *E. faecalis* is regarded as being a very recalcitrant microbe among the potential aetiological agents of persistent apical periodontitis.[31] It has the ability

to penetrate dentinal tubules, sometimes to a deep extent, can also enable them to escape from the action of instruments and substances used during treatment.[27] For all the above reasons this bacteria was selected for this study.

Elimination of endodontic infection is quite different from most other sites in the human body. Host measures that are sufficient to eliminate the infectious organisms in other sites do not suffice for complete elimination of endodontic infections, mainly because of the special anatomy and physiology of the tooth and the root canal. Hence, infections of endodontic origin are treated mainly by means of mechanical procedures aided by chemical substances.[22]

Eradication of endodontic infection enhances the success rate of the endodontic therapy. During endodontic treatment, bacterial reduction or elimination may be achieved by chemo-mechanical preparation. Chemo-mechanical preparation usually include two procedures the mechanical cleaning by instruments and the use of irrigation solution.[13]Chemo-mechanical debridement and obturation effectively reduce the bacterial load in the root canal system and allow periapical healing in about 80% of cases even though the apical bacterial biofilm survives in 88%.[31]

The goal of mechanical instrumentation is to remove all necrotic and vital organic tissue as well as some hard tissue from the root canal system and give the canal system a shape that allows easy debridement, predictable placement of locally used medicaments and a permanent root filling of high technical quality. Microbiologically, the goal of instrumentation is to remove all microorganisms in the root canal system. Bystrom and Sundqvist[22] reported a 100–1000 fold reduction in bacterial load after instrumentation with stainless steel hand files and irrigation with physiological saline.

Multiple endodontic instruments have been designed for the various procedures performed within the pulp chamber and root canal system. Manual root canal instruments were first introduced in the early to mid-nineteenth century and remained the primary devices of root canal preparation up until the late 1980s. The Kerr Company created the K-type instruments in the early 1900s, which reside as the oldest useful instruments for cutting and machining dentin. Structural limitations of steel instruments led to a high incidence of procedural accidents, and manual instrumentation prevailed as the primary mode of root canal preparation for almost a century. However, rotary-instrumentation of the root canal system was repopularized in the early 1990s with the introduction of nickel-titanium endodontic instruments. The alloy

proved to be more flexible and resistant to torsional fracture than stainless steel, allowing for greater instrument control in small, curved canals. These favorable characteristics have led to the creation of countless file systems exhibiting various designs and shapes. A variety of instrumentation techniques have also been advocated and are largely dependent on the file system employed.[3]

The ProTaper rotary file system was chosen for this experimentation for several reasons. First, the ProTaper system is relative popular among general dentists and endodontists alike, mainly due to its simplicity and efficiency. In fact, Yun and Kim [40] showed that the ProTaper system created acceptable shapes in significantly less time than GT rotary, ProFile, and Quantec instruments. Also, in cross-section, the ProTaper file exhibits sharp, triangular cutting edges and absence of radial lands that greatly enhances cutting efficiency and flexibility. Jeon et al showed that instruments with more active blades tend to shear dentin during cutting, producing a thin superficial layer of smear compared with the thicker, deep-penetrating smear layer produced by U-shaped blades.[40] It has been claimed that the progressive taper sequence of shaping files in the Protaper range the enhances flexibility in the middle to apical portion whereas decreasing taper

sequence of the finishing files enhance of the files whilst making them rather stiffer.[3]

The quality of apical shaping and cleaning is supposed to be affected both by the diameter and the taper of the last instrument used. Brunson et al[8] showed that an increase in apical preparation size from #35 to #45 and an increase in preparation taper from 0.02 to 0.08 resulted in an increase of the volume of irrigant being delivered to the apical areas of the canal. Hence, in this present study the apical size was prepared with #40 Protraper which has taper 0.09 and seems to maintain a good balance of tooth structure preservation and adequate volume of irrigation at the apical third. Also this enlargement help in placing the microcannula at the working length.[8]

Despite technological advances in the ability to shape root canals, at least 35 per cent of root canal surfaces still remain uninstrumented and cleaning of the canal in terms of soft tissue removal and elimination of bacteria relies heavily on the adjunctive action of chemically active irrigating solutions due to the anatomic complexity of the pulp space. Instrumentation of the root canal system must always be supported by the use of antimicrobial irrigating solutions. Irrigation is also necessary to suspend and rinse away debris created during instrumentation, to act

as a lubricant for instruments and to remove the smear layer that forms on instrumented dentine surfaces.[55]

The use of an inactive or neutral irrigant such as saline or water will only result in manual flushing of freely movable debris and does not provide an efficient means of bacterial reduction in the canal. In studies using culturing techniques, the use of water or saline was shown to be the least effective in achieving a negative bacterial culture.[10] Many different types of irrigants with antibacterial effect are employed in the practice of endodontics with different indications and uses.

Sodium hypochlorite (NaOCl) is the most commonly used antimicrobial irrigant. Eventhough its antibacterial effects are recognized, the exact mechanism of microbial killing is not well elucidated. When NaOCl is added to water, hypochlorous acid (HOCl) is formed, which contains active chlorine, a strong oxidizing agent. Substantial evidence suggests that chlorine exerts its antibacterial effect by the irreversible oxidation of -SH groups of essential enzymes, thereby disrupting the metabolic functions of the bacterial cell. Chlorine may also combine with cytoplasmic components to form N-chlorocompounds, which are toxic complexes which destroy the microorganism. However, the first contact oxidation reactions of chlorine with bacteria may lead to the rapid killing of bacterial cells

even prior to the formation of N-chlorocompounds in the cytoplasm.[45] It has been used in this study because of its well-known bactericidal action and to dissolve vital as well as necrotic tissue.

Clinical and laboratory studies have not demonstrated any significant differences in antibacterial effect between NaOCl concentrations ranging from 0.5% to 5.25% in the root canal wall.[2]

Marais and Williams found that 3.5% sodium hypochlorite was an effective antibacterial irrigation solution when tested on teeth contaminated with strict and facultative anaerobes such as *E. faecalis*. Their study found no colonies in samples taken immediately following irrigation or one week after sealing the non-medicated canals. Abdullah et al who compared the efficacy of 0,2% chlorhexidine gluconate, 17% EDTA and 3.0% NaOCl on E. *faecalis* biofilm. 3% NaOCl was the most effective agent and achieved 100% kills of *E. faecalis* after a two minutes contact time.[9] Ringel et al compared, in vivo, the effect of 2.5%NaOCl and 0.2% chlorhexidine gluconate on teeth with necrotic pulps and reported that the NaOCl solution was more effective.[9] So, 3% NaoCl was used in this study.

Materials that remain untouched or compacted into the root canal anatomy during instrumentation consist of both organic and inorganic components. In addition, the presence of biofilms in the uninstrumented

canal anatomy provides more material that can cause treatment failure. Any irrigants used for removal of these materials must address both these organic and inorganic components. Although sodium hypochlorite appears to be the most desirable single endodontic irrigant, it cannot dissolve inorganic component which comprised of dentinal debris which is formed during instrumentation.[28] This prevents the penetration of NaoCl in the dentinal tubules The removal of the inorganic component in the root canal is of primary importance because it allows penetration of the antimicrobial irrigants to areas of the dentin that may harbor bacteria.[44]

Ethylenediamine tetraacetic acid (EDTA) is a chelating agent that removes calcium ions to demineralise the inorganic component of dentine specifically at a concentration of 17%. In addition to their cleaning ability, EDTA has the property of reducing the hydrophobicity and surface free energy of root dentin and thereby influences the nature of bacterial adhesion, adhesion forces and biofilm formation of *E. faecalis* to dentin.[34] This may explain why EDTA irrigant proved to be superior to saline in reducing intracanal microbiota, despite the fact that its antiseptic capacity is relatively limited. Calt et al[12] showed that smear layer was as effectively removed from root canal walls by irrigation with 17% EDTA for one-minute and ten-minute intervals

followed by irrigation with 5.25% sodium hypochlorite. Hence, 17% EDTA was used in this study.

