AN IN VITRO EVALUATION OF THE ANTIBACTERIAL EFFICACY AND MECHANICAL PROPERTIES OF GLASS IONOMER CEMENT – FUJI IX INCORPORATED WITH THREE ANTIBIOTICS.

Dissertation submitted to

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

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

APRIL 2011

CERTIFICATE

This is to certify that this dissertation titled "**AN IN VITRO EVALUATION OF THE ANTIBACTERIAL EFFICACY AND MECHANICAL PROPERTIES OF GLASS IONOMER CEMENT – FUJI IX INCORPORATED WITH THREE ANTIBIOTICS."** is a bonafide record work done by **MD. OMAR FAROOQ B.**under our guidance during the study period between 2008-2011.

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 partially or full) for the award of any other degree or diploma.

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ACKNOWLEDGEMENT

I take this opportunity to sincerely thank my post graduate teacher and my guide, ***Dr. C. S. KARUMARAN, M.D.S., Professor*** *Department of Conservative Dentistry & Endodontics, Ragas Dental College, for his perseverance in motivating and supporting me throughout my study period.*

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

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

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

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

I extend my sincere thanks to ***Dr., M. Rajasekaran****,* ***Associate Professor, Dr. Revathi Miglani M.D.S, DNB, Associate Professor*** *for their constant encouragement throughout the completion of this work.*

I thank ***Dr. Veni Ashok M.D.S, Dr. A. D. Senthil Kumar M.D.S, Dr. Shankar Narayanan M.D.S****,* ***Dr. Durai Vel M.D.S, Dr. Venkatesan M.D.S, Dr. Poorni S., Senior lecturers*** *for their support and guidance.*

. I am profoundly thankful to ***Dr. Usha Anand Rao****, Director Research and Professor dept. of Microbiology Ragas Dental And Hospital for her guidance in Microbiology Study.*

My sincere thanks to ***Mr .O.S. Kumaran****, Manager – Production planning, The Madras Pharmaceuticals, Karapakkam, OMR, Chennai. for his guidance in High Performance Liquid Chromatography Analysis*

My sincere thanks to ***Mr. Naveen****, Central Institute Of Plastic Engineering and Tools, Chennai for his assistance in the Mechanical Testing.*

My sincere thanks to ***Mr. Manivannan****, Mohd Sathak college of Arts and Science, for his assistance in biostatics.*

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

Above all, I am thankful to the very God almighty, to have given me the strength and vision to choose the right path and to have given all these wonderful people in my life.

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INTRODUCTION

The quest for an ideal restorative dental material has been ongoing and this is nowhere evident than in the realm of adhesive dentistry. Tooth coloured adhesive materials include Glass Ionomer Cements, were invented by Wilson and Kent in 1972.[42] Glass Ionomer cements is usually the adhesive material of choice to treat high caries risk patients.[48] The factors contributing to patients presenting with high caries risk are poor diet, poor oral hygiene level, proportion of cariogenic bacteria in plaque, salivary flow, saliva buffering capacity, fluoride exposure, socioeconomic conditions.[7,18]

Glass ionomer cement acquires its name from its formulation of a glass powder and the materials adheres to the tooth structure by ionic bonding.[66] Bonding primarily involves chelation of carboxyl groups of the polyacids with the calcium in the apatite of the enamel and dentin[63]. The release of fluoride is one of the main advantages of GIC. It has an impressive anticariogenic property that helps in remineralization and makes the tooth more acid resistant.[12]

Fluoride is released as a result of the acid-base reaction of the material and is not a part of matrix formation. They freely move in and out of the cement.[31] Glass ionomers also serve as rechargeable reservoirs due to its ability to take up fluoride from various sources and hence continue to release the same throughout the life of the restoration.[38] However, the amount of fluoride release varies with curing material based on their composition, setting reaction and amount of fluoride incorporated.[8]

Atraumatic restorative treatment (ART) is a minimal intervention approach in which demineralized tooth tissue is removed using hand instruments manually. Atraumatic restorative treatment is indicative for use in the posterior teeth, it is critical that the type of restorative material shows strong physical properties.[46]One of the materials to which cariostatic and bactericidal properties are attributed is glass ionomer, a adhesive filling material used in the ART technique.[51]

Cavities treated by ART may have residual infected dentin and if a GIC is unable to arrest the carious process, the restoration could fail. Clinical studies appear to support this

concern by showing the viability of residual bacteria under GIC restorations. The therapeutic benefits may therefore be gained in combining antibacterial agents with GIC materials.[6]

Streptococci are gram positive, non motile, non sporing and facultative anaerobic organism. Viridans Streptococci or oral Streptococci, comprising of Streptococcus mutans are chiefly involved in the production of dental caries.[65]

Lactobacillus casei are common inhabitants of the oral cavity. It is a facultative anaerobic, gram positive, non sporing organism growing in chains. Lactobacillus casei are nonpathogenic and contributes to the progression of dental caries.[65]

In the past there are several studies reporting that fluoride itself has antibacterial properties[18,6,28,] to inhibit the bacterial popularion.

Previous studies have been reported using Glass ionomer cement (Fuji IX) incorporated with Chlorhexidine, antimicrobial agents and Hydroxyapatite to evaluate its antibacterial effect

against Streptococus species and Lactobacilus species.[21,32,38]The Glass ionomer for the use in the ART approach require an optimum amount of antibacterial agents that should not jeoparadize the basic properties of the parent material.

Only few studies have been cited in the literature with incorporation of antibiotics to the Glass Ionomer cement to enhance its therapeutic benefits.

It has been reported that the addition of antibacterial agents to Fuji IX cement creates a GIC material with significant antimicrobial action which is dependent on concentration and type of antibacterial agent.[6]

Metronidazole, Ciprofloxacin and Minocycline are primarily active against obligate anaerobic bacteria, gram negative bacteria and gram positive bacteria respectively.

The aim of this present study was to evaluate the antibacterial Efficacy of Glass ionomer cement (Fuji IX) incorporated with three antibiotics Metronidazole, Ciprofloxacin

and Minocycline against gram positive organisms Streptococus mutans and Lactobacillus casei.

The objectives of this study were

1. To compare the antibacterial activity of set and unset glass ionomer cement against Streptococcus mutans and Lactobacillus casei.
2. To quantify the amount of drug released from experimental glass ionomer cement at the end of 24 hours and 7 days.
3. To evaluate the influence of these drugs on the mechanical properties like compressive strength and shear bond strength to dentin.

REVIEW OF LITERATURE

Sato et al [37](1993) clarified the antibacterial efficacy of mixed antibacterial drugs on bacteria of carious and endodontic lesions of human deciduous teeth in vitro. The antibacterial drugs used in this study were mixtures of ciprofloxacin, metronidazole, plus a third antibiotic: amoxicillin, cefaclor, cefroxadine, fosfomycin or rokitamycin. When carious and endodontic lesions on split surfaces of freshly extracted teeth were covered overnight with alpha-tricalcium phosphate cement containing a mixture of ciprofloxacin, metronidazole and cefaclor , no bacteria were recovered from the lesions. No bacteria were recovered from carious and endodontic lesions when these lesions were immersed in a solution of the mixture (200 micrograms each/ml; 5 cases).

Seppa et al [38](1993) investigated whether the release of fluoride and antimicrobial effect of freshly mixed glass ionomers could be prolonged by application of fluoride on aged material.It was concluded that fluoride release from old

gless ionomers and antimicrobial effect could be significantly increased by application of fluoride gel on the material.

Hoshino et al [17](1996) clarified the antibacterial effect of a mixture of ciprofloxacin, metronidazole and minocycline, with and without the addition of rifampicin, on bacteria taken from infected dentine of root canal walls.. Bacteria ranging in number from 10(2) to 10(6) occurred in samples of infected root dentine (27 cases). However, none was recovered from the samples in the presence of the drug combination at concentrations of 25 micrograms ml-1 each. The respective drug alone (10, 25, 50 and 75 micrograms ml-1) substantially decreased the bacterial recovery, but could not kill all the bacteria. Bacteria taken from carious dentine (25 cases) and infected pulps (12 cases) were also sensitive to the drug combination.

Shalhav et al [39](1997) evaluated antibacterial activity of a recently introduced glass ionomer endodontic sealer, Ketac Endo(KE), compare to a commonly used ZOE based endodontic sealer, Roth's cement(RC).The authors concluded that KE possesses a short – acting very potent and diffusible

antibacterial activity,whereas RC extends its effect over 7 days after setting.

Frencken et al [18](1999) discussed the effectiveness of ART in the management of dental caries. The authors concluded that ART should be considered a caries treatment modality that benefits people and educational courses need to be organized before the approach is applied in the clinic.

Almuammar et al [1](2001) compared the shear bond strength of a conventional GIC, a resin modified GIC(Fuji II LC), a composite resin and three compomer restorative materials(Compoglass, Hytac and Dyract AP). Conventional GIC, Ketac Molar aplicap showed the lowest mean shear bond strength and the composite resin, Heliomolar showed the highest mean bond strength. It was concluded that the compomer restorative materials show higher shear bond strength than conventional GIC and resin modified GIC but less than composite resin.

Sharanbir et al[40] (2001),studied the biocompatibility of glass –ionomer cement materials.The authors concluded that

unfavourable initial reactions, if present,resolved with time if a bacterial layer under the restoration and pulp exposures were prevented.

Burrow et al [9](2002) compared the microtensile bond strength of a conventional GIC (Fuji IX), a resin modified GIC (Fuji II LC) and two resin based dentin adhesives (Prime and Bond NT with NRC and Single Bond).He demonstrated no differences among the bond strengths to primary and permanent dentin for the materials tested in the study. It was concluded that the Fuji IX bond strengths were significantly lower than other systems tested and the FE-SEM observations showed hybrid like layer formation for the GICs and hybrid layer formation for the resin based adhsesives.

Pereira et al [34](2002) evaluated the mechanical properties and bond strength of GICs and resin modified GICs that are indicated as restorative materials for the ART technique. Ketac-Fil, Ketac Molar, Fuji IX and Fuji PLUS were used in the study. The results demonstrated that the RM-GIC had the highest diametral tensile strength with no changes between

the test periods and the highest tensile bond strength for both enamel and dentin.

Yap et al [56](2002) investigated the mechanical properties of two restorative reinforced GICs namely, GC Fuji IX GP and Miracle Mix. Results demonstrated that the mechanical properties generally increased with time for both cements, hardness at 1 day was significantly lower than that at 1 week and 1 month. It was concluded that the diametral tensile strength of Fuji IX was however greater than that of Miracle Mix at all time intervals and Fuji IX GP may serve as a potential substitute for Miracle Mix.

Frencken et al [17](2003) investigated the various aspects of ART and highlighted the tissue preservation treatment concept as being less painful and therefore more patient friendly than conventional treatments. He concluded that there was no difference in survival results between single surface ART restorations and comparable amalgam restorations in the permanent dentition after 3 years and also stressed the importance of ART sealants using high viscosity GICs.

Lucas et al [28](2003) investigated the improvement in mechanical strength of GIC (Fuji IX GP) by the addition of Hydroxyapatite and studied its effect on the fracture toughness, bonding to dentin and fluoride release properties. The results demonstrated a significantly higher fracture toughness after 15 minutes and 24hrs after mixing in the GIC specimens containing Hydroxyapaptite. He concluded that hydroxyapatite-added GICs has a potential as a reliable restorative material with improved fracture toughness, long term bonding to dentin and unimpeded ability of sustained fluoride release.

Xu et al [53](2003) studied the compressive strength and recharge profiles of 15 commercial fluoride releasing restorative materials. The materials include GICs (Fuji IX, Ketac Molar, Ketac Silver and Miracle Mix), Resin modified GICs (Fuji II LC improved, Photac-Fil and Vitremer), Compomers (Compoglass, Dyract AP, F 2000 and Hytac) and Composite resins (Ariston pHc, Solitaire, Surefil and Tetric Ceram). He discussed that restorative materials with high fluoride release have lower mechanical properties. It was concluded that materials with higher initial fluoride release

have higher recharge capability which seem to be of paramount importance in the clinical scenario.

Yap et al [57](2003) investigated the hardness, strength (Compressive and Tensile) and wear resistance of a Fast Set highly viscous GIC (Fuji IX GP Fast). The results demonstrated that there was no significant difference in hardness, compressive and diametral tensile strength between Fuji IX GP and Fuji IX GP Fast at 1 day. It was concluded that besides being harder, the fast set highly viscous GIC restorative offers no other physico-mechanical advantage over its regular set counterpart.

Botelho et al [6](2003) investigated the compressive strength of GICs combined with oral antibacterial agents- Chlorhexidine hydrochloride, Cetylpyridinium chloride and Cetrimide were added to the Powder and Benzalkonium chloride was added to the liquid of Fuji IX GIC. These were prepared to concentrations of 1%,2% and 4% of the GIC and compared to the Fuji IX with no antibacterial agent added. He concluded that increasing the concentration of the antibacterial agent had increasing adverse effects on the

physical properties. The addition of antibacterials to the Fuji IX reduces the seven day compressive strength which may affect the clinical performance of the material.

Kleverlaan et al [24](2004) assessed the influence of externally applied command set applications on the mechanical properties of GICs namely Fuji IX Fast, Fuji IX, Ketac Molar Quick and Ketac Molar cured using standard curing, Ultrasonic excitation and by an external Heat source. The results demonstrated an increase in strength going from standard curing to ultrasonic curing to Heat curing. It was concluded that an increase in strength was found especially at the early curing time and enhanced material properties at early curing time can improve the survival rate of GICs in the clinical situation.

Palmer et al [33](2004) investigated the use of an experimental GIC as a carrier for the release of chlorhexidine acetate(CHA) at included concentrations ranging from 0.5% to 13% of CHA by weight.In general, compressive strengths were found to be decreased indirect proportion to quantity of CHA added, while working and setting times increased.

Yap et al[58] (2004) compared the hardness and the modulus of the continuum of direct tooth coloured restoratives using a depth sensing micro indentation approach. The six materials selected were an Ormocer, a Giomer, a Compomer, a minifill Composite, a resin modified GIC and a highly viscous GIC. He concluded that the hardness and modulus of some GICs may be comparable or even superior to minifill and ormocer composites.

Alonso et al[2] (2005) evaluated the shear bond strength of different sealant and filling materials used in minimally invasive dentistry to enamel. Enamel specimens were assigned into seven groups based on the materials- Fluoroshield, Clinpro, Dyract AP, F 2000, Vitremer, Fuji IX and Vidrion F. Fluoroshield resin sealant and Vitremer resin modified GIC showed statistically higher shear bond strength values than the conventional GICs.

Botelho et al[5] (2005) investigated the application of GIC to antibacterial conditioned dentin without rinsing and determined whether there is an affect on the material's Bond

strength. Chlorhexidine acetate, Benzalkonium chloride and Cetrimide were added to dentin conditioner at 1% and 5% concentrations. He concluded that only the 5% Benzalkonium chloride Dentin conditioner left in situ affected the bond strength of Fuji IX to Dentine.

Duque et al [15](2005) evaluated the antibacterial activity of GICs- Vitrebond, Ketac Molar, Fuji IX against Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus and Actinomyces viscosus using the Agar diffusion test. He confirmed significant antibacterial activity for two conventional GICs and one resin modified GIC. The resin modified GIC Vitrebond presented the best antibacterial activity against Streptococcus mutans and Srteptococcus sobrinus. It was concluded that Vitrebond >0.2% CHX > Ketac Molar > Fuji IX in terms of best Antibacterial activity to the least.

Pinheiro et al[35] (2005) assessed the total viable bacteria in infected dentin after sealing with glass-ionomer cement containing 1% metronidazole, 1% ciprofloxacin and 1% cefaclor. The glass-ionomer cement with 1% of

metronidazole, 1% of ciprofloxacin and 1% of cefaclor showed a significantly greater reduction in microbiota in the infected dentin in comparison to the reduction with the conventional ionomer cement (P< 0.01), with a mean reduction of 98.65% of all viable bacteria. The infected dentin after sealing with glass-ionomer cement with antibiotics showed, using scanning electron microscopy, the presence of bacterial aggregates, intertubular dentin with exposure of collagen fibers, and dentin tubules.

Trushkowsky et al [47](2005) investigated the history, characteristics and contributions of Atraumatic Restorative Treatment for use in preventing and controlling Dental Caries. He concluded that given the limitations of the ART technique, more research on this approach should be encouraged with the aim of improving the technique's effectiveness based on strength characteristics and antibacterial properties.

Marczuk – Kolada et al [30](2006) investigated the fluoride ion release and the antibacterial activities of GIC Fuji IX and compomer Dyract AP using direct potentiometry with an

Orion Fluoride ion selective electrode. He evaluated the antibacterial activity against the bacteria Streptococcus mutans, Streptococcus salivarius, Streptococcus sanguis, Lactobacillus casei. It was concluded that both materials released ion fluorides and after 24 hrs of bonding there was inhibition of bacterial growth by Fuji IX whereas Dyract AP did not show similar activity.

Peez et al [36](2006) evaluated the time dependence of physical-mechanical performance of GICs, namely the Ketac Molar Easymix and 4 handmix glass ionomer restoratives. The Compressive strength, Flexural strength, Acid erosion and Solubility was studied. His results demonstrated that Ketac Molar Easymix had the superior strength properties when compared to Fuji IX,Ionofil Molar, Vidrion R and Vitro Molar. He concluded that the high Flexural strength combined with the lowest susceptibility for acid attack and solubility in water made Ketac Molar Easymix (3M ESPE) the best performing material.

Wang et al [50](2006) investigated the failure modes of various types of GICs by Hertzian indentation test. Specimens of

10mm diameter x 2 mm thickness were prepared for six GIC products including silver reinforcement, ceramic reinforcement, resin modified type and without reinforcement. It was concluded that the type of GIC controls its failure mode and the inclusion of metallic or ceramic filler has little effect on increasing the load bearing capacity of GIC.