Albeit never shown in a randomized clinical trial, an alternating irrigating regimen of NaOCl and EDTA may be more sufficient in reducing bacterial loads in root canal systems than NaOCl alone.[28] Yamada et al, Bystrom et al, [11] Johal et al also proved that combination of NaOCl and EDTA is effective in debriding and disinfecting root canals than using the irrigant alone. Hence in this present study combination of 3% NaOCl and 17% EDTA was used as final irrigation.

According to Chow et al[13] for the solution to be mechanically effective in removing all the particles, it has to (a) reach the apex, (b) create a current force and (c) carry the particles away. For this a proper delivery system is needed to deliver the irrigants.

Conventional irrigation with syringes has been advocated as an efficient method of irrigant delivery before the advent of newer techniques. Irrigation with syringes is still widely accepted by both general practitioners and endodontists. The technique involves dispensing an irrigant into a cannula through needles/cannula of variable gauges, either passively or with agitation.[21]

Nevertheless, the mechanical flushing action created by conventional hand-held syringe needle irrigation is relatively weak. After conventional syringe needle irrigation, inaccessible canal extensions and irregularities are likely to harbour debris and bacteria, thereby making thorough canal debridement difficult.[21]

Smaller-gauge needles/cannulas might be chosen to achieve deeper and more efficient irrigant replacement and debridement. However, the closer the needle tip is positioned to the apical tissue, the greater is the chance of apical extrusion of the irrigant. Slow irrigant delivery in combination with continuous hand movement will minimize NaOCl accidents.[21]

Past studies have shown that conventional irrigation methods are effective at cleaning root canals coronally but less effective apically. Thus, it would be advantageous to develop improved delivery systems that increase dentin tubular penetration depths. This ensures more thorough debridement of the prepared canals, while minimizing apical extrusion to eliminate the cytotoxic effects of canal irrigants such as NaOCl on the periapical tissues.

Numerous investigations have been performed to evaluate the effectiveness of instruments, instrumentation techniques, and irrigants and methods of irrigation in canal debridement. These studies have all demonstrated that debris remain in the root canal system after instrumentation and irrigation. To aid in root canal debris removal, a few attempts have been described that use cotton wrapped around an endodontic file or a broach or the use of an Endobrush. The former study indicated that a cotton wrapped around a file or broach was not able to clean the canal properly especially the irregularities, whereas, the latter study demonstrated a better cleaning effect when the Endobrush was used with hand instrumentation compared with that of instrumentation alone.[1]

Recently, a 30 gauge irrigation needle covered with brush (NaviTip FX) has been introduced in the market. The design of the NaviTip FX allows it to reach upto the apex and at the same time can be used to actively scrub the canal wall while concomitantly delivering the irrigant.[47] A study by Goel et al[18] demonstrated almost complete removal of smear layer and debris at the apical third with no significant difference between the apical, middle and coronal third. So, it was used in this study.

Max-I-Probe is a irrigation needle with side vented and close ended, which delivers the irrigant laterally. A review of literature revealed that study reported by Hauser et al, have advocated that such a design improves the hydrodynamic activation of an irrigant and reduces the chances of apical extrusion. Vinothkumar et al[54] showed that irrigation with safety ended needles with single port such as Max-i-probe are efficient in mechanically removing the bacteria from the instrumented roots canals. Hence it was used in the study

Historically, irrigation has been achieved by using a **positive pressure technique** whereby irrigant is expressed under positive pressure into the root canal system. However, the effectiveness and safety in delivering the irrigant have been questioned. In conventional needle irrigation, replenishment and exchange of irrigant in the apical third and the effectiveness of chemical debridement are dependent on the depth of penetration.[8] Boutsioukis et al[5] showed in a computational fluid dynamic model that the exchange of irrigant only occurs 1–1.5 mm past a side-vented needle, and the irrigant beyond that point remains stagnant. Chow et al [13]also found that the exchange of irrigant does not extend much beyond the tip of the irrigating needle. Vapor lock that results in trapped air in the apical third of root canals might also hinder

the exchange of irrigants and affect the debridement efficacy of irrigants. Studies have shown that conventional needle irrigation is less effective in cleaning the apical areas compared with the coronal areas of root canal systems.[50]

Recently, the use of **negative pressure irrigation technique** has been reported to be superior to positive pressure irrigation. Negative pressure irrigation systems have been shown to deliver irrigant to the apical portions of the root canal system in a safe and effective manner. It has also been suggested that negative pressure irrigation achieves better microbial control than traditional irrigation delivery systems. Endovac (Discus Dental, Culver City) is a commercially available negative pressure irrigation system.[8]

The Endovac system consists of Hi-Vac adapter assembly that connects to the high volume evacuation hose in the dental operatory at one end and has a 'T' connector at the other end .It is mainly composed of 3 basic components: a) **master delivery tip**: delivers and evacuates the irrigant concomitantly; b) the **macrocannula**: made of plastic with an open end of 0.55 mm in diameter and a 0.02 taper, used to suck irrigants up to the middle segment of the canal. It is used to remove the coarse debris, c) **microcannula**: which is made of stainless steel and has 12 microscopic holes disposed in 4 rows of 3, laterally positioned at the

apical 1 mm of the cannula. Each hole is 0.1 mm in diameter, the first one in the row is located 0.37 mm from the tip of the microcannula, and the distance between holes is 0.2 mm. The microcannula has a closed end with external diameter of 0.32 mm and should be taken to the working length (WL) to aspirate irrigants and debris. During treatment a master delivery tip delivers irrigant to the pulp chamber and macrocannula or microcannula is used simultaneously with it .When microcannula or macrocannula kept inside the canal it exerts a negative pressure that pulls the irrigant from its fresh supply in the chamber, down the canal to the tip of the cannula, into the cannula, and out through the suction hose .Thus a constant flow of fresh irrigant is being delivered by negative pressure to working length.[7]

Considering the irrigation Walton and Torabinejad stated that "Perhaps the most important factor is the delivery system and not the irrigating solution per se.[26] Hence, the aim of the present study was to compare the efficacy of three different irrigation systems in reduction of *E. faecalis*.

In this present study fifty five single rooted teeth were selected to standardize root canal anatomy and minimize the anatomical variations. Conventional access cavities were done by using #2 round burs. Patency with an #10 stainless steel K-file was achieved, and the working length

was set at 1 mm back from the total root length. Teeth exceeding 20mm in length were adjusted to 20 mm by incisal reduction. To standardize the apical constriction size, root canals were instrumented at the apical foramen up to a K-type file #25 in reaming action, under irrigation with saline. The apical foramen was sealed with Type II GIC.

The teeth were mounted vertically up to the cervical region in blocks made of a self cure resin. This makes handling and identification of the samples easier and also closely resemble the clinical situation acting in which the tooth's foramen and outer surface are sealed by the periodontal ligament and further embedded in alveolar bone. The blocks containing the teeth were sterilized in autoclave for 20 minutes at 121^0c.

Each root canal was filled with 0.1ml of *E. faecalis* suspension which was made by adding 1 ml of a pure culture of *E. faecalis* (ATCC 29212) to 5 ml of fresh Trypticase soy broth (TSB) by using sterile 1-ml syringes. Blocks were then placed inside a rectangular surgical tray and incubated at 37^oC for 7 days in 100% humidity. After 7 days of experimental contamination, teeth were randomly divided into 3 experimental groups of 15 teeth each according to the irrigation technique and a control group consisting of 10 teeth. Instrumentation of root canal was done apically by Protaper rotary instrument (S1, S2, F1,F2,F3,F4) in a crown down technique as per manufactures

recommendation in all the groups. In all the groups 20 ml syringe was used to deliver the irrgants.

Group I: NaviTip FX (Ultradent)

The canal was irrigated with 3% NaOCl (2ml) after each instrumentation with 30-gaugeNaviTip FX in active scrubbing in and out motion according to the manufacturer's instruction. After the instrumentation the canal was irrigated with 3% NaOCl (2ml) for 30s and left the irrigation solution in canal for 1 minute .Final irrigation done with 3% NaOCl for 30sec, 17 % EDTA for 30sec, 3 % NaOCl for 30sec. During irrigation, needle was kept at 2mm from working length.

Group II: Max I probe (Dentsply)

The canal was irrigated with 3% NaOCl (2ml) after each instrumentation with 30-gauge Max I probe. After the instrumentation the canal was irrigated with 3% NaOCl (2ml) for 30s and left the irrigation solution in canal for 1 minute. Final irrigation done with 3% NaOCl for 30sec, 17 % EDTA for 30sec, 3 % NaOCl for 30sec. During irrigation, needle was kept at 2mm from working length.

Group III: Endovac (Discus Dental)

The canal was irrigated with 3% NaOCl (2ml) after each instrumentation with master delivery tip. After the instrumentation the canal was irrigated with 3% NaOCl (2ml) for 30s with macrocannula and left the irrigation solution in canal for 1 minute. Final irrigation done with 3% NaOCl for 30sec, 17 % EDTA for 30sec, 3 % NaOCl for 30sec using microcannula. During irrigation, microcannula was kept at working length according to the manufacturer's instruction. The manufacturers and inventors of the Endovac recommend final irrigation regimens to be performed for a time interval of thirty seconds.

Group IV (Control group)

The canal was irrigated with saline after each instrumentation using 20 ml syringe and conventional needle (27 gauge needle).

NaOCl require an adequate working time to reach the potential. A study by Retamoza et al[39] proved that long exposure time is needed for elimination of *E. faecalis*.

In this present study design contact time was only the factor that could be standardized. Volume cannot be standardized, as comparison were made between different delivery systems with different

mechanisms of action and different volumes displaced at a given time. This in accordance with Heilborn et al[23] who proved that variations on volume inherent to the delivery system. During manual instrumentation, 2 ml of sodium hypochlorite was arbitrarily chosen as a realistic amount of irrigation solution to be delivered between file transitions for all the groups.

To simulate the clinical situation with a normal irrigation method, the irrigating needle used in this study was placed at 2 mm from working length for the **positive irrigation techniques** (Max-i-probe& Navitip FX). Because of the inherent differences between these irrigating techniques, the variable of cannula or needle compared with working length was not held constant and represents the possible advantage of the Endovac system, namely, safe irrigation at working length. Desai et al [14] compared the safety of different irrigation systems and concluded that Endovac is safe to work at working length. Hence in this present study microcannula was placed at working length. Two millimeters represents a distance from the working length that is potentially the closest that most practitioners place an ordinary needle during irrigation.[32] Thus, this distance is a best-case scenario for needle irrigation to compare with the Endovac system.

After finishing irrigation in all the sampled teeth, the canals were flushed with 10% sodium thiosulfate to neutralize the NaOCl. Hedstrom instrument #40 is used to the dentinal walls to obtain dentinal shavings and canal contents were aspirated with a 1-ml syringe and then placed to tubes containing 1 ml of saline .Three paper points also#40 were placed at WL to soak up the canal contents. Paper points were transferred to the tubes containing 1 ml of saline. It is vortexed for 1 minute 10-fold serial dilutions in saline, aliquots of 0.1 ml were plated onto Brain Heart infusion agar plates and incubated at 37^{o}C for 48 hours. The colony-forming units (CFUs) grown were counted and then transformed into actual counts based on the known dilution factors.

All the tested irrigation techniques showed a significant reduction in *E. faecalis* population when compared to the control group. The results are in accordance with the study of Brito et al[7] who concluded that three experimental groups (Endovac, Endoactivator, Navitip needle) with NaOCl and EDTA as irrigant were significantly more effectively than the conventional irrigation with saline.

Among the tested group the Endovac group showed few number of bacterial colonies in the present study. This is in accordance with the study by Hockett et al[24] who compared the incidence of canals positive for growth of *E. faecalis* after the use of either the Endovac system or

needle irrigation and concluded that Endovac had potential to achieve better microbial control.

The effectiveness at producing in clean dentinal surfaces may be attributed to its apical negative pressure approach. Placement of macrocannula at middle–apical third of the canal followed by the placement of the microcannula directly at the apical end enables an irrigant to be suctioned in sufficient volume and flow to displace the debris. Additionally the orfices of the microcannula provide a portal of exit for canal debris from the apical end.

Lastly the increasing volume of sodium hypochlorite delivered by the Endovac may also contribute to an enhanced microbial effect. Sodium hypochlorite dissolves necrotic tissue and organic debris by breaking down proteins into amino acids. It provides continuous tissue dissolution under the condition that free chlorine is available in solution. This free chlorine is depleted during the tissue dissolution requiring frequent replenishment of sodium hypochlorite. Neilsen et al (2007) [32] showed that the volume of irrigant delivered by the Endovac system was significantly higher than the volume delivered by conventional syringe needle irrigation during the same time period. Sedgley[41] et al showed that 6 ml of sodium hypochlorite is significantly more effective than 3 ml at removing bacteria in root canals.

In group I (Navitip Fx) showed reduction in the bacterial load because the brush covered needle was mechanically activated in an active scrubbing action during the irrigation process to increase the efficiency. This is in accordance with studies by Al-Hadlaq et a1[1] and Zemner et al[56] who proved that brush covered irrigation needle was more effective in removing the debris from the root canal.

In group II (Max -i-probe) there was reduction in bacteria load compared to control group. The probable reason may be attributed to its design, closed- ended, side vented channel, which tends to deliver the irrigant laterally. This unique design produce upward turbulence that enhances the complete cleaning of the root canals. This may have significantly removed the more bacteria when compared to conventional needle irrigation. This finding is similar with results of Vinothkumar[54] et al study who concluded that irrigation using safety needles with single side port was significantly more effective.

In group IV (control group) showed highest number of bacteria when compared to experimental group. This in accordance with Bystrom et al[11] who proved that combination irrigants effective in reduction of bacteria from the root canal.

This study showed that the current systems though clearly reduce the bioburden within the canal space in vitro, it is still not effective in complete elimination of bacteria in the canal system.

This present study showed that apical negative pressure irrigation has the potential to achieve better microbial control than traditional irrigation delivery system.

A recent study by Benjamin et al (2007)[32] showed that the volume of irrigant delivered by the Endovac system was significantly higher than the volume delivered by conventional syringe needle irrigation during the same time period. His study also supported that the use of the Endovac system resulted in significantly more debris removal at 1 mm from the working length than needle irrigation.

Apart from being able to avoid air entrapment, the Endovac system is also advantageous in its ability to safely deliver irrigants to working length without causing their undue extrusion into the periapex which was in accordance with Desai and Himel[14] who compared the extrusion of Endovac irrigation with manual irrigation with Max-I-Probe needle, EndoActivator irrigation, ultrasonic needle irrigation, and Rinsendo irrigation. They found that the Endovac did not extrude irrigant, whereas the EndoActivator had minimal extrusion out the apex,

and the Manual, Ultrasonic, and Rinsendo groups had a significantly greater amount of extrusion.