Wang et al [49](2006) examined the effect of early water exposure on the Shear bond strength of Fuji II [FT] GC, Fuji II LC[FL], Fuji IX GP Fast[FN] GC, Ketac Molar Quick and Ketac Molar Cements and concluded that no significant difference in shear strength was observed, with the Ketac Molar and Ketac Molar Quick being the strongest restorative materials after shear punch testing. He also added that contrary to current teaching, early exposure to water did not weaken GICs and a marginal increase in strength was actually observed for some materials.

Yamazaki et al [54](2006) evaluated the viscoelastic behaviour of six glass ionomer cement and determined whether there was a correlation to fracture toughness. Three conventional

GICs namely Alpha – Fil, Alpha - Siver , Ketac – Molar and three resin modified GICs namely Vitremer, Fuji II LC and Photac – Fil Quick were evaluated using measurements of compressive strength, flexural strength and diametral tensile strength. The test specimens for the compressive strength and diametral tensile strength were standardized to 4mm x 6mm and 4mm x 2mm respectively. He demonstrated that there was no statistical difference in fracture toughness amoung the GICs tested although the resin modified GICs displayed higher fracture toughness.

Czamecka et al [11](2007) studied the bonding of GICs to carious and non carious dentine using Fuji IX GP, Fuji IX capsulated, Fuji IX fast capsulated(all GC Japan), Ketac Molar, Ketac Molar Aplicap(3M-ESPE Germany).He concluded that the shear bond strength to sound dentine was found not to differ statistically from carious dentine for the automix cements whereas for hand mix cements the shear bond strength to sound dentine was found to be higher to carious dentine stressing the importance of interaction of GIC with the tooth in developing strong bonds.

Da silva et al [12](2007) studied the in vitro antibacterial activity of four GIC (Fuji IX, Ketac Molar, Vidrion R and Vitromolar) indicated for ART against Stretococcus mutas, Stretococcus sobrinus, Lactobacillus acidophilus and Actinomyces viscosus and concluded that Fuji IX and Ketac Molar presented the most effective antibacterial activity considering the ART approach.

Davidovich et al [13](2007) evaluated the antibacterial properties of restorative materials – three GICs and Zinc oxide eugenol in vitro.Streptococcus mutans, Actinomyces viscosus and Enterococcus faecalis were the test microorganisms using the direct contact test of the freshly prepared and one week aged materials. It was concluded that conventional GICS used in ART showed antibacterial surface properties against cariogenic bacteria for atleast one week and has crucial importance in preventing secondary caries.

Gama-Teixeira et al [19](2007) aimed to study, in vitro, the potential to inhibit secondary caries of restorative materials namely GIC, Amlagam, light cured composite resin, ion releasing composite and light cured fluoride containing

composite resin. The specimens were thermocycled and exposed to a cariogenic challenge using Streptococcus mutans. It was concluded that the restorative materials GIC, Amalgam and ion releasing composite may reduce secondary caries formation.

Knight et al [25](2007) evaluated the measure of caries at the dentin restoration interface of bonded composite resin and auto cured GIC (Riva Fast, Fuji IX Fast, Ketac Molar Quick and Fuji VII) restorations and measured the amount of surface degradation occurring in the restorative materials. The specimens were disinfected and placed in a continuous culture of Streptococcus mutans for two weeks. It was concluded that placing a GIC restoration into dentine protects the surrounding tooth from caries but degradation of the restoration surface occurs.

Lopes et al [27](2007) investigated the shear bond strength to enamel of rest seats made with a GIC cement Fuji IX GP Fast, resin modified GIC Fuji II LC and a composite resin. Under monotonic and cyclic loading. It was demonstrated that Fuji IX GP Fast promoted the lowest shear bond strength fatigue

limit. He concluded that fatigue testing can provide a better means of estimating the performance of rest seats made with dental restoratives.

Mallmann et al [29](2007) evaluated the compressive strength of two GICs, a conventional one Vitro Fil – DFL and a resin modified material Vitro Fil LC – DFL, using two test specimen dimension , 6mm x 4mm and 12mm x 6mm. He concluded that the resin modified GIC Vitro Fil LC – DFL obtained the best results irrespective of the specimen dimensions and for both GICs , the 12mm x 6mm matrix lead to higher compressive strength than the 6mm x 4mm matrix.

Silva et al [43](2007) evaluated the surface micro hardness of four GICs (Fiji IX, Ketac Molar, Vidrion R,Vitromolar) and a composite resin (Z 250).Ten specimens of each GIC with 8mm diameter and 5 mm height and micro hardness measurements were taken at 1 day and 1 week at initial setting reaction. It was concluded that values of microhardness increased after 1 week with the exception of Fuji IX.

Badet et al [3](2008) observed a strong correlation between the saliva Lactobacillus count and Dental Caries, the higher the DMF Index, the higher the number of Children harbouring a high Lactobacillus count. He concluded that a better understanding of the Lactobacillus Species and its Effect on Caries dynamics could allow the development of new tools for prevention.

Dowling et al [14](2008) evaluated three GI restorative systems to determine if encapsulated GI restoratives performed more favourably than the hand-mixed equivalents prepared with powder contents progressively decreased from that recommended by manufacturers in 10% increments for a constant weight of liquid which are routinely employed in clinical practice. The authors concluded that encapsulated GI restoratives are a potential solution to the operator induced variability associated with hand-mixed GI restoratives.

Hoszek et al [21](2008) assessed if the addition of chlorhexidine gluconate to glass-ionomer cement adds an effect that enables it to be used as a varnish for the temporary coating of surfaces at risk for caries. It was concluded that

the addition of CHX and CHX-TA adds antibacterial properties to GI and the release of fluoride is decreased.

Yesilyurt et al [59](2008) evaluated the influence of irradiation on the dentine shear bond strength of two conventional GICs. Half the Specimens were irradiated, while the other half served as non irradiated controls. It was concluded that Irradiation may have an Adverse effect on bond strength of GICs depending on the application sequence.

Bonifacio et al [4](2009) evaluated mechanical properties of glass ionomer cements (GICs) used for atraumatic restorative treatment. In this study wear resistance, Knoop hardness (Kh), flexural (F(s)) and compressive strength (C(s)) were evaluated. The GICs used were Riva Self Cure (RVA), Fuji IX (FIX), Hi Dense (HD), Vitro Molar (VM), Maxxion R (MXR) and Ketac Molar Easymix (KME).He concluded that KME and FIX presented the best in vitro performance. HD showed good results except for early-term wear.

Koenraads et al [26](2009) tested the compressive strength of two newly developed glass-ionomer materials for use with the

Atraumatic Restorative Treatment(ART) approach in class II cavities. The authors concluded that class II ART cavities restored with the newly launched Glass-carbomer and Ketac Molar Easymix were not significantly more fracture resistant than comparable restorations using the conventional glass-ionomer Fuji IX.

Nakajo et al [32](2009) evaluated the inhibitory effects of GIC on the acid production of caries-related oral streptococci, and to identify the components responsible for the inhibition. The author concluded that the GIC elute used in his study inhibits the acid production of caries-related oral streptococci at acidic pH and that the effect is due to fluoride derived from the GIC. Thus, adjacent to GIC fillings, bacterial acid production and the subsequent bacterial growth may decrease, establishing a cariostatic environment.

Yesilyurt et al [60](2009) evaluated the antibacterial effects, physical properties and bonding strengths of conventional glass-ionomer cements (GICs) containing antibiotics and determined the optimal concentration of antibiotics addition for use with the ART approach. Fuji IX GIC was used as a

control. Three antibiotic mixtures, ciprofloxacin, metronidazole and minocycline, were added to powdered GIC (Fuji IX) to obtain concentration ratios of 1.5, 3.0 and 4.5% w/w. The antibacterial activity of each GIC was evaluated against Streptococcus mutans or Lactobacillus casei using agar-diffusion methods.All tested groups showed a significantly greater inhibition with growth of the selected bacteria in comparison to the control groups ($p < 0.01$). However, the 3% and 4.5% concentration ratios of antibiotics had significantly lower compressive strength and lower bond strength to dentin than the control group ($p = 0.003$). The GIC-containing antibiotics were effective in inhibiting S Mutans and L Casei. The addition of a 1.5% antibiotic mixture was optimal to giving appropriate physical and bonding properties.

Zhang et al [62](2009) analysed the count of Streptococcus mutans (S. mutans) and Lactobacilli (LB) in different groups and the cases in dental caries and to research the synergistic effect of Streptococus mutans and Lactobacillus in the process of dental caries. He concluded that the pathopoiesis capability of Streptococus mutans and Lactobacillus enhanced

when the extent of caries increased. In the older group, their synergism role play a lead position. In evolution period and arrested caries, Streptococus mutans and Lactobacillus were difference only in quantity and their solo cariogenic potential all enhanced in active stage, but there were not correlation on pathopoiesis capability and active or stationary phase.

Carvalho et al [10](2010) evaluated the shear bond strength of three glass ionomer cements (GIC) to enamel and dentine Twenty four specimens of each GIC: Fuji IX (FJ - GC), Ketac Molar Easymix (KM - 3M ESPE) and Maxxion (MX - FGM) were prepared according to the Atraumatic Restorative Treatment (ART) (12 enamel and 12 dentine), in a bonding area of 4.91 mm and immersed in water (37 degrees C, 24h). The shear bond strength was tested in a universal testing machine. He concluded that Ketac Molar has the best adhesion to both enamel and dentine, followed by Fuji IX and Maxxion.

MATERIALS AND METHODS

Materials used:

1. Streptococcus mutans strains ATCC 25175 (Himedia Labs) – 2 vials
2. Lactobacillus casei strains ATCC 393 (Himedia Labs) – 2 vials
3. Blood agar (Himedia Labs) – 100gms
4. BHI broth (Himedia Labs) – 100 gms
5. Lactobacillus de man Rogosa Sharpe (MRS) agar (Himedia Labs) – 100 gms
6. Lactobacillus de man Rogosa Sharpe (MRS) broth (Himedia Labs) – 100 gms
7. Glass Ionomer Fuji IX (GC Gold Label, Tokyo, Japan) –15gms– 4nos
8. Teflon Moulds 10 mm x 2 mm – 4 slabs
9. Teflon tubes 6 mm x 4 mm – 20 nos
10. Plastic tubes 4 mm x 3 mm – 20 nos
11. Borosilicate test tubes – 25 nos.
12. Disposable culture plates – 20 nos.
13. Aluminium foil – 1 nos

14. Cotton roll – 1 nos
15. Distilled water
16. Minocycline RM 9231(Himedia Labs) – 300 gms
17. Metronidazole (CEEAL Analtycal Labs) – 300 gms
18. Ciprofloxacin (CEEAL Analtycal Labs) – 300 gms

Armamentarium used :

1. U V Irradiation sterilizer.
2. Autoclave.
3. Incubator.
4. High Performance Liquid Chromatography unit. (Shimadzu, Prominence, Isocratic LC 20 AT – Japan)
5. Instron Universal Testing Machine (LR 100 K Lloyd Instruments, UK)

I. PREPARATION OF ANTIBACTERIAL CEMENT

- A Conventional restorative glass ionomer cement (Fuji – IX, Tokyo, Japan) was used as the control group.
- In the experimental groups three antibiotics Metronidazole (CEEAL Analytical Lab), Ciprofloxacin (CEEAL Analytical Lab)

and Minocyline (Himedia Lab) were added to the powder in 0.75%, 1.5%, 3.0% w/w.

- In group I - 75mg of powder was removed from 10g of GIC powder and the three antibiotics were added in the combination of 25mg Metronidazole + 25mg of Ciprofloxacin + 25mg of minocylcine to obtain 75mg of the drug mixture and added to the GIC powder to get the first experimental group of 0.75% w/w.
- In group II -150mg of powder was removed from 10g of GIC powder and the three antibiotics were added in the combination of 50mg of Metronidazole + 50 mg of Ciprofloxacin + 50mg of minocycline to obtain 150mg of the drug mixture and added to the GIC powder to get the second experimental group of 1.5% w/w.
- In group III - 300mg of powder was removed from 10g of GIC powder and the antibiotics were added in the combination of 100mg of Metronidazole +100 mg of Ciprofloxacin + 100mg of minocycline to obtain 300mg of the drug mixture and subsequently added to the GIC powder to get the third experimental group of 3.0% w/w.

All the three bottles were mixed in a mechanical agitator to get a homogenous dispersion of the drugs in the GIC powder.

II. ANTI BACTERIAL ACTIVITY SCREENING TESTS:

The antibacterial effect of the three groups of cements along with control groups was tested against streptococcus mutan (Himedia Lab) and Lactobacillus casei (Himedia Lab).The study was further carried out as set cement subgroup and unset cement subgroup for Streptococcus mutans and Lactobacillus casei bacterial strain.

In the streptococcus groups the strains were stored at -20°C and were cultured on Blood agar (Himedia) at 37°C for 24 hours in 50% CO_2. Single colonies from plates were transferred into Brain Heart Infusion Broth (Himedia) and incubated for 37°C for 24 hours.Suspensions of the strains were prepared in Phosphate Buffer Solution at ca 1.5 x 10^8 organisms/ml by using the McFarland 0.5 turbidity tube. The blood agar culture plates were flood inoculated by the prepared turbid suspension of Streptococcus strains.

In the Lactobacillus group, the strains were stored at -20°C and were cultured on Lactobacillus de mann Rogosa Sharpe (MRS) Agar (Himedia) plates at 37°C for 24 hours.

Suspension of the strains were prepared in Phosphate Buffer Solution at ca 1.5 X 10^8 organisms/ml by using the McFarland 0.5 turbidity tube. The Lactobacillus de mann Rogosa Sharpe (MRS) Agar (Himedia) plates were flood inoculated by the prepared turbid suspension of Lactobacillus strains. Before placement of the set and unset specimens, the surface of the plates was air dried by leaving the specimens at 37°C for 15 minutes.

Teflon molds were prepared with 10mm internal diameter and 2mm depth walls on a Teflon slab.

For the set group cement discs with the following specification was prepared – 10mm as dm and 2mm thick by mixing powder and liquid from each group in a ratio of 3.6:1.The mixed cement was poured onto the wells of the Teflon moulds of 10mm diameter and 2mm thickness and was allowed to set for 30 minutes at room temperature. After 30 minutes the specimens were UV sterilized. The set specimens of each group were then placed on the blood agar plate for Streptococcus mutans and Lactobacillus MRS Agar plates for Lactobacillus casei set group respectively.

For the unset cement group, disc specification were, 10mm dm and 2mm thick wells were cut from the agar plates by using a sterile-glass made pipette. Cements from each experimental group were mixed in a powder and liquid ratios of 3.6:1.The freshly mixed cement was now poured in the wells cut on the corresponding agar plates.

The set cement disc specimens and unset specimens of both the subgroup of Streptococcus mutans placed in anareobic jar and Lactobacillus casei were incubated for 48 hours at 37°C.

After the incubation period of 48 hours, zones of inhibition around the specimens were measured.The sizes of the inhibition zones were calculated by substracting 10mm (diameter of wells) from the average diameter of the zones for each specimen and control group. Five specimens were tested for each experimental groups.

III. RELEASE OF ANTIBACTERIAL DRUGS

The Teflon molds of 10mm internal diameter and 2mm thick were used in the preparation of the specimens. The specimens were prepared with a powder liquid ratio of 3.6:1. The mixed cement was poured into the wells of the Teflon mold and was allowed to set for 30 minutes to get

a round disc-shaped GIC Specimens.The prepared samples, after 30 minutes were completely dissolved in 2.5ml distilled water and stored at 20°C for 24 hours and 7 days.

Sample concentration analyses were done in Shimadzu, Prominence Isocratic LC 20 AT High performance liquid chromatography (HPLC) system. The chromatographic reversed-phase column used for analysis was ODS (Octa Decyl Silane) column C18 (Phenominex, Gemini 25mm x 4.6mm) Rheodyne & 10 millipore millimeters of phosphate buffer solution (ph 2.6 60:40) were used for the mobile phase at a flow rate of 1ml / minute detector wavelength Metronidazole (282 nm), Ciprofloxacin (276nm) & minocycline (268 nm).Five specimens were tested from each experimental groups.

IV. COMPRESSIVE STRENGTH EVALUATION:

Teflon molds with 4mm internal diameter and 6mm height were prepared for sample preparation of the experimental groups for compressive strength evaluation,. The cements were mixed from each experimental groups in the powder and liquid ratio of 3.6:1 and packed into the molds to get cylindrical shaped GIC specimens. The specimens were stored at 37°C in 100% humidity for 1 hour after mixing and

placing into the molds. The specimens were then stored in distilled water for 24 hours and 7 days.

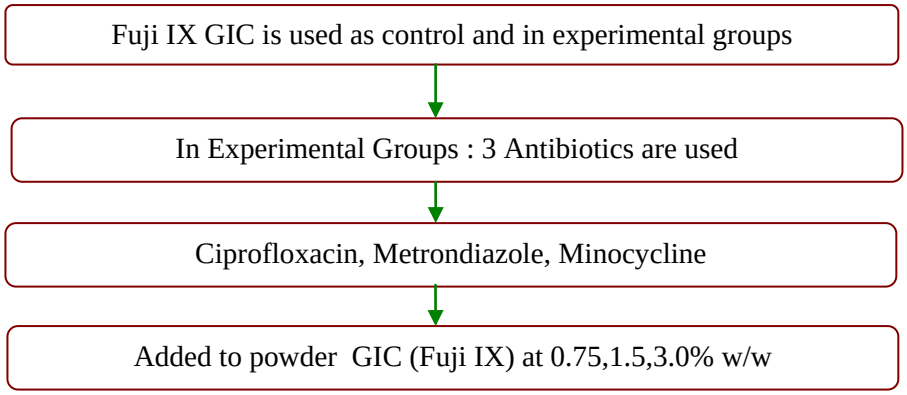
The compressive strength testing was performed by applying compressive load using Instron Universal Testing machine (LR 100 K Lloyd Instrument Ltd) at a crosshead speed of 1mm/minute^{-1}. The compressive strength for each specimens was determined. Five samples were tested in each experimental group.