Therefore, the use of Endovac system has to be recommended as a newer irrigation system in order to enhance the canal disinfection. However increasing the apical preparation size might be difficult or even unfeasible in thin and curved roots which lead to transportation of the canal system and possibly perforation of the root, the use of Endovac system in these conditions is limited.

E. faecalis grown as a biofilm was more resistant to than the same strain grown in planktonic suspension. Trials investigating the effects of these regimens on a mixed bacterial community in the clinical set up are required to determine the method that best provides predictable disinfection of infected root canals of teeth with apical periodontitis. Additionally further investigations are also necessary to evaluate the efficacy of these irrigation systems in vivo for improved cleanliness of the canal wall in chemo-mechanical preparation.

SUMMARY

The purpose of this an ex-vivo study is to evaluate the efficacy of three irrigation techniques in reduction of *E. faecalis* in root canal. 55 intact maxillary anteriors were used in this study. Root length was standardized to 20mm. Teeth were mounted in self cure resin and autoclaved . Teeth were contaminated with *E. faecalis* and stored for 7 days.

After contamination, teeth were randomly distributed into 3 experimental groups of 20 teeth each and control group of 10 each. **Group I:** irrigated with NaviTip FX; **Group II:** irrigated with Max-i-probe, **Group III,** irrigated with the Endovac system. **Group IV** (Control group) irrigated with 20 ml syringe and using 27 gauge needle. 3% NaOCl and17% (EDTA) were the irrigants used in all experimental groups and control group was irrigated with saline solution. 20ml syringe was used to deliver irrigant for all the groups. Root canal were instrumented by Protaper rotary instrument till size F5. After chemo-mechanical procedures bacterial samples taken from the root canal were cultured, and incubated for 2 days in Brain heart infusion agar and the colony-forming units (CFUs) were counted.

All the experimental groups showed significant bacterial reduction when compared to group IV (control group). Among experimental groups, Group III (Endovac) showed less number of bacterial colonies. There was no statistical significance between the Group I (Navitip FX) and Group II (Max I probe).

CONCLUSION

Under the limitations of the present study it can be concluded that:

1. All three newer irrigation delivery system (Navitip FX, Maxi-i-probe, Endovac) have been found to be effective in the reduction of *E. faecalis*.
2. Among the three irrigation systems Endovac showed maximum reduction of colony forming units.
3. Between Navitip FX and Max-i-probe delivery systems which were used under positive pressure, showed no statistical significance in reduction of *E. faecalis* population.
4. The results of this study confirmed that apical negative pressure technique has high potential to achieve better antimicrobial effect compared to the traditional irrigation delivery system.

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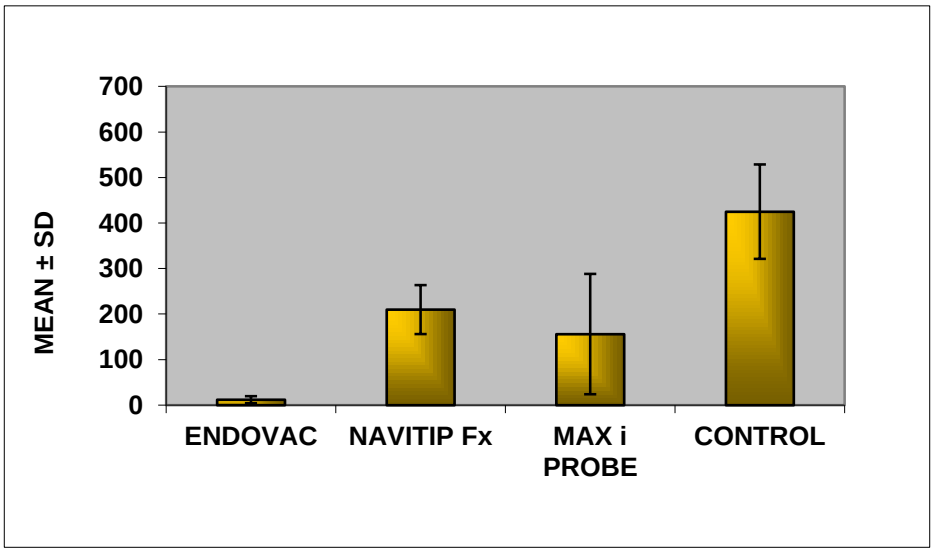
Table 1: Comparing Mean and Standard Deviation Values For All the Groups.

Groups	Mean	SD	P-value
Endovac	12.00	7.746	.000
Max-i-probe	156.00	34.056	
NaviTip FX	210.00	53.852	
Control	425.00	32.770	

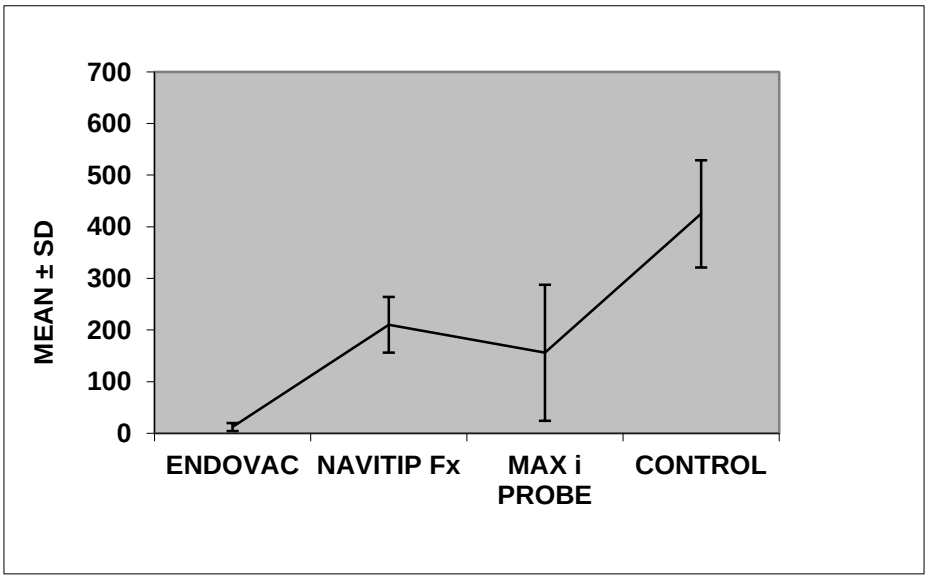
Table 2: Tukey HSD for Specific Inter Group Comparison

Group		P –value
Endovac	Navitip FX	.000
	Max-i-probe	.000
	Control	.000
Navitip FX	Endovac	.000
	Max-i-probe	.329
	Control	.000
Max-i-probe	Endovac	.000
	Navitip FX	.329
	Control	.000
Control	Endovac	.000
	Max-i-probe	.000
	Navitip FX	.000

Graph 1: Bar graph showing mean and standard deviation of *E .faecalis* CFU counts after using different irrigation systems



Graph 2: Line graph showing the mean and SD *E. faecalis* CFU counts after using different irrigation systems



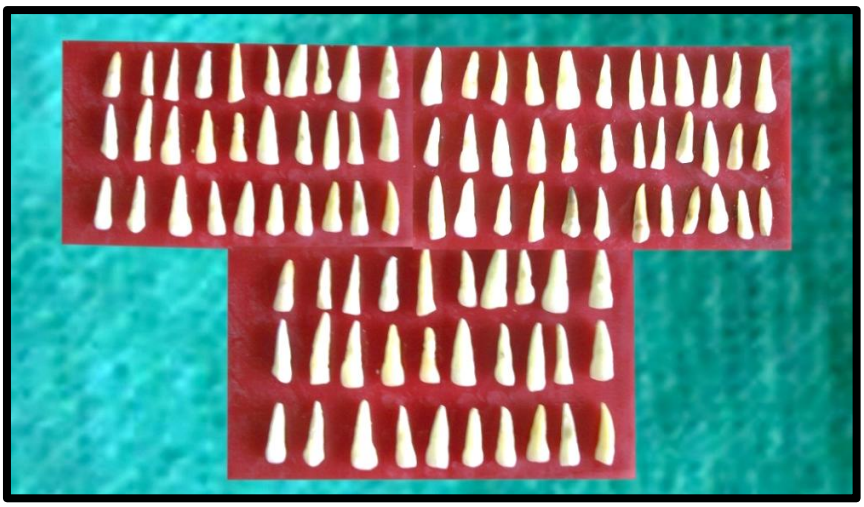


Fig.1: MAXILLARY ANTERIORS

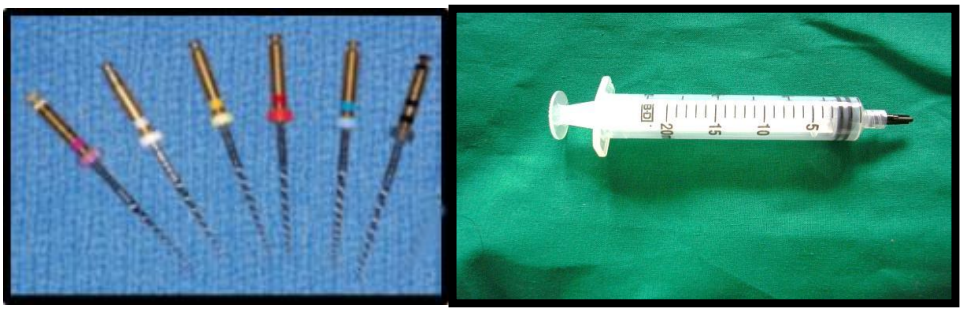


Fig.2: ARMAMENTARIUM USED FOR TOOTH PREPARATION AND IRRIGATION.

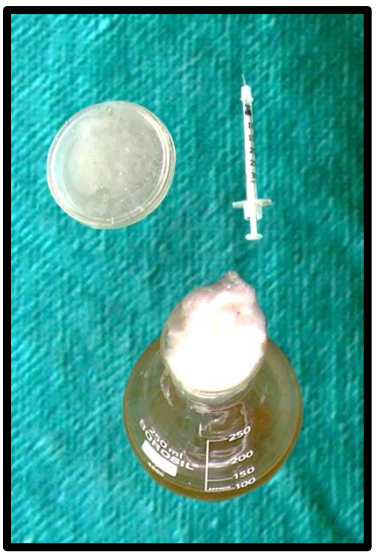


Fig 3: ARMAMENTARIUM USED FOR BACTERIAL CULTURE AND BACTERIAL INOCULATION

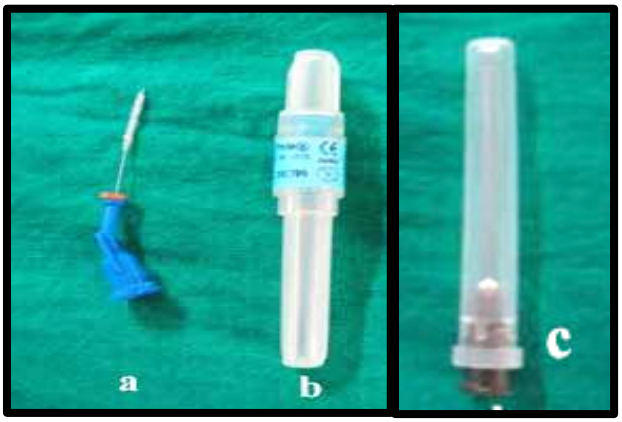


Fig 4a: NAVITIP FX

Fig 4b: MAX I PROBE

Fig 4c: SYRINGE NEEDLE

ENDOVAC SYSETM

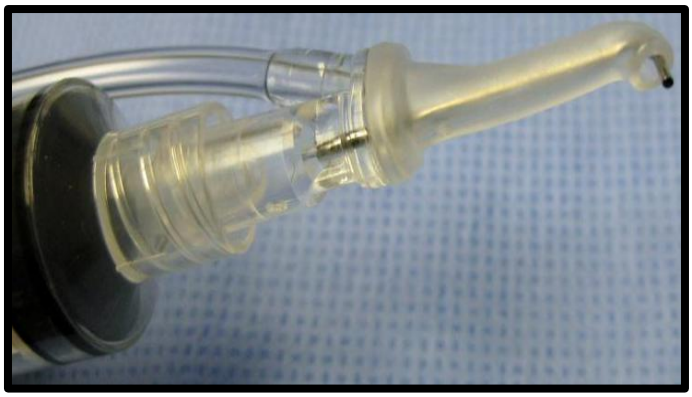


Fig 5: MASTER DELIVERY TIP (MDT)

Fig 6: MACROCANULA AND TITANIUM HAND PIECE

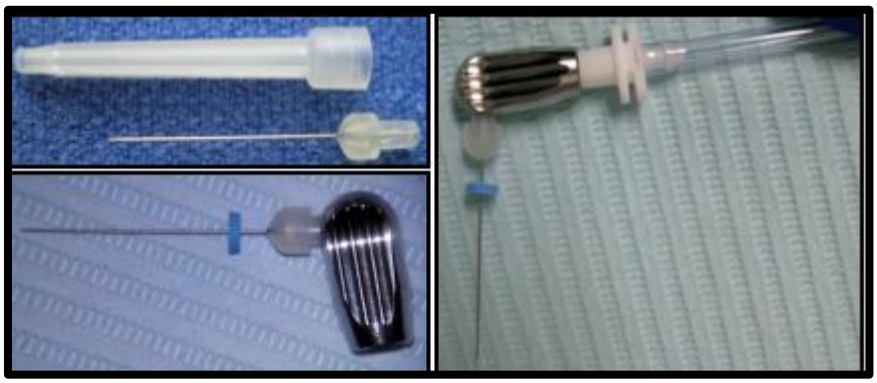


Fig 7: MICROCANNULA AND FINGER PIECE

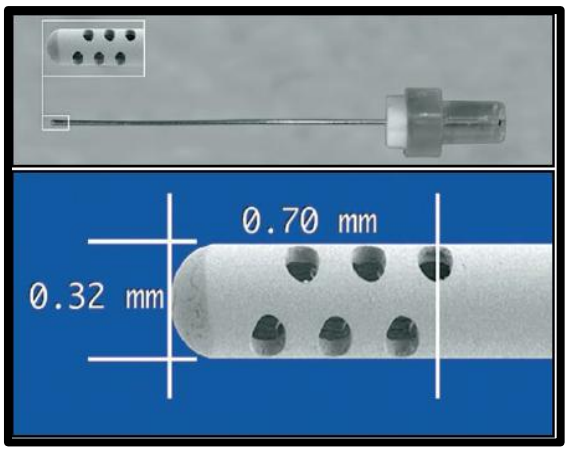


Fig 8: MAGNIFIED SPHERICAL, WELDED-END OF MICROCANNULA ILLUSTRATING MICRO-HOLES.