V. SHEAR BOND STRENGTH TO DENTIN EVALUATION:

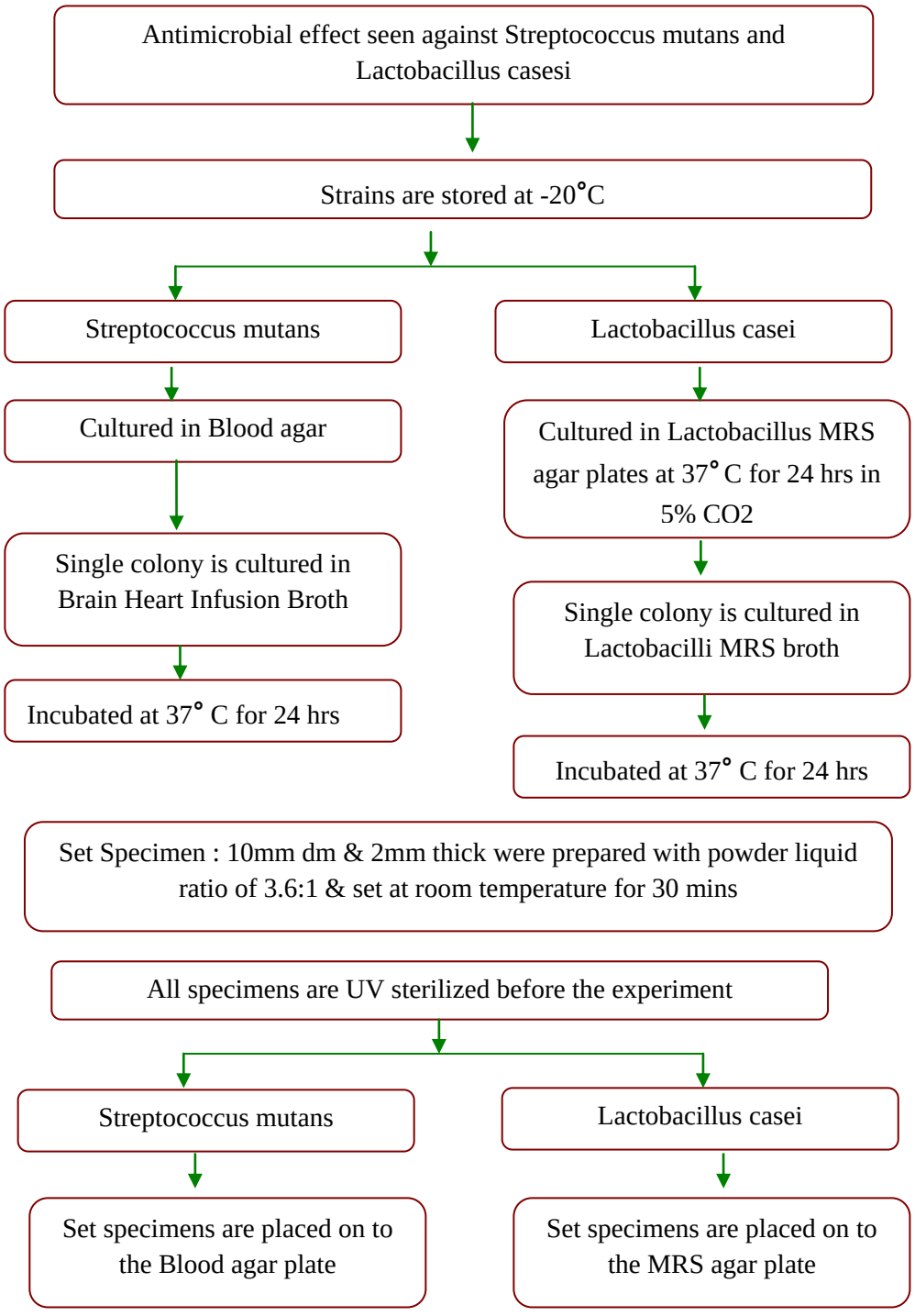
Plastic tubes were prepared with 3mm internal diameter and 4mm height for sample preparation of the experimental groups for Shear Bond Strength evaluation, Twenty mandibular first human molars whose occlusal dentin was reduced to 2 mm beyond the DEJ was polished flat with 200,400,600 grit silicon carbide papers to expose the flat surface. The dentin surface was conditioned with a polyacrylic acid (cavity conditioner ph 1.65) for 10 seconds. After completion of the surface procedures the cement was mixed in a powder and liquid ratio of 3.6:1. The plastic tubes of 3mm internal diameter and 4mm height was placed into the center of the prepared dentin surfaces. The mixed cement is then packed into the cylindrical tubes.

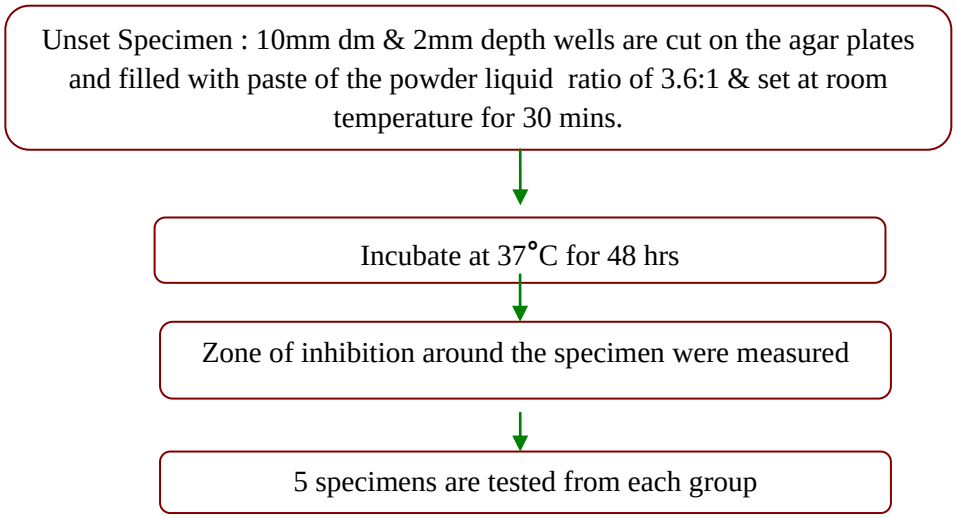
The prepared specimens were stored at 37°C and 100% for 24 hours. The specimens were tested for shear bond strength using a Universal Testing Machine (LR 100 K Llyod Instrument Ltd) at a crosshead speed of 1mm/minute^{-1}. The shear bond strength was determined for each specimen.Five specimens were tested for each group.

I. ANTIBACTERIAL CEMENT PREPARATION

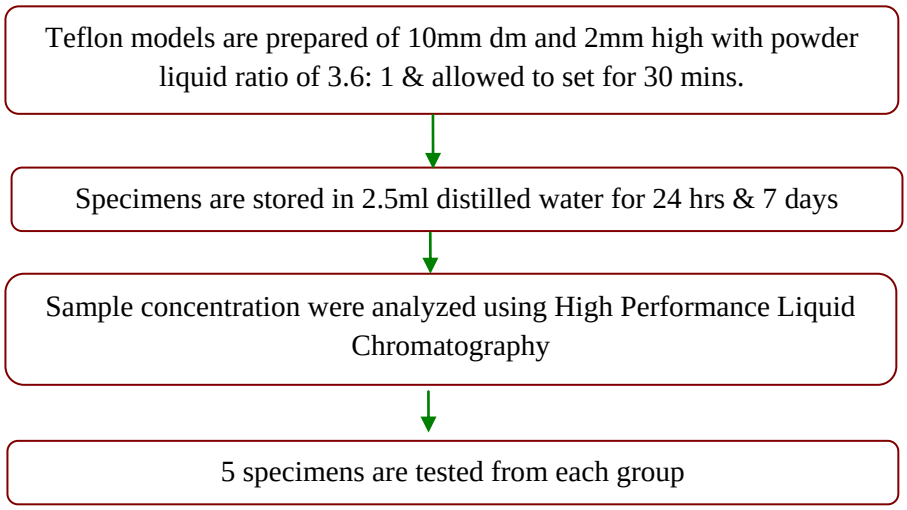


II. ANTIMICROBIAL ACTIVITY SCREENING TESTS:

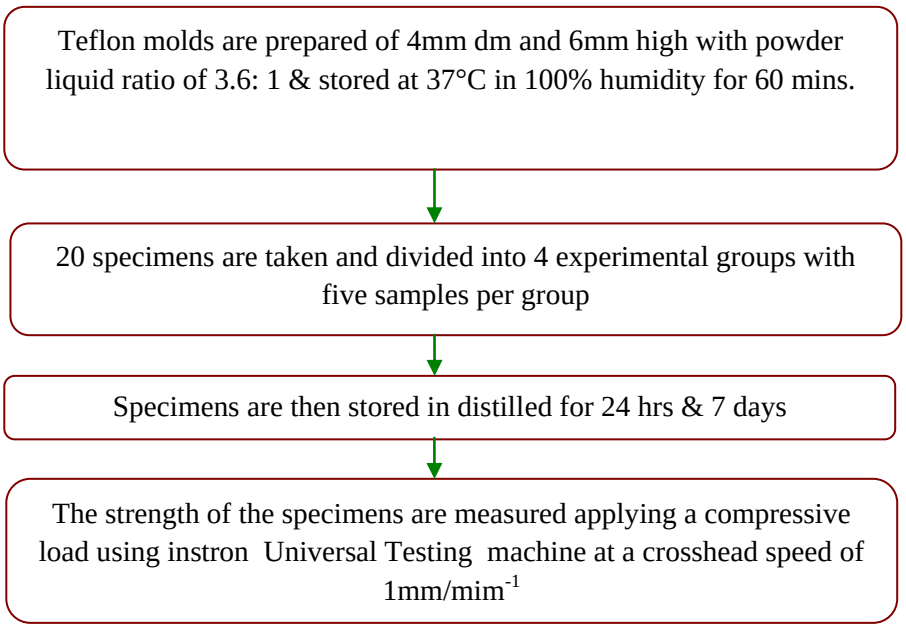



Unset Specimen : 10mm dm & 2mm depth wells are cut on the agar plates and filled with paste of the powder liquid ratio of 3.6:1 & set at room temperature for 30 mins.
Incubate at 37°C for 48 hrs
Zone of inhibition around the specimen were measured
5 specimens are tested from each group

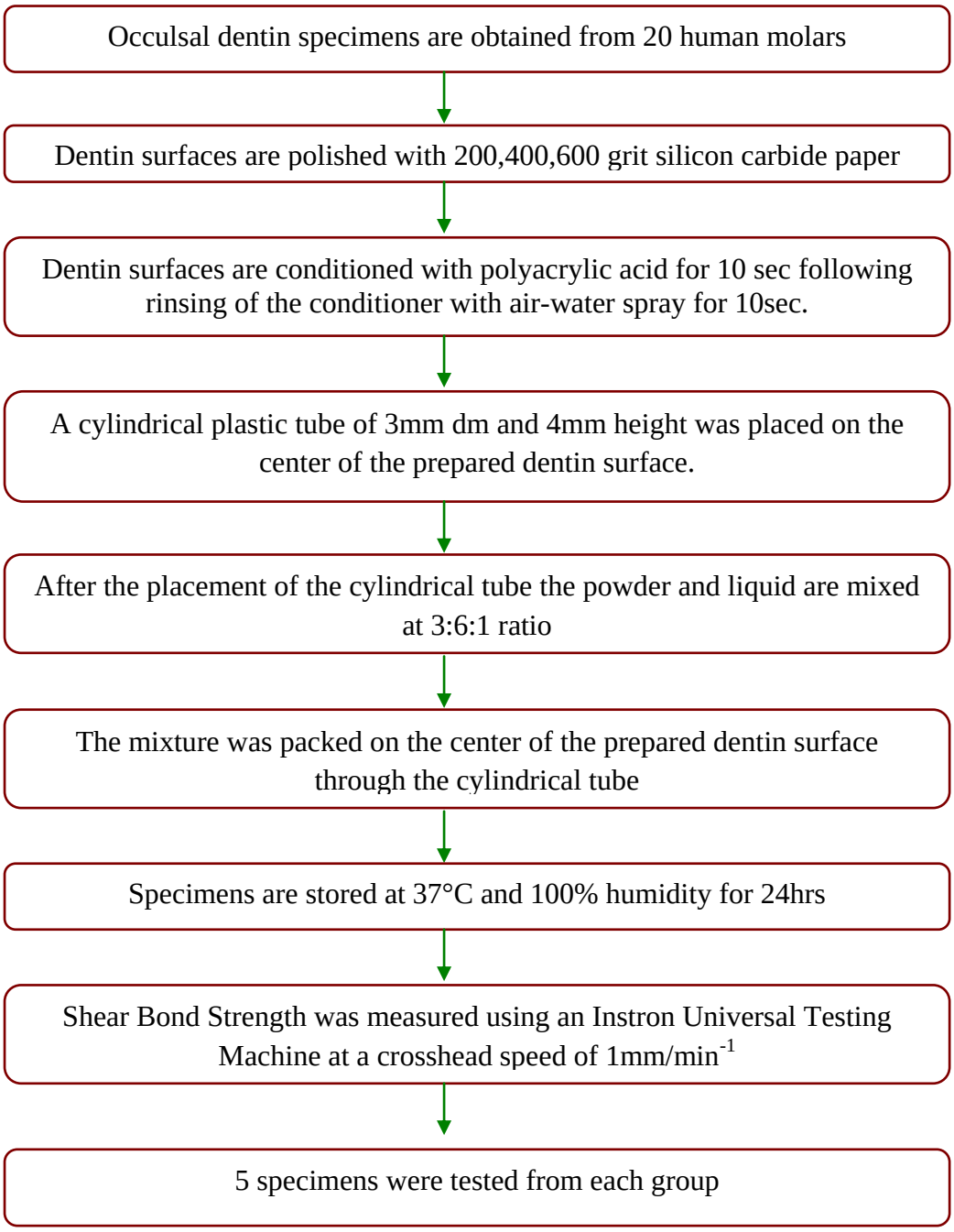
III. RELASE OF ANTIBACTERIAL DRUGS



IV. COMPRESSIVE STRENGTH



SHEAR BONDING STRENGTH:



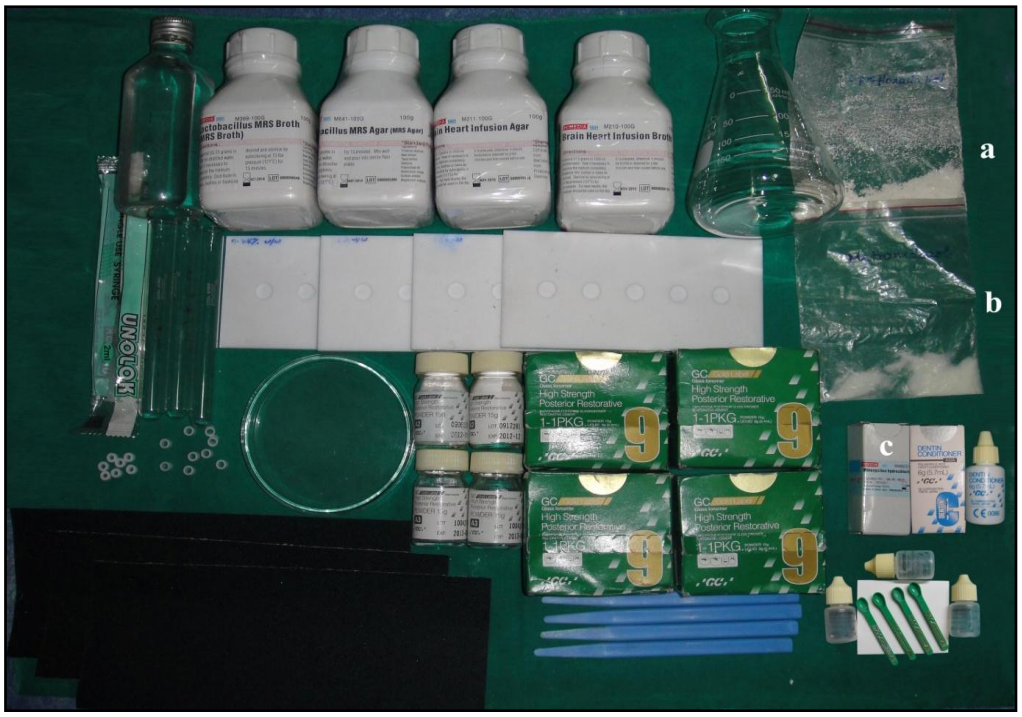


Figure 1: Armamentarium a) Ciprofloxacin b)Metronidazole c) Minocycline

Figure 2 : Streptococcus mutans and Lactobacillus casei bacterial strains

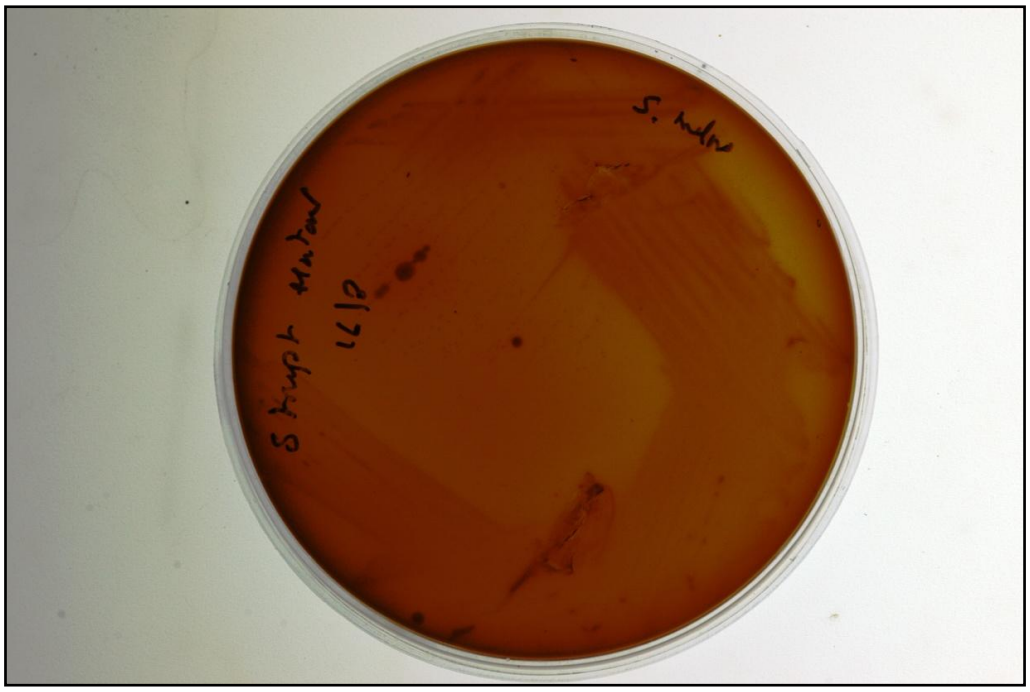


Figure 3: Individual colonies growth of Streptococcus mutans

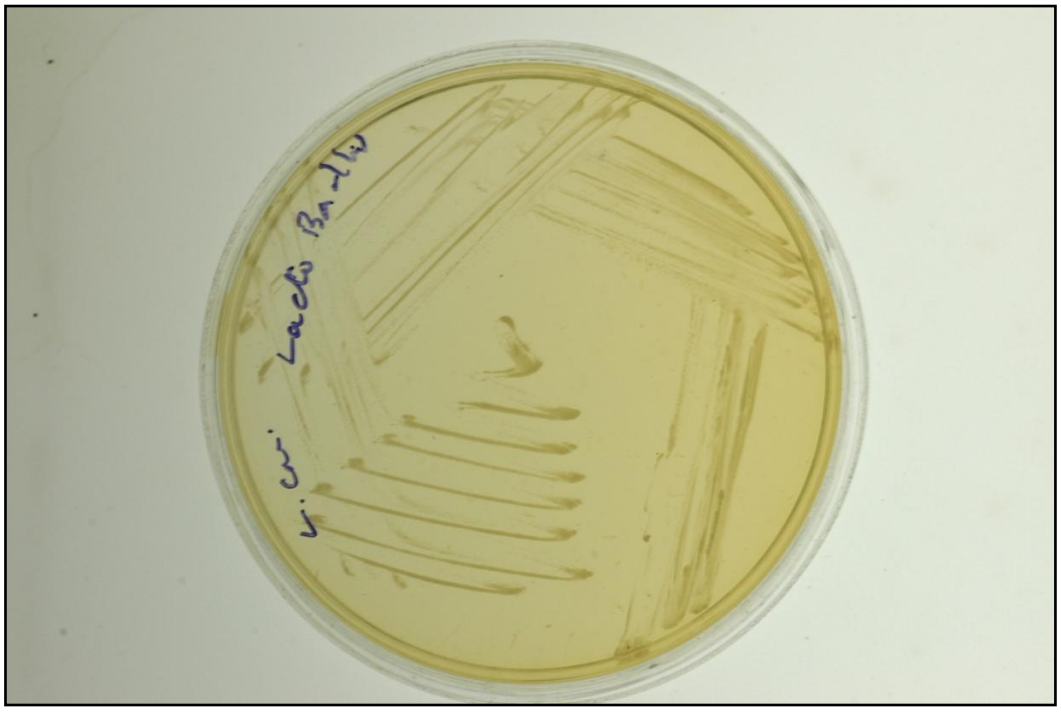


Figure 4: Individual colonies growth of Lactobacillus casei

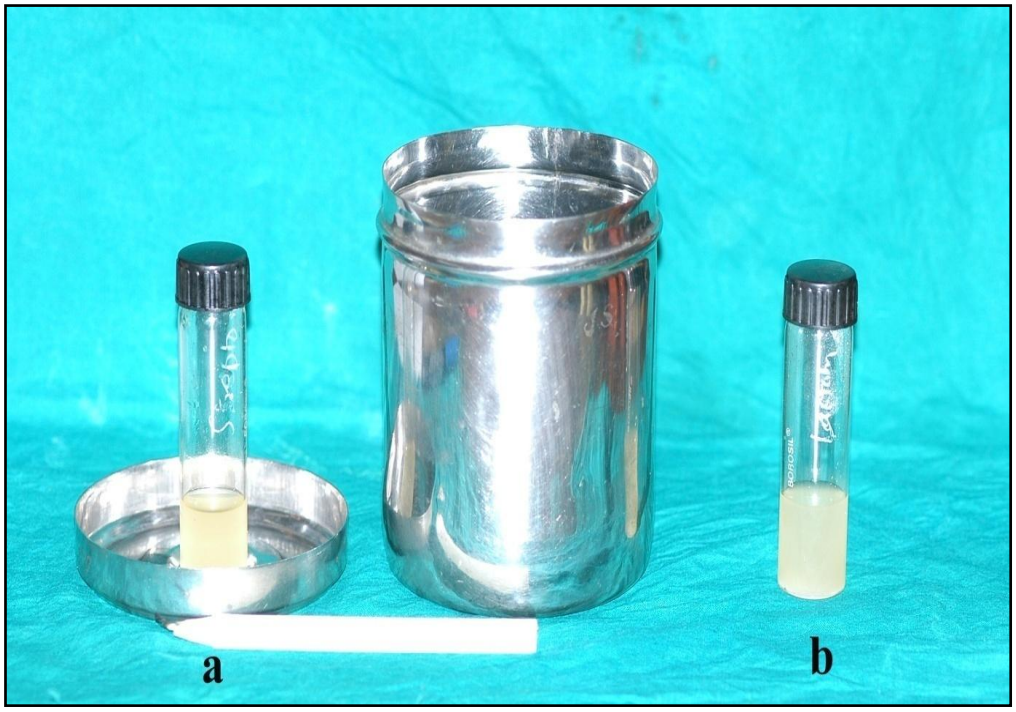


Figure 5: a) Streptococcus mutans broth

b) Lactobacillus casei broth

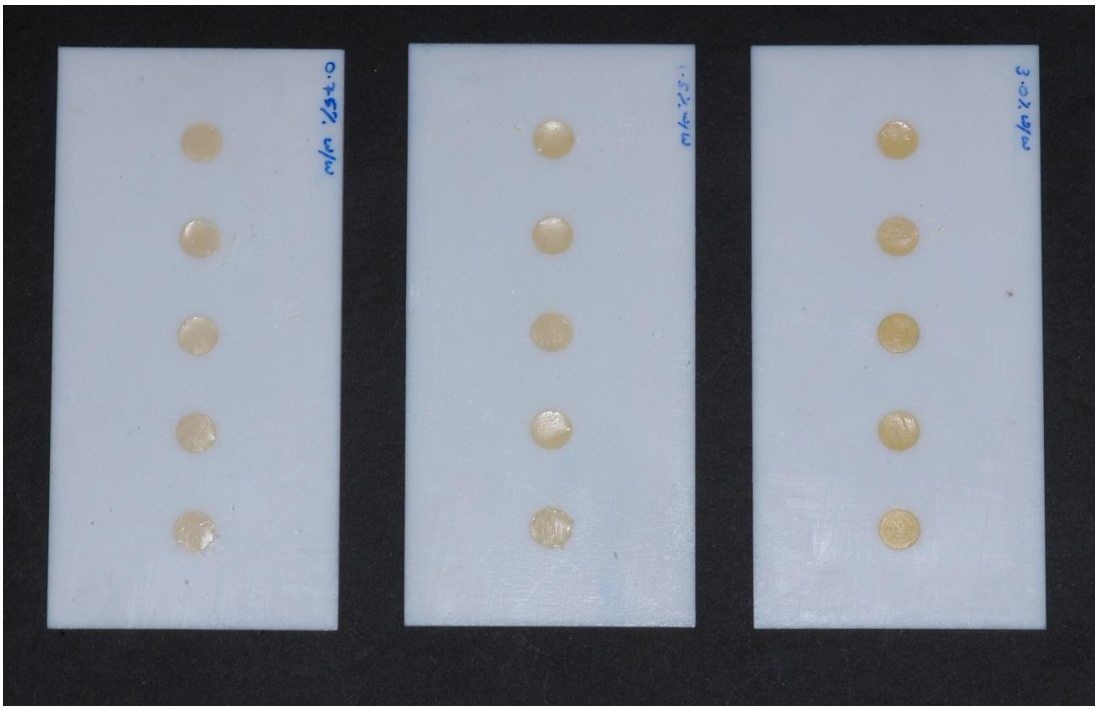


Figure 6: Prepared samples for microbial test

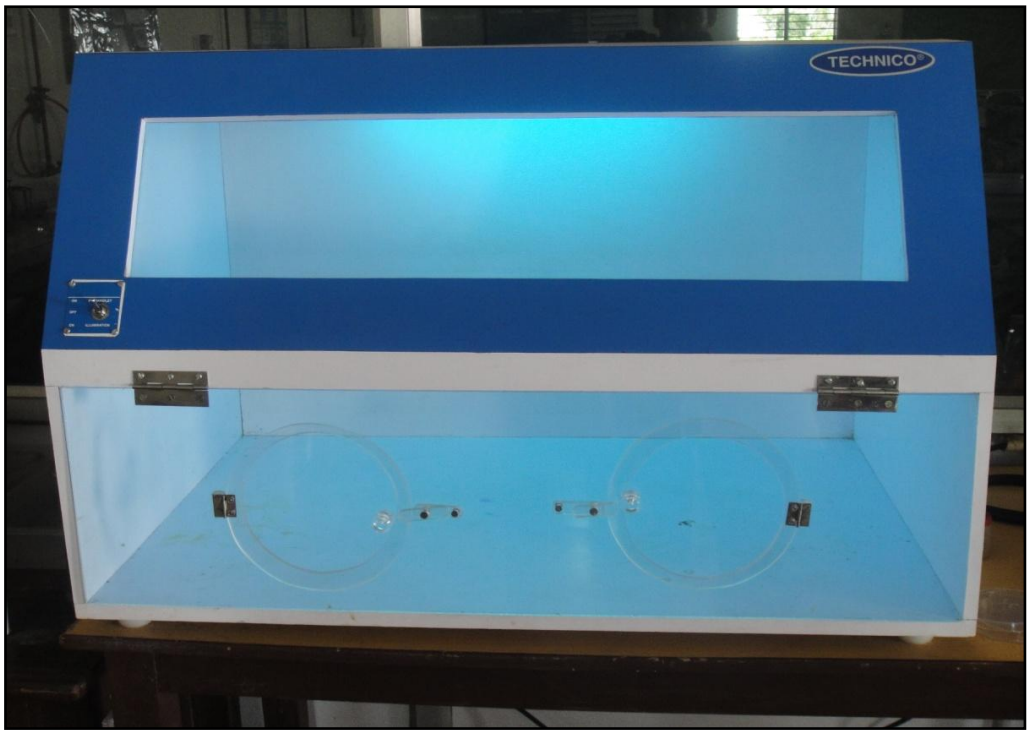


Figure 7: Ultraviolet sterlizer

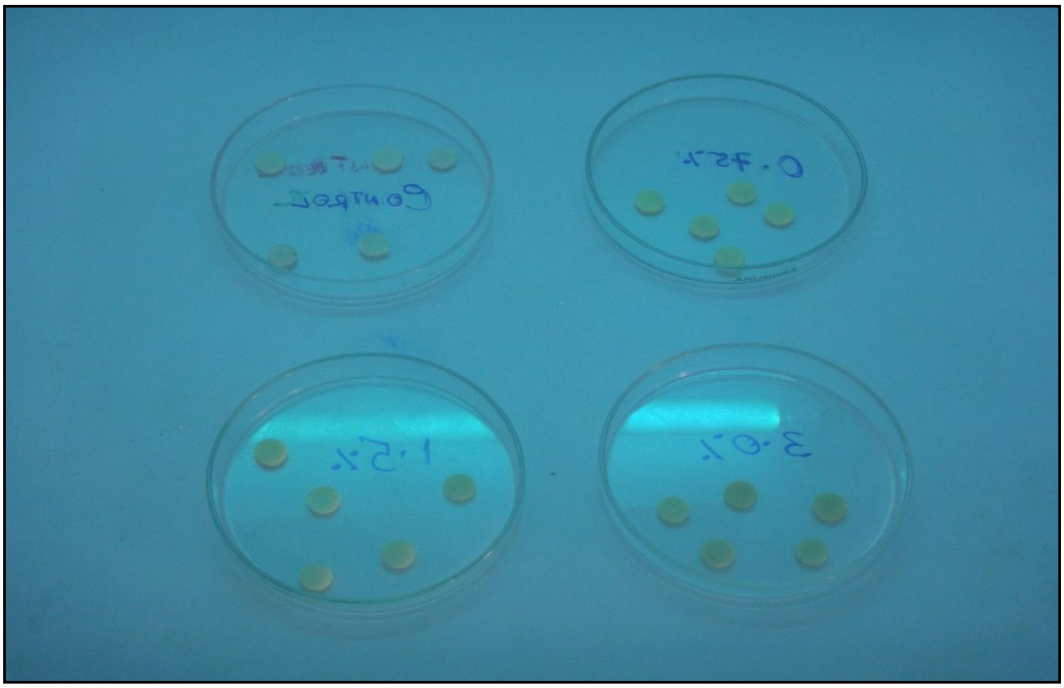


Figure 8: ultraviolet sterilized samples

Figure 9: Incubator

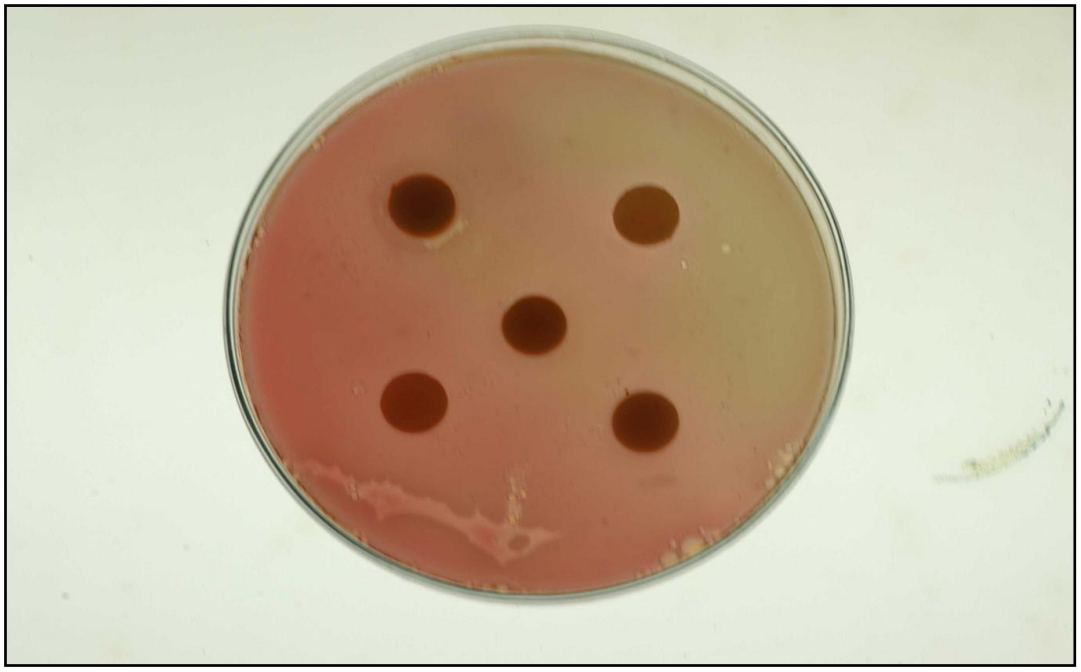


Figure 10: Control group - set specimens in S.mutans showing zone of inhibition

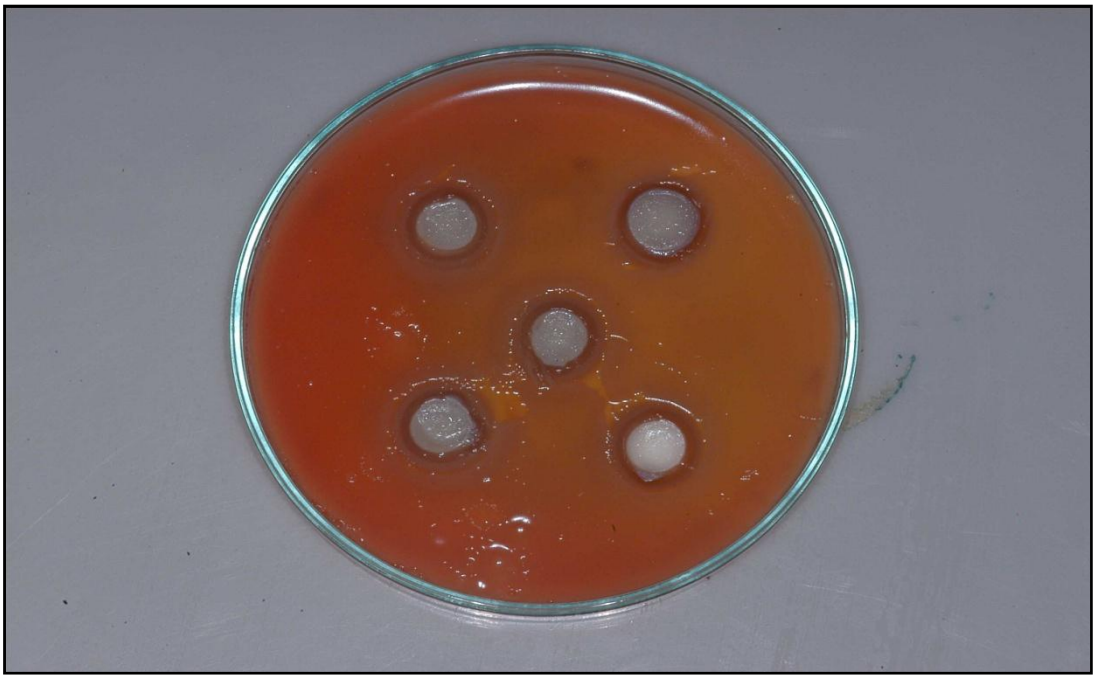