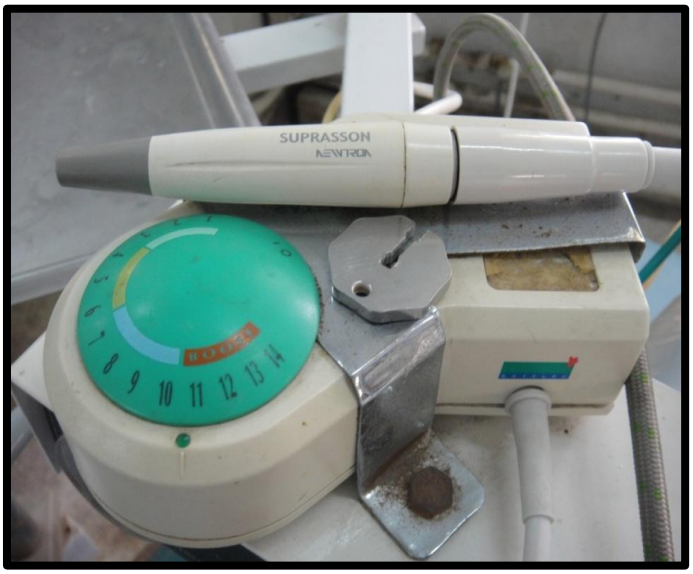


Fig 9: ULTRASONIC SCALER UNIT (SATELEC)