Figure 11: Control group -unset specimens in S.mutans showing zone of inhibition

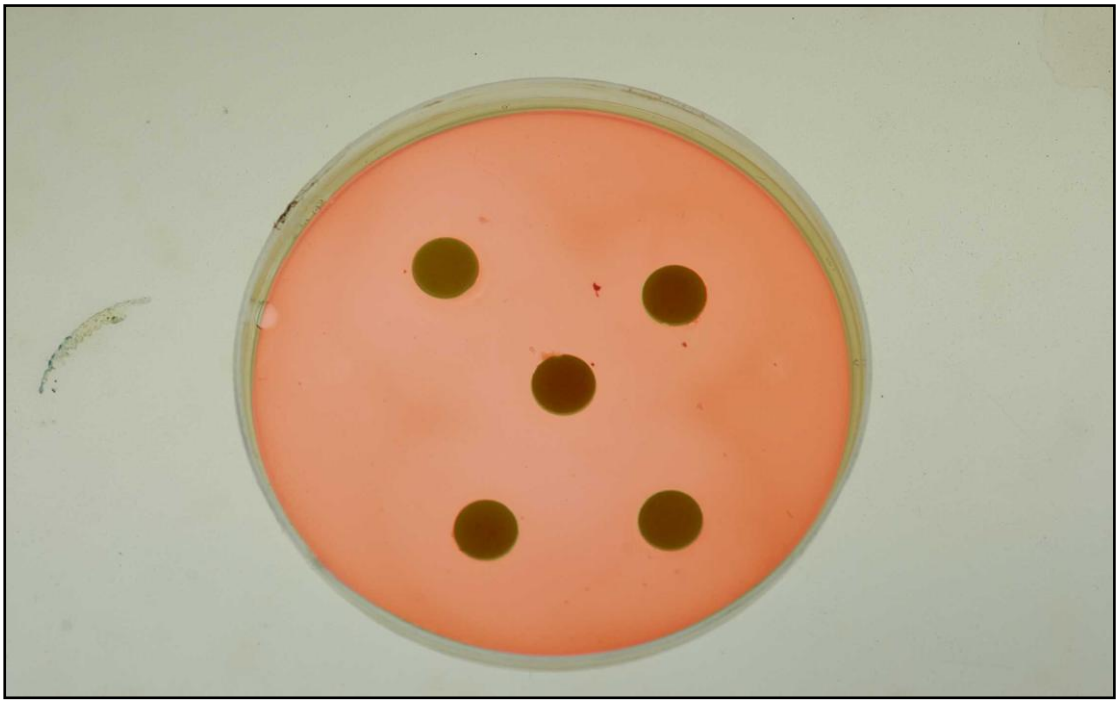


Figure 12: Group I - set specimen in S.mutans showing zone of inhibition

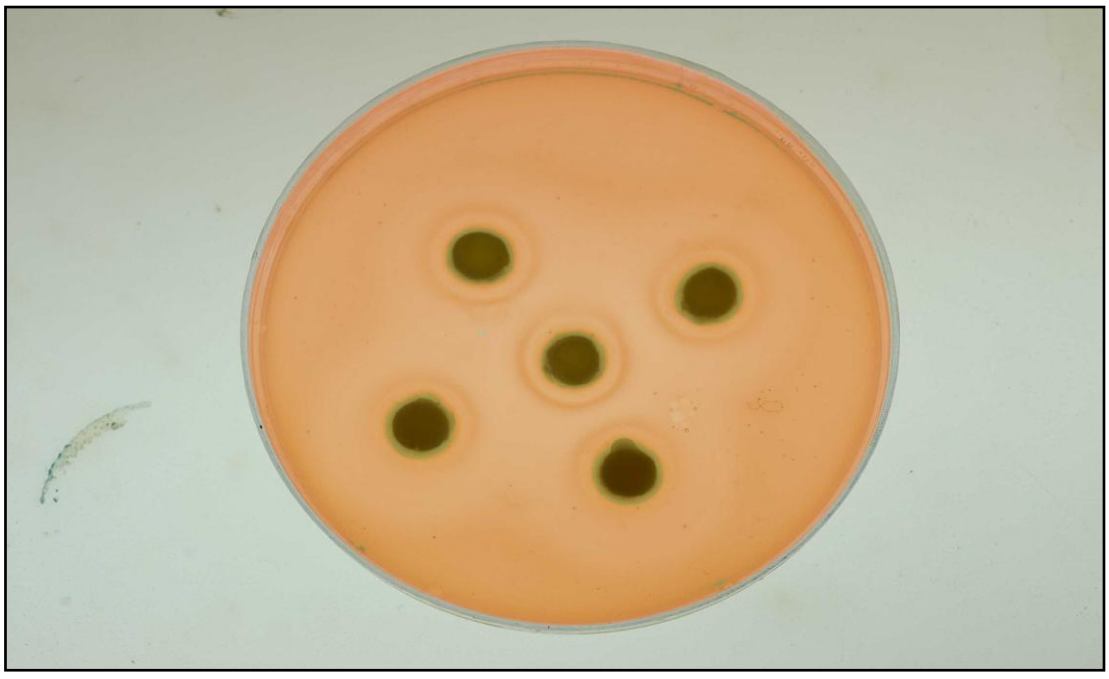


Figure 13: Group I - unset specimen in S.mutans showing zone of inhibition

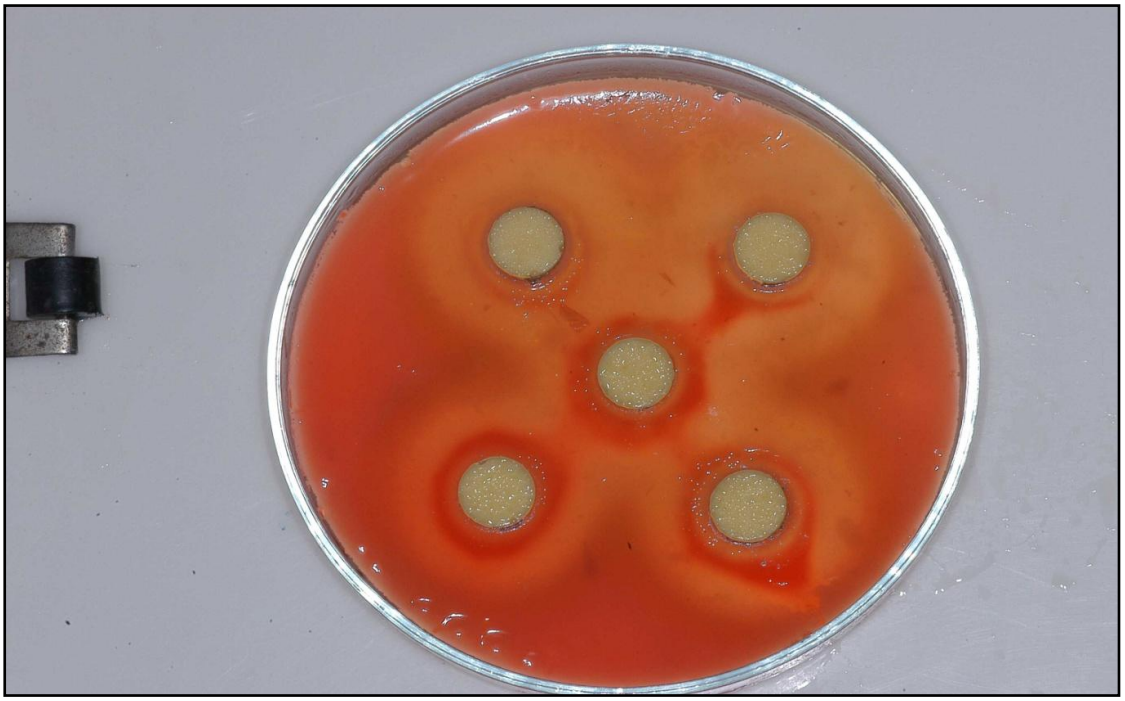


Figure 14: Group II - set specimen in S.mutans showing zone of inhibition

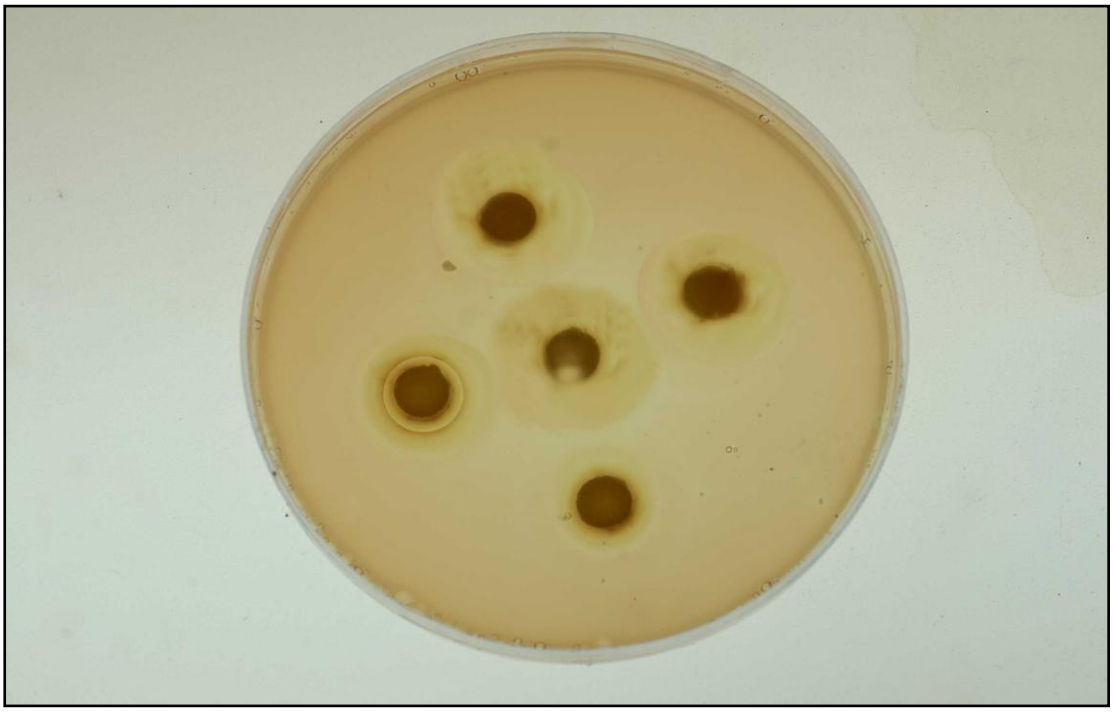


Figure 15: Group II - unset specimen in S.mutans showing zone of inhibition

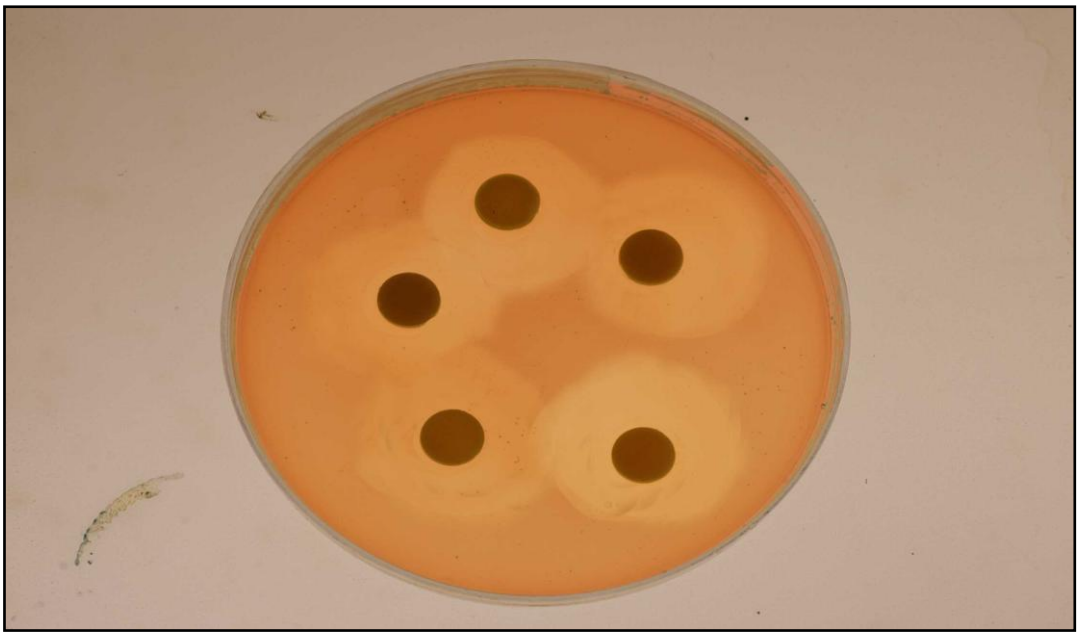


Figure 16: Group III - set specimen in S.mutans showing zone of inhibition

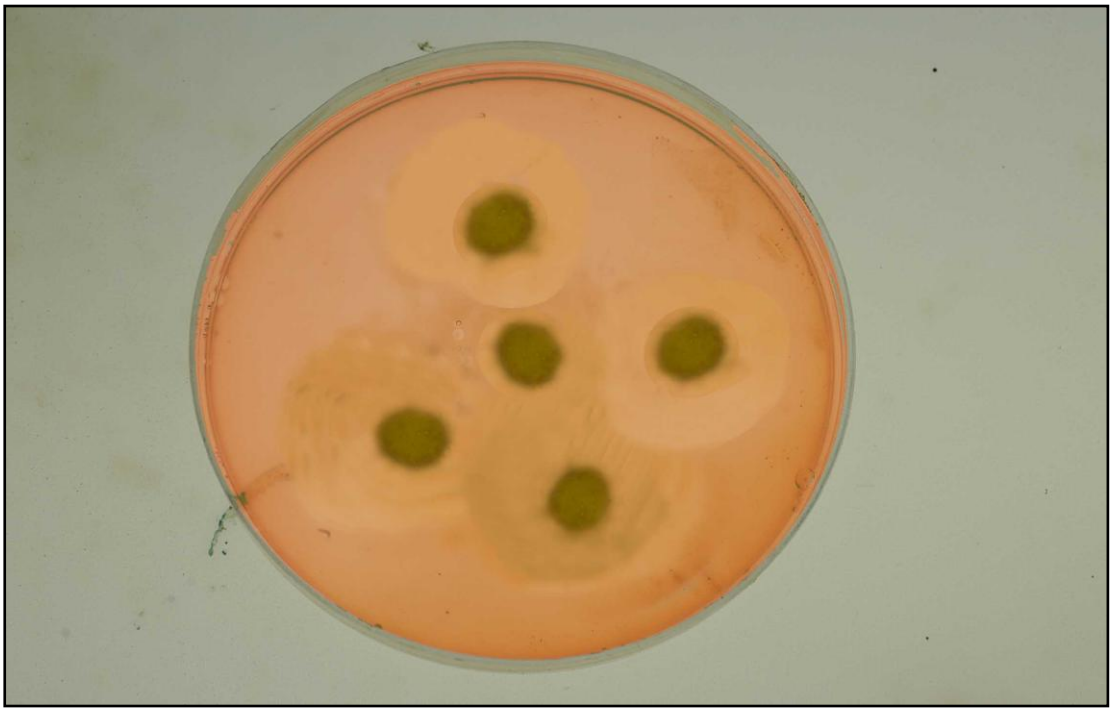


Figure 17: Group III - unset specimen in S.mutans showing zone of inhibition

Figure 18: control group - set specimen in L.casei showing zone of inhibition

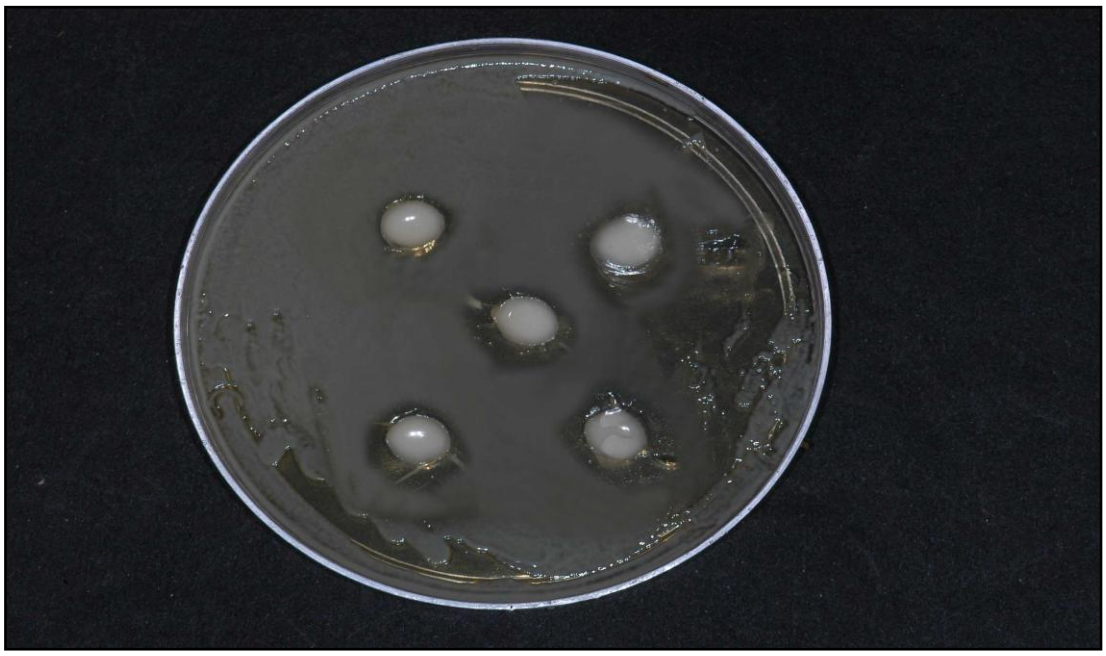


Figure 19: control group - unset specimen in L.casei showing zone of inhibition

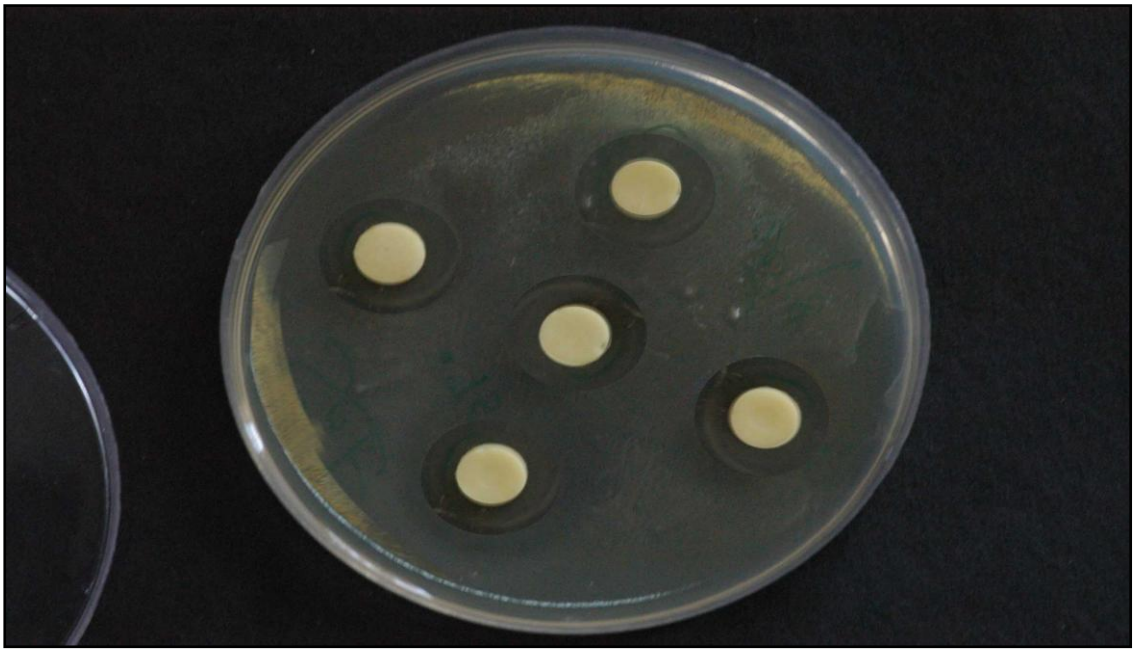


Figure 20: Group I - set specimen in L.casei showing zone of inhibition

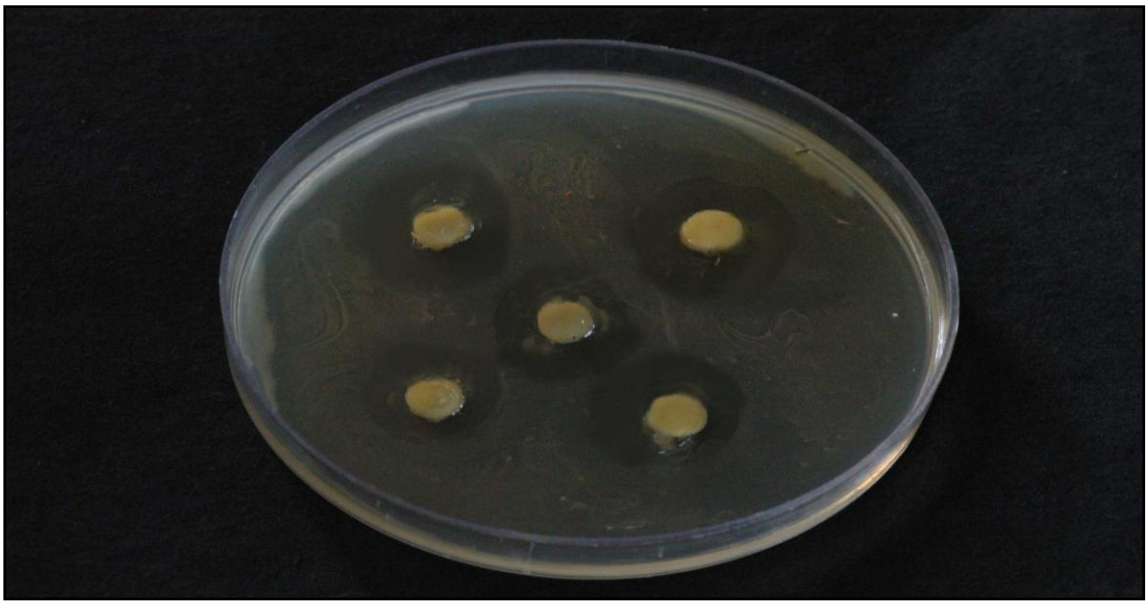


Figure 21: Group I - unset specimen in L.casei showing zone of inhibition

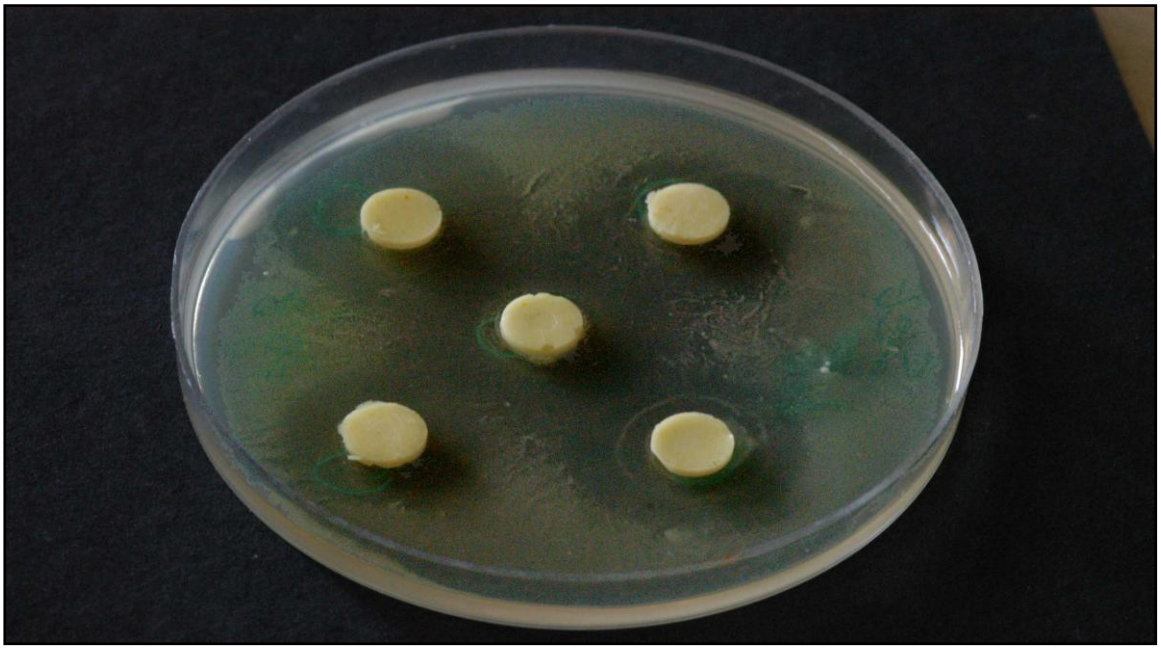


Figure 22: Group II - set specimen in L.casei showing zone of inhibition

Figure 23: Group II - unset specimen in L.casei showing zone of inhibition

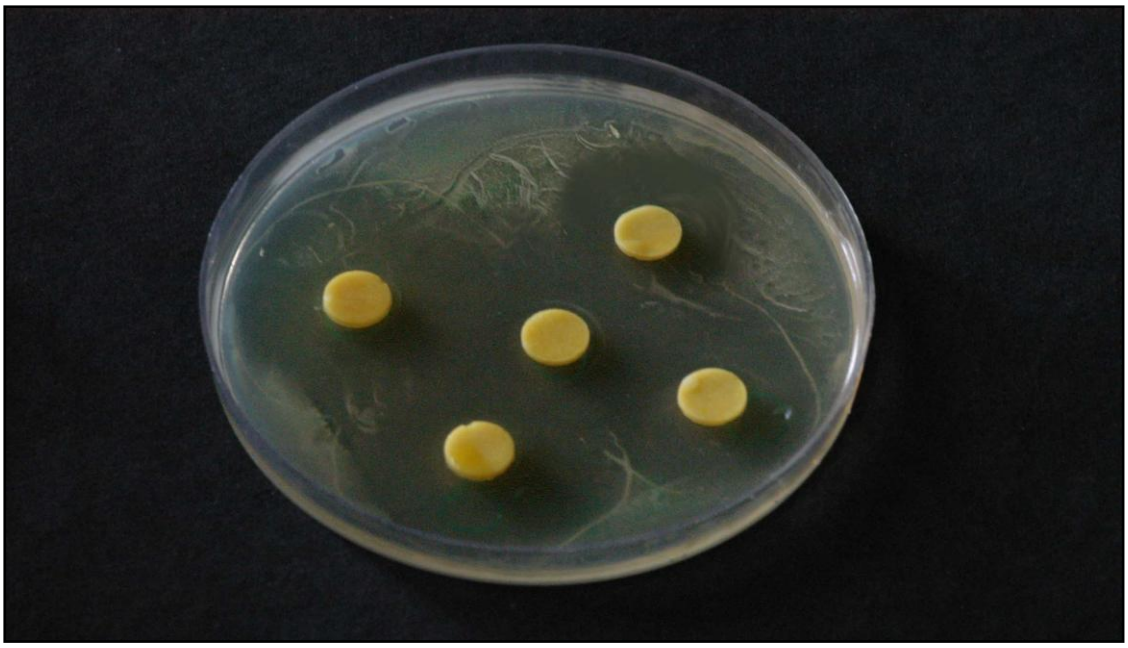


Figure 24: Group III - set specimen in L.casei showing zone of inhibition

Figure 25: Group III - unset specimen in L.casei showing zone of inhibition

Figure 26: High Performance Liquid Chromatography Unit Shimadzu, LC 20AT - Japan

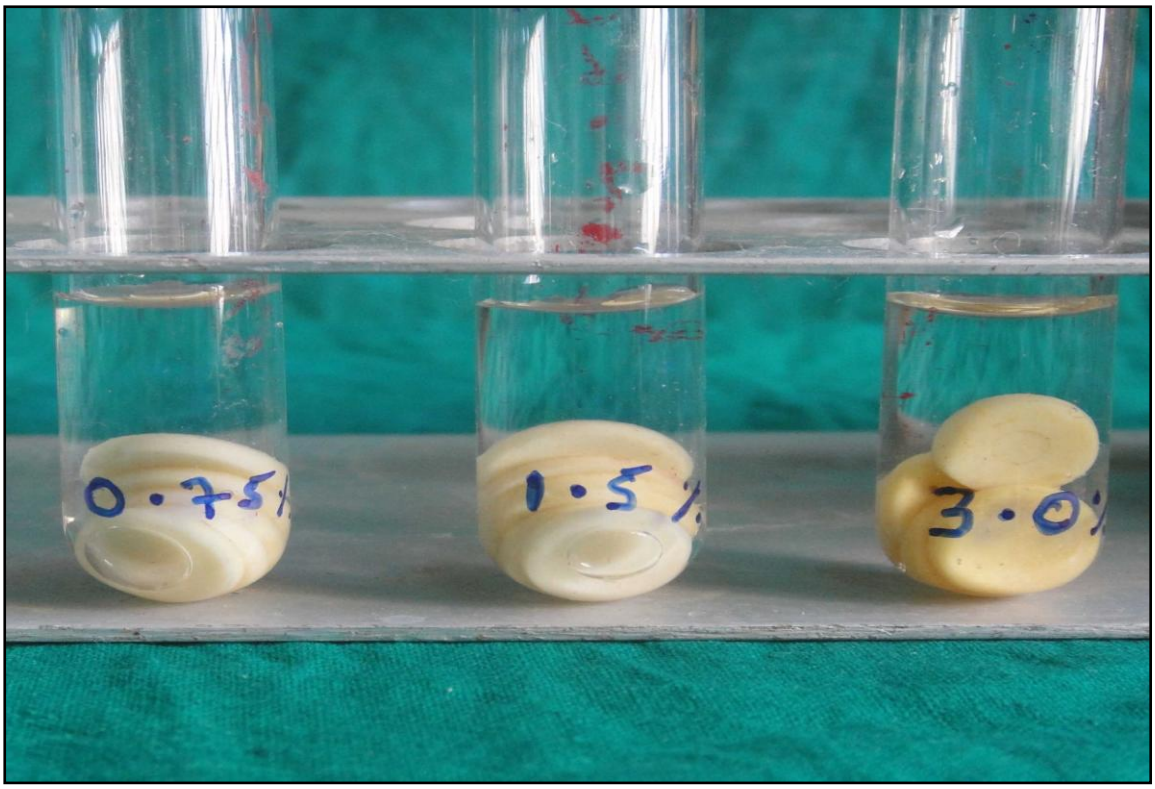


Figure 27: HPLC Samples

Figure 28: C18 Octadecyl silane column

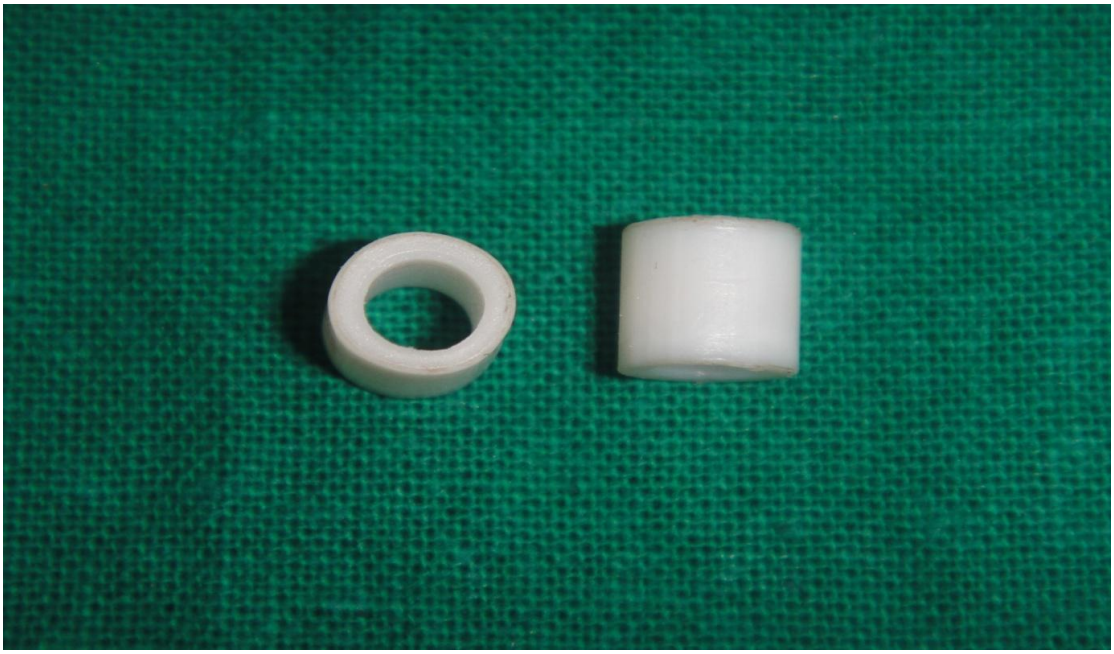


Figure 29: Teflon tubes (internal diameter 4mm x height 6mm)