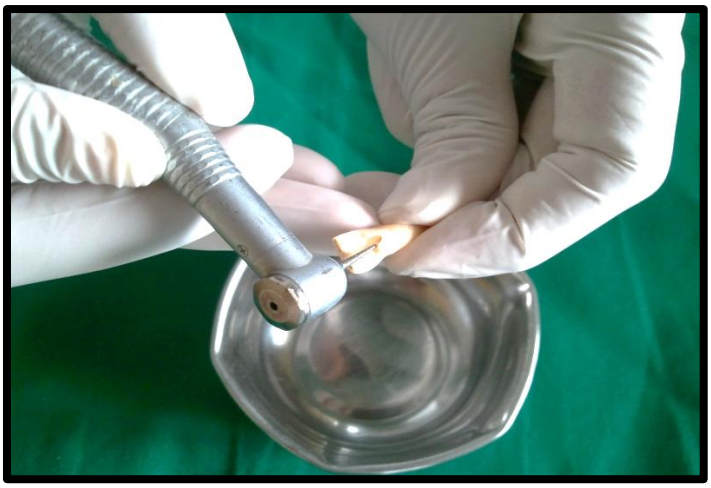


Fig 10: ACCESS OPENING OF THE TOOTH

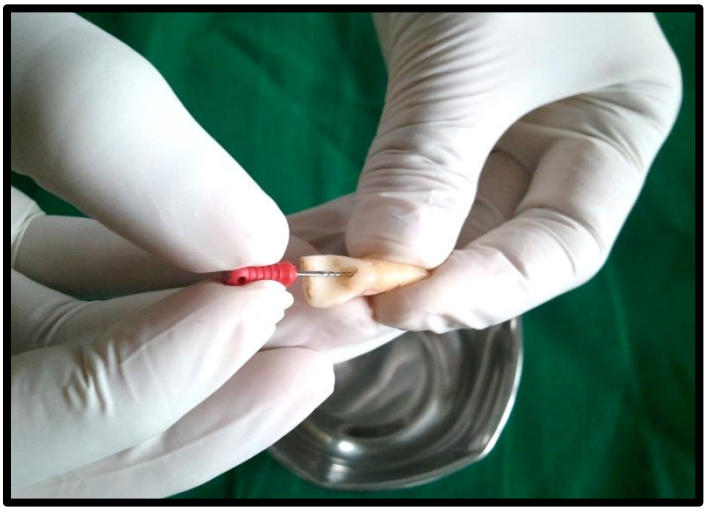


Fig 11: PRELIMINARY PREPARATION OF THE ROOT CANAL UPTO # 25

Fig 12: TOOTH MOUNTED IN SELF CURE ACRYLIC BLOCK

Fig 13: INOCULATION OF E.FAECALIS

Fig 14:INCUBATOR

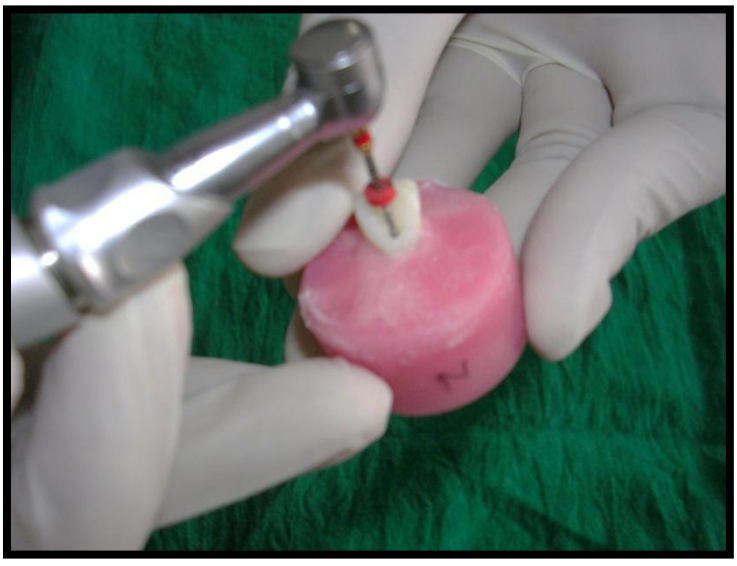


Fig 15: PREPARATION OF TOOTH SAMPLES WITH PROTAPER ROTARY SYSTEM

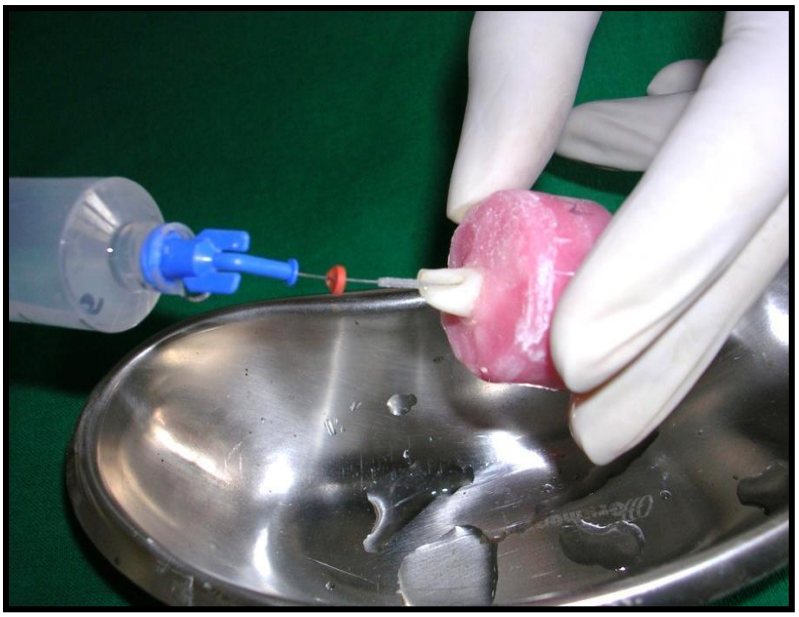


Fig 16: IRRIGATION WITH NAVITIP-FX

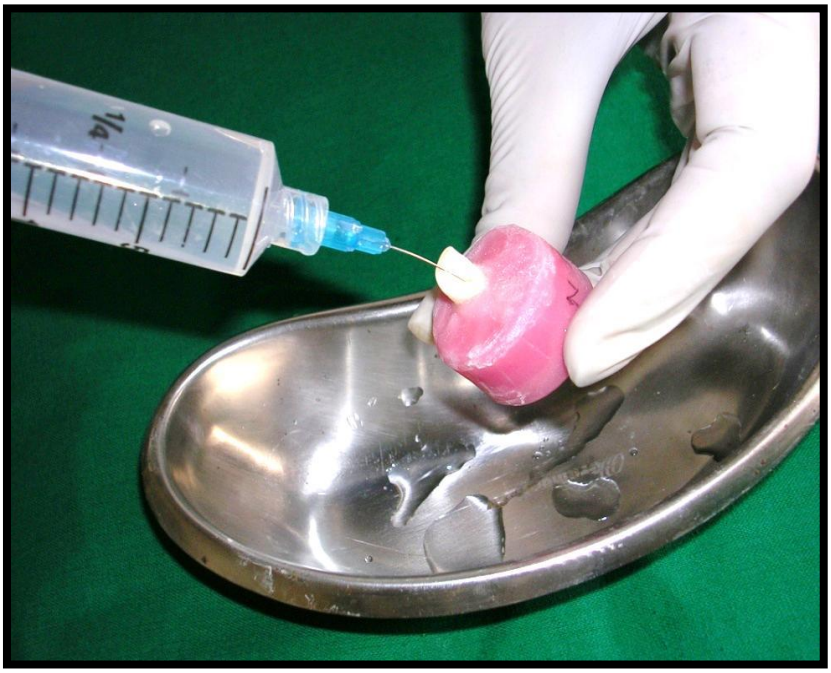


Fig 17: IRRIGATION WITH MAX I PROBE

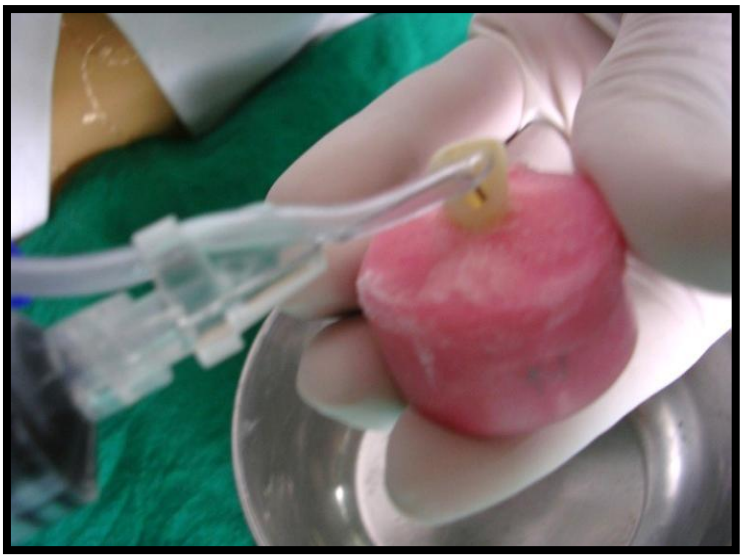


Fig 18: IRRIGATION WITH MASTER DELIVERY TIP OF ENDOVAC SYSTEM

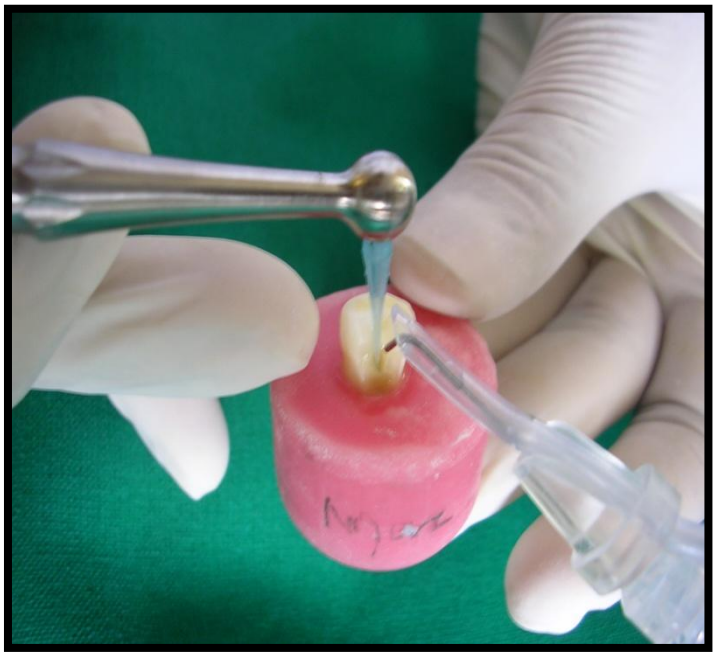


Fig 19: IRRIGATION WITH MACROCANNULA OF ENDOVAC SYSTEM

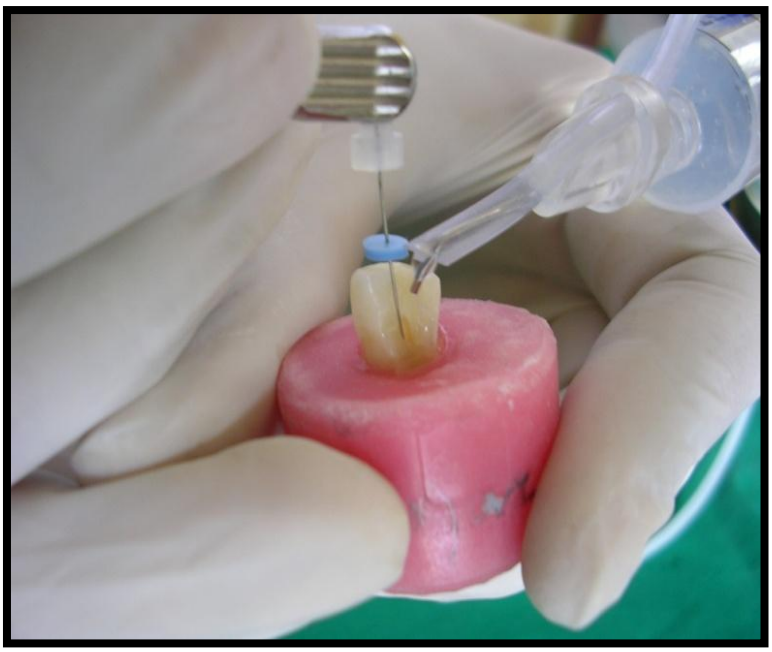