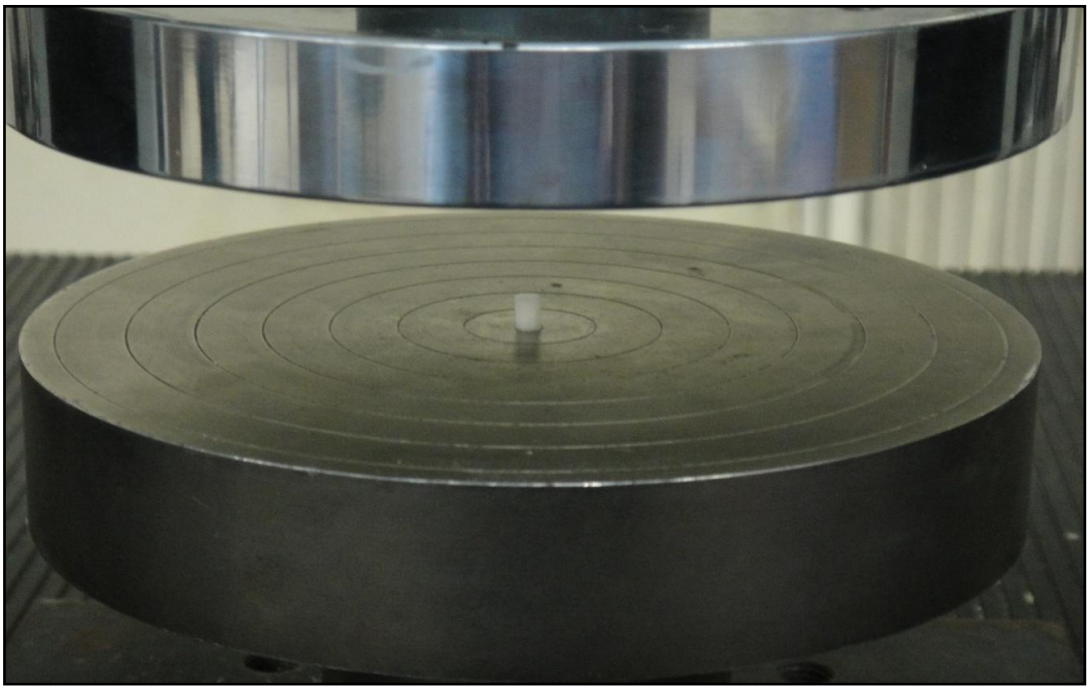


Figure 30: Sample tested under Instron
Universal Testing Machine LR 100K Lloyd Instruments, UK

Figure 31: 10% Polyacrylic acid – Dentin Conditioner

Figure 32: Tooth samples conditioned with 10% polyacrlyic for shear bond strength evaluation

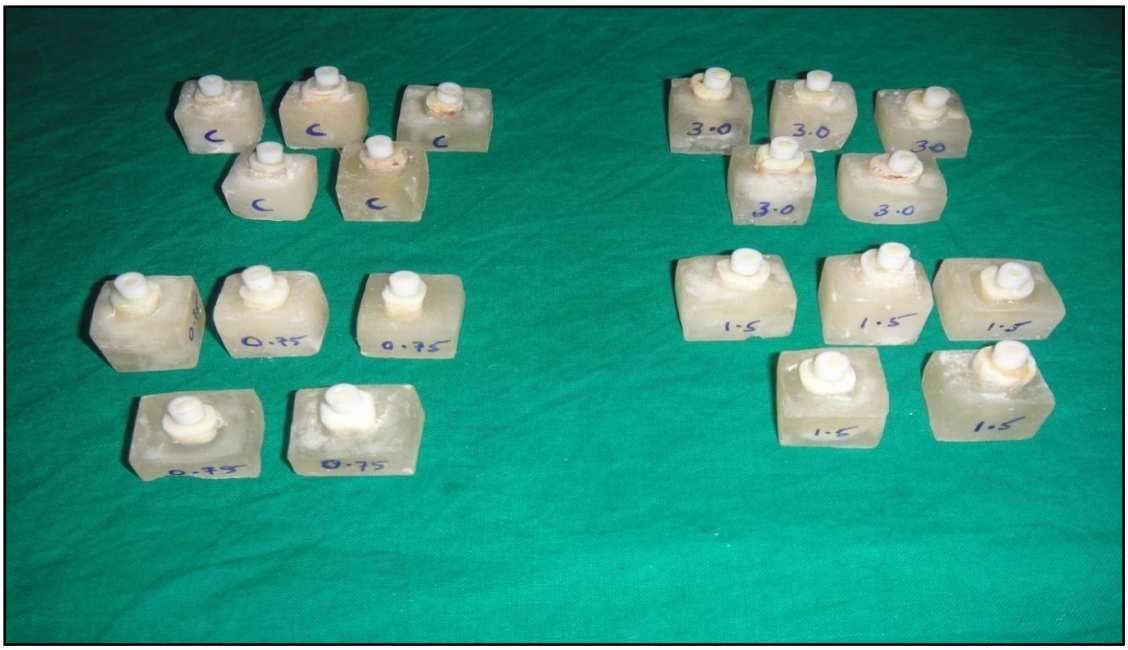


Figure 33: Samples prepared for Shear Bond Strength Evaluation

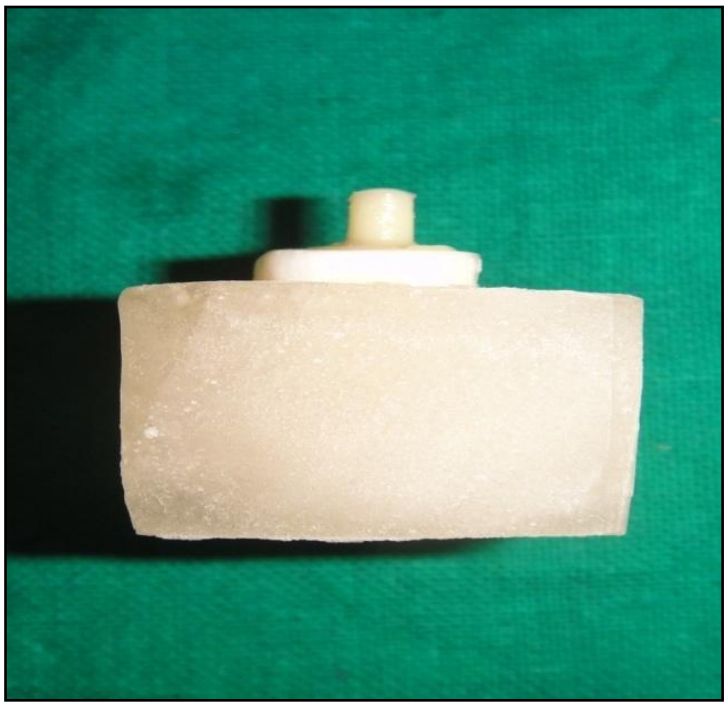


Figure 34: Individual sample for shear bond strength testing

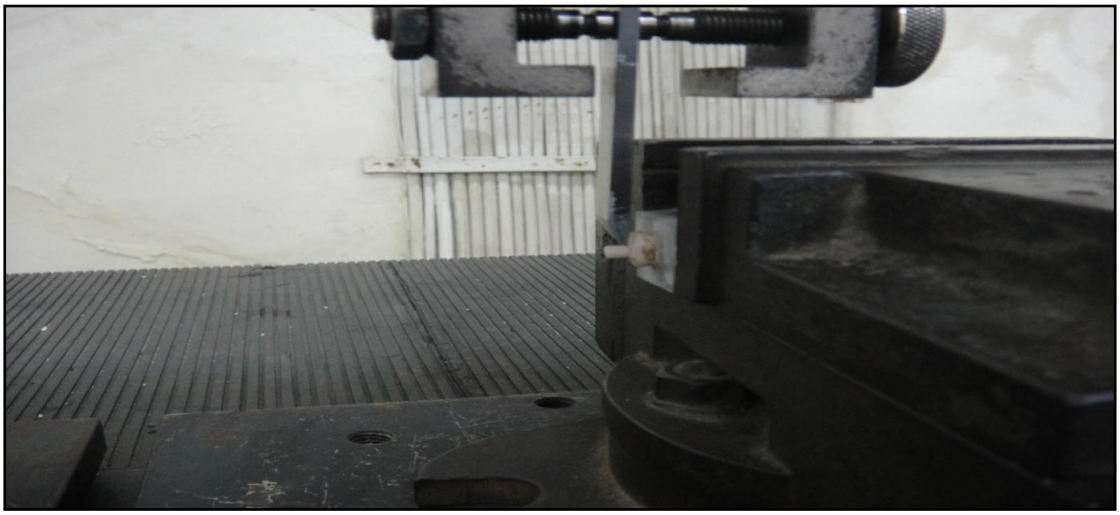


Figure 35: Sample being tested for shear bond strength

RESULTS

I ANTI BACTERIAL ACTIVITY SCREENING TESTS

Table 1: Results of antibacterial activity of antibiotic mixture (set) against *Streptococcus mutans*

Groups	Zone of inhibition (mm)					
	Sample 1	**Sample II**	**Sample III**	**Sample IV**	**Sample V**	**mean±SD**
Control	15	17	14	14	16	15.2±1.30 [a]
I (0.75%)	28	20	18	17	23	21.2±4.43 [b]
II (1.5%)	28	29	25	24	27	26.6±2.07 [c]
III (3.0%)	30	30	28	30	29	29.4±0.89 [d]

Values mean ± standard deviation from 5 samples in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

Table 2: Results of antibacterial activity of antibiotic mixture (unset) against *Streptococcus mutans*

Groups	Zone of inhibition (mm)					
	Sample 1	**Sample II**	**Sample III**	**Sample IV**	**Sample V**	**mean±SD**
Control	15	17	20	15	19	17.2±2.28[a]
I (0.75%)	25	20	23	28	22	23.6±3.04[b]
II (1.5%)	35	31	28	28	33	31±3.08[c]
III(3.0%)	31	34	31	31	33	32±1.41[c]

Values mean ± standard deviation from 5 samples in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

Table 3: Results of antibacterial activity of antibiotic mixture (set) against *Lactobacillus casei*

Groups	Zone of inhibition (mm)					
	Sample 1	**Sample II**	**Sample III**	**Sample IV**	**Sample V**	**mean±SD**
Control	15	15	13	13	14	14±1.00 [a]
I (0.75%)	29	20	29	22	26	25.2±4.08 [b]
II (1.5%)	30	25	30	26	28	27.8±2.28 [b]
III (3.0%)	33	34	33	37	35	34.4±1.67 [c]

Values mean ± standard deviation from 5 samples in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

Table 4: Results of antibacterial activity of antibiotic mixture (unset) against *Lactobacillus casei*

Groups	Zone of inhibition (mm)					
	Sample 1	**Sample II**	**Sample III**	**Sample IV**	**Sample V**	**mean±SD**
Control	15	17	20	15	18	17.0±2.28[a]
I (0.75%)	22	20	30	32	26	26±5.09 [b]
II (1.5%)	27	28	30	29	29	28.6±1.14 [b]
III (3.0%)	40	27	42	32	36	35.4±6.06 [c]

Values mean ± standard deviation from 5 samples in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

Fig: 1 Results of antibacterial activity for set and unset specimens

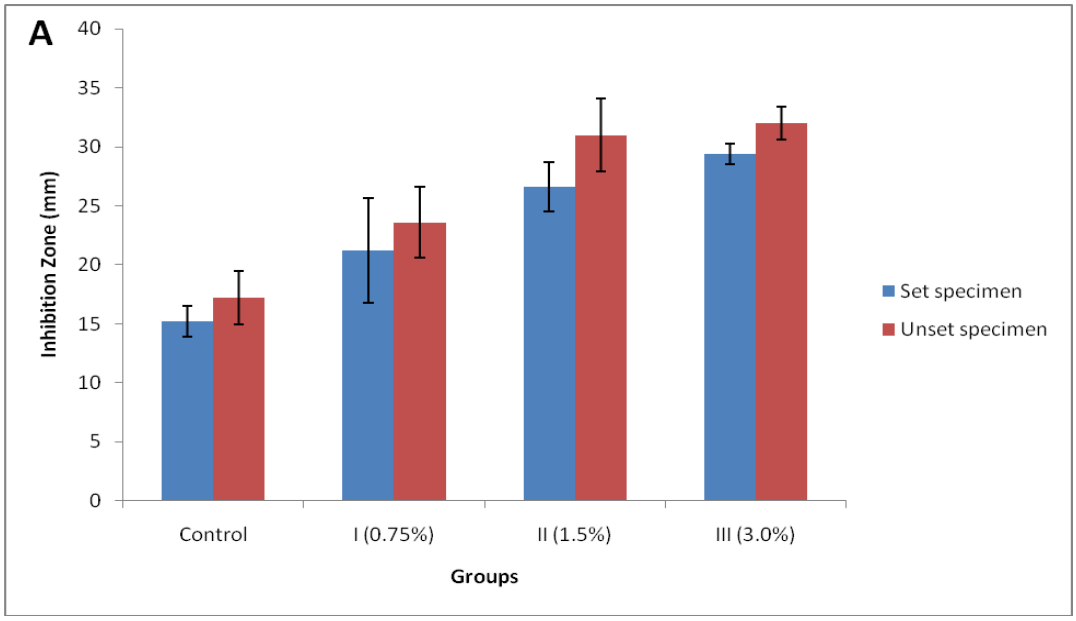


Fig 1A: Streptococcus mutans

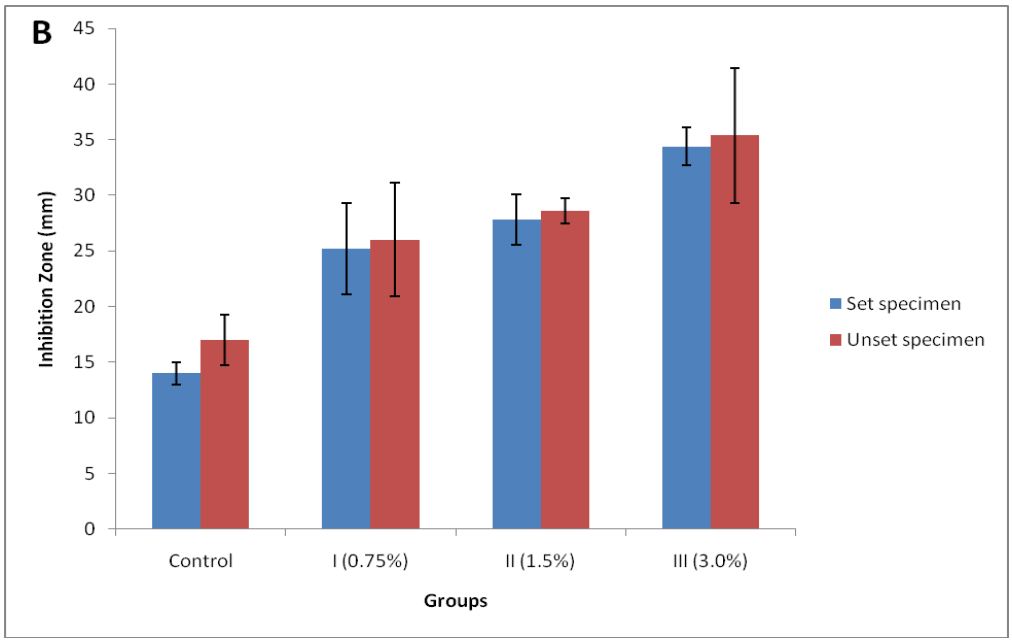


Fig 1B: Lactobacillus casei

For Fig.1A & 1B, the verticle bar indicates the standard deviation for five samples.

II RELEASE OF ANTIBIOTIC DRUGS EVALUATION

Table 5 : Amount of Antibiotic Drug Released at the end of 24 Hrs and 7 Days evaluated using High Performance Liquid Chromatography

Groups	Release of Antibiotics at 24hrs (mg)			Release of Antibiotics at 7 days (mg)		
	Metro	Cipro	Mino	Metro	Cipro	Mino
I (0.75%)	6.676	0	0.052	17.58	0.46	0.139
II (1.5%)	12.3	0	0.081	33.94	1.345	0.246
III (3.0%)	21.3	0	0.137	24.48	1.382	0.132

III COMPRESSIVE STRENGTH EVALUATION

Table 6: Compressive strength at the end of 24 Hrs

Groups	Compressive Strength (MPa)					
	Sample 1	Sample II	Sample III	Sample IV	Sample V	mean±SD
Control	103.8	105.04	104.67	105.47	105.74	104.94 ± 0.75^{a}
I (0.75%)	102.87	103.37	104.08	103.86	104.31	103.69 ± 0.57^{a}
II (1.5%)	56.61	42.64	48.93	54.87	45.51	49.712 ± 5.96^{b}
III (3.0%)	39.86	38.68	37.15	35.99	43.77	39.09 ± 3.00^{c}

Values mean ± standard deviation from 5 samples in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

Table 7: Compressive strength at the end of 7 days.

Groups	Compressive Strength (MPa)					
	Sample 1	**Sample II**	**Sample III**	**Sample IV**	**Sample V**	**mean±SD**
Control	111.14	111.39	110.09	111.97	112.29	111.37±0.85[a]
I (0.75%)	108.7	109.86	110.61	110.87	109.12	109.83±0.93[a]
II (1.5%)	80.48	79.34	81.33	84.96	78.81	80.984±2.42[b]
III (3.0%)	48.16	56.85	54.39	49.65	52.71	52.352±3.51[c]

Values mean ± standard deviation from 5 samples in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

Compressive Strength Evaluation

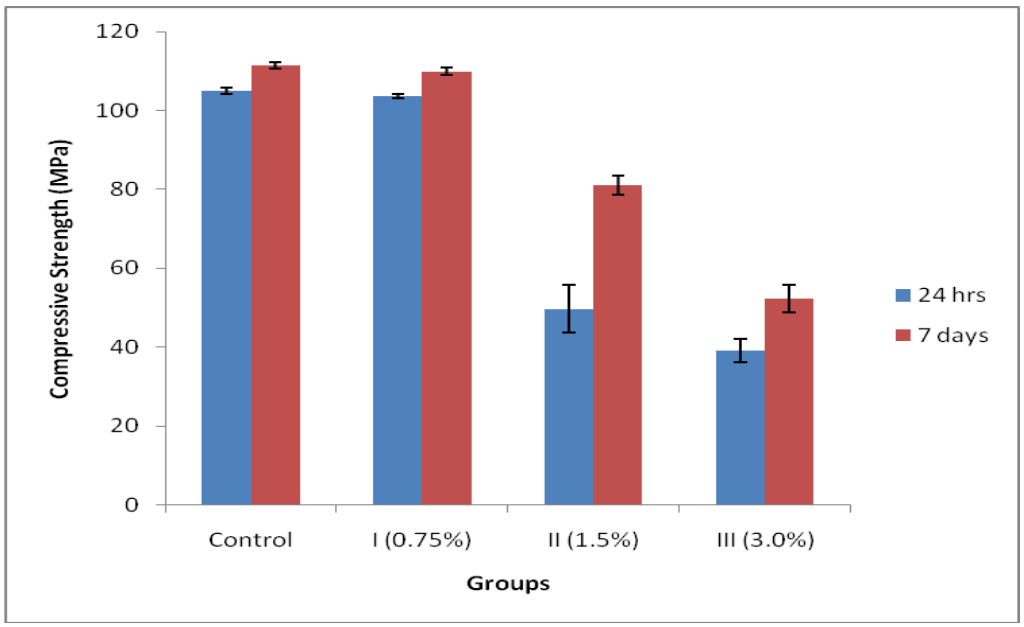


Figure 2 The verticle bar indictes the standard deviation for five samples.