Fig 20: IRRIGATION WITH MICROCANNULA OF ENDOVAC SYSTEM

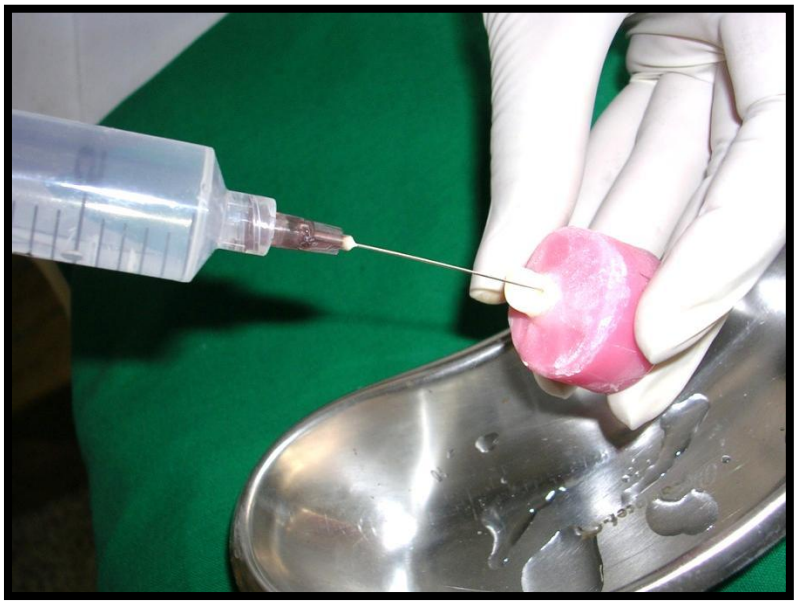


Fig 21: IRRAGATION WITH SYRINGE NEEDLE

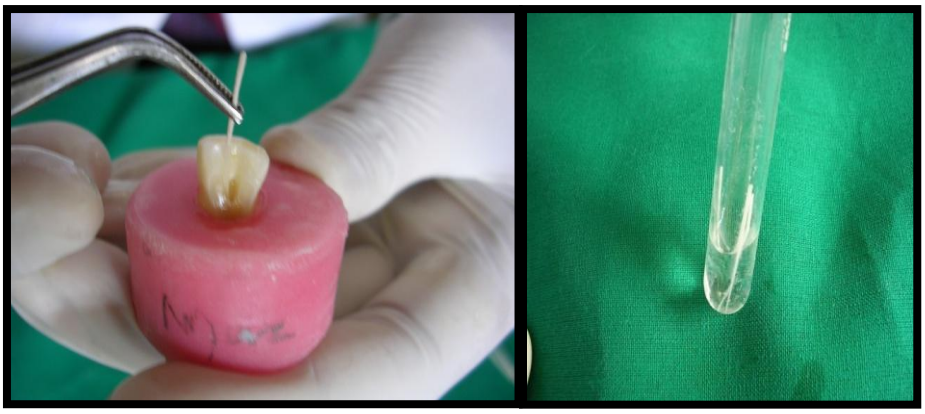


Fig 22: SAMPLE FROM ROOT CANAL COLLECTED AFTER CHEMO-MECHANICAL PROCEDURE USING PAPER POINTS AND TRANSFERRED TO TEST TUBE

Fig 23: TEST TUBE IN VORTEX MACHINE

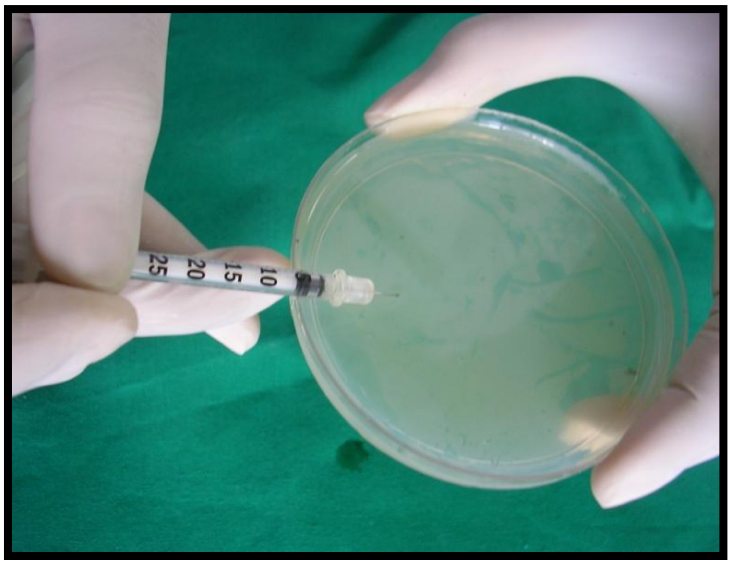


Fig 24: INOCULATION OF BACTERIA INTO AGAR

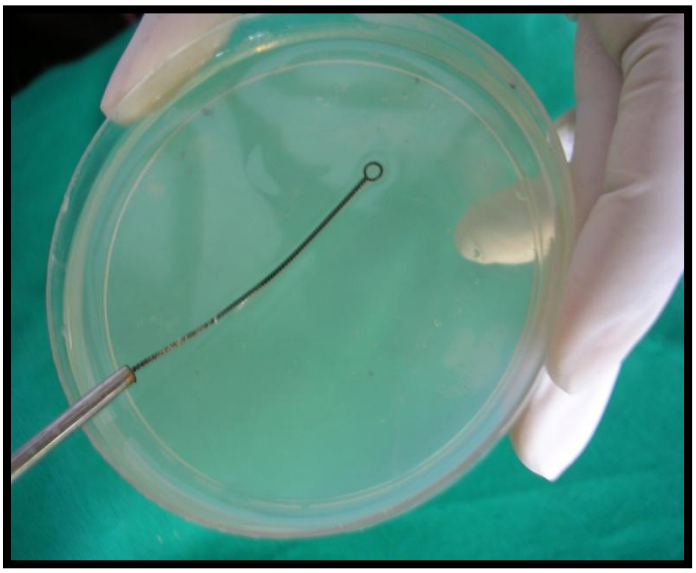


Fig 25: SPREADING THE BACTERIA USING LOOP

Fig 26: AGAR PLATE SHOWING COLONING FORMING UNIT FOR NAVITIP-FX

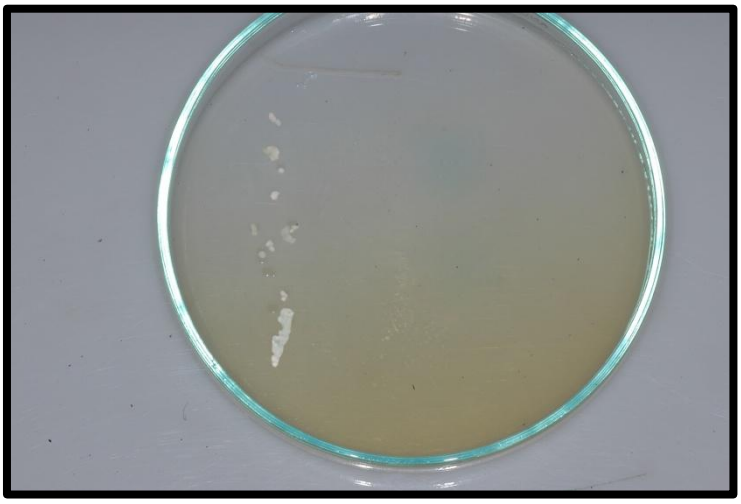


Fig 27: AGAR PLATE SHOWING COLONING FORMING UNIT FOR MAX-I-PROBE

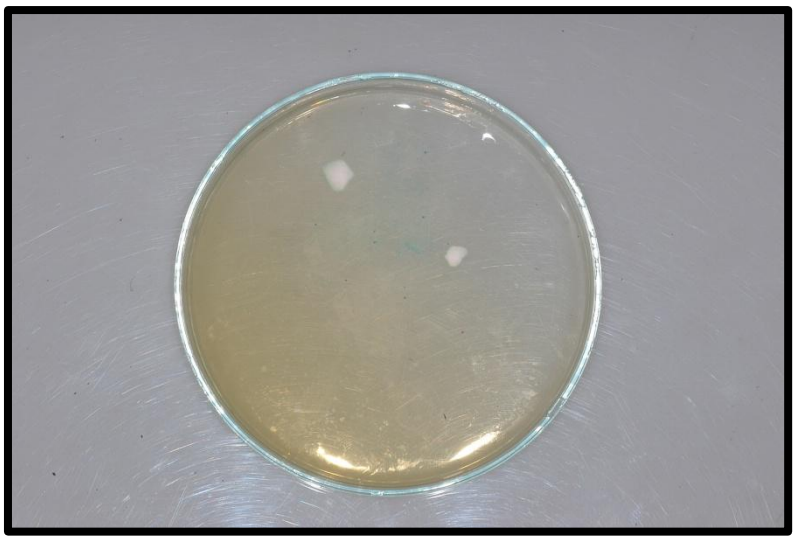


Fig 28: AGAR PLATE SHOWING COLONING FORMING UNIT FOR ENDOVAC

Fig 29: AGAR PLATE SHOWING COLONING FORMING UNIT FOR CONTROL GROUP