IV SHEAR BOND STRENGTH EVALUATION

Table 8: Shear bonds strength evaluation at the end of 24 Hrs

Groups	Shear Bond Strength (MPa)					
	Sample 1	**Sample II**	**Sample III**	**Sample IV**	**Sample V**	**mean±SD**
Control	4.68	4.26	4.39	4.39	4.78	4.5±0.21[a]
I (0.75%)	4.09	3.5	3.62	3.96	3.79	3.79±0.24[b]
II (1.5%)	2.69	2.83	2.67	2.84	2.82	2.77±0.08[c]
III (3.0%)	2.05	2.13	2.09	2.29	2.09	2.13±0.09[c]

Values mean ± standard deviation from 5 samples in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

Shear Bond Strength Evaluation

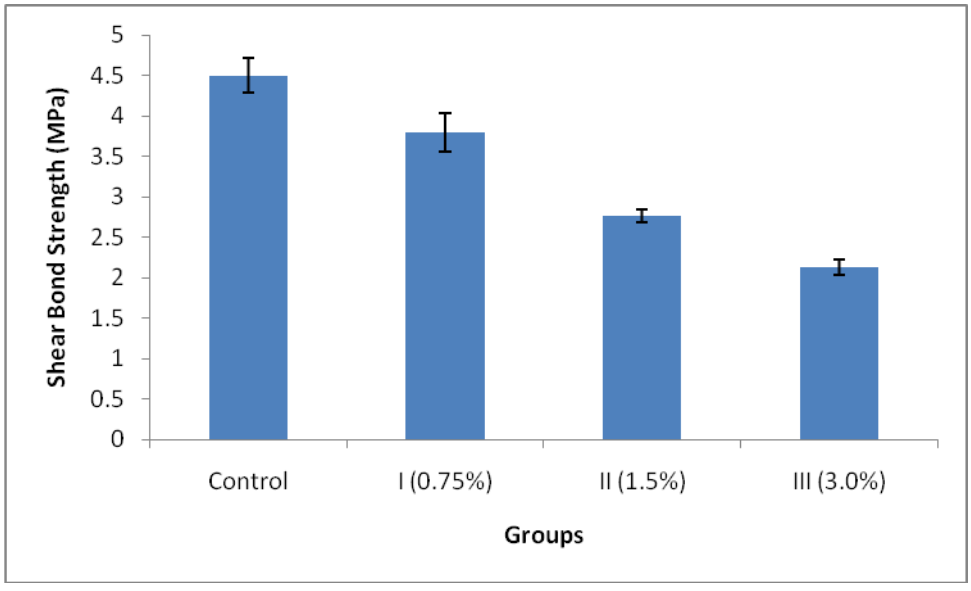


Figure 3 The verticle bar indictes the standard deviation for five samples.

Statistical analysis:

Statistical analysis was made by analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). Results are expressed as mean ± standard deviation from 5 samples in each group. $p < 0.05$ were considered to be significant.

RESULTS

ANTIMICROBIAL ACTIVITY SCREENING TESTS:

The mean values (mm) of the growth inhibition zones for the control and experimental groups are shown in Figures IA and IB. In set specimens, the size of the inhibition zones was significantly smaller than in the unset specimens against all bacteria tested. The size of the inhibition zone was dependent upon the amount of added antibiotic mixture.

When S mutans and L Casei were compared, significant difference existed in the size of the inhibition zones produced among the control and experimental groups in the set specimens [for S mutans, ($p < 0.05$) and for L casei, ($p < 0.05$)]. Significant differences in the size of the inhibition zones produced among all the groups were observed in testing with S mutans and L casei in unset specimens ($p < 0.05$).

RELEASE OF ANTIBACTERIAL DRUGS:

Release of the antibiotics metronidazole, ciprofloxacin and minocycline from the experimental groups after 24 hours and 7 days is shown in Table 7 except for ciprofloxacin. The amount of antibiotic drugs that were released increased as the concentration of drug mixture incorporated to the cement

increased. The levels of drug released were at 7 days were greater than at 24 hours for all the experimental groups except for metronidazole in group III at the end of 7days. Significant differences were observed among the groups of experimental GICs ($p < 0.05$) for each group).

COMPRESSIVE STRENGTH EVALUATION:

The mean compressive strength of the control and experimental groups after 24 hours and 7 days of storage in water is shown in Figure 2. The compressive strength values at 7 days were greater than at 24 hours and 7 days. However, no significant differences existed between the control and Group 1 at 24 hours and 7 days. No significant differences were observed among the experimental groups at 24 hours. However, a significant difference was observed between Group I, Group II and Group III after 7 days.

SHEAR BOND STRENGTH EVALUATION TO DENTIN:

The shear bonding strengths for the control and experimental groups are shown in Figure 3 ($p < 0.05$). The shear bonding strengths of Group II and III to dentin were significantly lower than that of the control group (Figure 3). No difference in bonding strength existed between the control and Group I.

DISCUSSION

Glass ionomer cements (GICs) are considered the material of choice when dental caries and cavity preparation are managed only with the use of dental hand instruments when performing atraumatic restorative therapy (ART).[5,32] The glass ionomer cements used in earlier field trials were not specifically developed for the atraumatic restorative therapy technique, and the relatively high failures found may have been partly related to case selection the material and to the technical skills of the operator. Recently several more viscous esthetic conventional glass ionomer cements with improved handling and physical properties, largely due to smaller mean particle size, have been marketed specifically for the atraumatic restorative therapy approach.44

However, the use of dental hand instruments are known to be insufficient at removing the infected dentine, and in such situations, residual caries is likely to be restored over. Thus, cavities treated by atraumatic restorative therapy may have residual infected dentin and if a glass ionomer cements is unable to arrest the carious process, the restoration will fail.[6]

The main advantages are relatively ease of use chemical bonding to the tooth, long term fluoride ion release, low coefficient of thermal expansion and acceptable esthetic quality.[61]

Therefore the use of an antibacterial agents along with GIC may help to reduce or eliminate cariogenic bacteria that may contribute to secondary caries and failure of the restoration.[5]

The past several years have seen renewed interest in minimal intervention techniques in the management of dental caries. The use of chemical agents to slow down or arrest caries progression without surgical removal of the lesion has been documented in the literature for many years.[23]

Streptococcus and Lactobacillus species were used as they are commonly associated with dental caries.[21,6,32]

Streptococcus mutans are considered to be the most important group of bacteria initiating caries lesion. The number of salivary Streptococcus mutans in the oral cavity is

correlated to the formation of new caries lesion and it is generally accepted that reducing the number of them also reduces the caries activity.[21,7]

Lactobacilli population involved in dental caries are generally recognized to be associated with the caries progression. They are considered to be secondary invaders rather than initiators of the caries process.[3]

GIC having an antibacterial property was reported by Nakajo et al that the population of S.mutans was reduced, due to the fall of ph to 4.8 – 5.0pH after sucrose fermentation. GIC inhibit the acid production of S.mutans markedly at 5.5 pH and subsequently decrease the bacterial population. It also neutralizes acidic condition and has the buffering potential of the released elements to neutralize acids.[32]

The role played by fluoride in arresting caries is as follows :

Fluoride is bacteriostatic and bactericidal to oral streptococci at concentrations of 15.8 – 160 mM.[32] A strong

fluoride effect may inhibit bacterial activity and arrest the process in unintentionally left carious dentine. Furthermore, it may be expected that the fluoride will activate the remineralization of uninfected inner dentine and dimineralized enamel.[55] Fluoride has been shown to depress metabolic activity in plaque bacteria by inhibiting glucose transport into the cells, translocation of sugars, cation transport and accumulation of intercellular phosphates. The antimetabolic effects of fluoride are favored by low ph that enhance cell permeability by the ion and reduces glycolysis.[45] Hence Glass ionomer cement Fuji IX was chosen for this study to evaluate the antibacterial and physical properties like compressive strength and shear bond strength to dentin.

The Agar Diffusion Test is commonly used test and is insensitive as the results are highly dependent on molecular size and the diffusion constant of the antimicrobial component, inoculum size, incubation and degree of material/agar contact. [39, 55]

A relatively small specimen size was chosen to simulate clinical dimensions of restorations. The rationale of using set

specimens was to standardize the size of the test materials and therefore the amount of antibacterial effect. Set or aged dental materials are known to have less antibacterial action than freshly mixed specimens.[6,55,39]

Unset specimens exhibited production of greater inhibition zone compared with corresponding set specimens against all bacterial strains. When applied directly into the agar wells it had antibacterial effects which exhibited a two fold larger inhibition. Freshly mixed GICs may alter the metabolism of Streptococcus mutans.[46,39,38]

Liquid Chromatography (LC) is a separation technique based on the difference in the distribution of components between two non – miscible phase in which liquid mobile phase elutes through a stationary phase in a column. Three forms of high performance liquid chromatography most often used are based on the mechanism of partition, adsorption and ion exchange. Ion exchange chromatography, also referred as ion chromatography, is an analytical technique for the separation and determination of ionic solutes i.e. inorganic cations, organic anions, low molecular weight (water soluble)

organic acids and bases etc. The separation of ionic solutes takes place on the basis of ion exchange on stationary phase with charged functional groups. The functional groups typically are quaternary ammonium groups for anion exchange and negatively charged groups like sulphonates for cation exchange. The corresponding counter ions are located in vicinity of the functional groups and can be exchanged with other ion of the same charge in the mobile phase. Thus various ionic components of the sample can be separated based on their differential affinities towards the immobilized stationary and the liquid mobile phase.[69]

Takahashi et al[46] in his study investigated the concentration of the eluted chlorhexidine using High performance Liquid Chromatography.

Thus in the present study High performance liquid chromatography evaluation was undertaken to quantify the amount of drug released at the end of 24 hrs and 7 days.

Compressive strength is important for dental cements when they are used as posterior restorative cements because

of masticatory forces encountered during function. During compressive testing, cracks propagate uniformly and twist out of their orientation parallel to the compression axis so that failure occurs by the slow development of many cracks to form a crushed zone.[16]

Glass ionomer cements bonds strength have been studied extensively in sound and carious dentine using conventional shear bond testing methods.

The bond strength strength of glass ionomer cements have been reported in the range of 3 – 4 MPa.[52] Yip H.K. et al reported that there is a higher bond strength due to the use of smaller specimen cross sectional area.[43]

ANTIMICROBIAL ACTIVITY SCREENING TEST:

The results of the current study demonstrated that GIC – containing antibiotic mixture was effective in inhibiting bacterial growth. Both set and unset specimens containing antibiotics exhibited inhibitory effect against Streptococcus mutans and Lactobacillus casei compared with the control specimens. Moreover, all the antibiotic containing set

specimens showed less antibacterial activity than the unset specimens against both the bacteria tested.[60] The results of the present study are similar to the previous studies which shows that Unset specimens exhibited production of greater inhibition zones compared with the set specimens.[6,46,38,55,39] The zone of inhibition of unset antibiotic mixture was larger and statistically significant compared to the set antibiotic mixture when tested against S.mutans and L.casei respectively.

In the control group (pure glass ionomer cement) showed zones of inhibition in both S.mutans (15.2 mm-set,17.2mm-unset) and L.casei (14.0 mm-set, 17.0 mm-unset) in set and unset specimens which were marginally significant among the control group.

The results of this present study Contradicts with the results of the study of Yap et al, who found conventional glass ionomer to have no antibacterial effect when the specimens are set.[6]

However in the experimental, groups, antibacterial activity seen against S. mutans showed that as the concentration of the antibiotic drug mixture increased, the size of the inhibition zone increased in both set and unset specimens from group I (21.2 mm-set,23.6 mm-unset), group II (26.6 mm-set,31.0 mm-unset) and group III (29.4 mm-set,32.0 mm-unset) which were statistically significant.

The results of the present study was in correlation to the study conducted by Botelho and Nakajo et al where the inhibitory effect of the antibacterial test specimens showed a dose dependent response with increase in concentration.[6,32]

Similarly, antibacterial activity seen against L. casei also showed that as the concentration of the antibiotic drug mixture increased, the size of the inhibition zone increased in both set and unset specimens from group I (25.2 mm-set,26.5 mm-unset), group II (27.8 mm-set,28.6 mm-unset), group III (34.4 mm-set, 35.4 mm-unset) which were statistically significant.

The possible reason for this was related to the property of GIC for having low pH during the initial setting, fluoride release or other chemical components present in the powder of these materials.[32,38,15,30] It could be assumed that, as there is an initial fluoride release there are possibilities that the antibacterial drugs are also released along with fluoride.

Yap et al(1999), found that most dental materials proved to be bactericidal while setting ie, so long as chemical reaction is proceeding, which holds good for glass ionomer cement.[55] Nakajo et al stated that the population of S.mutans was lower, due to the fall of ph to 4.8 – 5.0ph after sucrose fermentation. Glass ionomer cement inhibit the acid production of S.mutans markedly at 5.5 ph and subsequently decrease the bacterial population.[32]

Botelho Michael G. et al, incorporated Chlorhexidine hydrochloride, Cetylpyridinium chloride, Cetrimide and Benzalkonium chloride in Fuji IX and stated that apparent greater potency of the antibacterial glass ionomer cement material may possibly be due to high elution rates of the antibacterial agents from the glass ionomer cement or due to

synergistic interactions between the antibacterial agents and glass ionomer cement.[6]

Pinherio et al suggested that glass inomer containing antibiotic mixture may be used for the treatment of carious lesions, reducing total viable bacteria .[35]

Sato et al investigated the efficiency of drug combinations of Metronidazole, Ciprofloxacin and Minocycline and found that this approach was very effective in the sterilization of carious lesions, necrotic pulps and infected root dentin of deciduous teeth.[37]

Hoshino et al investigated the efficacy of these drugs, alone and in the combination and these three drugs combination where able to consistently sterilize the micro organisms.[20]These previous studies where taken into consideration in the present study for incorporation of Metronidazole, Ciprofloxacin and Minocycline into Glass ionomer cement – Fuji IX..

The setting glass ionomer cement materials are more soluble and therefore better able to diffuse in agar gel than in the set material.[60,21] The antibiotic compounds were solids that were easily mixed with the GIC powder.[60] For all the groups examined, the agar diffusion tests showed that the size of the inhibition zones produced in the presence of Streptococcus mutans and Lactobacillus casei were dependent upon the quantity of the antibiotic incorporated to the GIC.[6] There are limitations associated with the Agar Diffusion Test, like the inability to distinguish between bacteriostatic and bactericidal effects.[6]

In the previous dental literature, extensive studies have been done on various combinations of bacterial strains and the action of glass ionomer cement fluoride release against [S. Mutans[21,38,40,19], S. mutans, S sobrinus, L casei[55] , S. mutans, S. sanguinis [32]], Fuji IX with antibacterial agents against [S.mutans, L. casei, L. acidophilus, Actinomyces odontolyticus , Actinomyces naeslundii [6]], Fuji IX alone against [S. mutans, S sobrinus, L. acidophilus, A. viscosus ,[15,12] S .mutans, S. sanguinis, S.salivarius, L. Casei[30] , S. mutans, A. viscosus, E.faecalis[13]]

Silver fluoride/Stannous fluoride against S.mutans, L.casei .[23] All stated that the primary mechanism of bacterial inhibition is achieved by the initial fluoride release, followed by, synergistic action of the drugs incorporated in the glass ionomer cement.

Knowing that a large number and variety of bacteria play a role in caries development, the use of a mixture of antibiotics is probably a better choice than the use of a single antibiotic.[60] Therefore considering the previous studies, it can be said that fluoride is released along with which the drug mixture to bring about a wider spectrum of antibacterial activity.

In this present study as the concentration of the antibiotic combination increased, the antibacterial activity also showed an increase, 0.75% showed zone of inhibition lesser than 1.5 % in both the bacterial specimens tested in set and unset samples and 1.5% had lesser zone of inhibition than 3.0%. 3.0% showed the maximum inhibition zone but the size was not significantly different for the both the bacterial specimens tested in set and unset samples. 1.5% showed a

marked statistical difference in both the bacterial specimens tested in set and unset specimens.

RELEASE OF ANTIBIOTIC DRUGS:

The release of antibiotic drug was monitored using High Performance Liquid Chromatography and was observed to change in relation to concentration and time. The amount of antibiotic that was released increased as the amount of antibiotic that was added increased.[60] It is well known that release of agents from restoratives jeopardizes physical properties.[46]

In the present study, all the 3 groups released the Metronidazole and Minocycline except for ciprofloxacin at the end of 24hrs.

At the end of 7 days release period all 3 groups released Metronidazole, Ciprofloxacin and Minocycline.

However the release of Metronidazole at the end of 24 hrs, increased from group I, group II, group III as 6.67 mg, 12.3 mg, 21.3 mg. At the end of 7 days release it showed an

increase, from group I, group II and group III as 17.5 mg, 33.9 mg, 24.4 mg respectively.

There was no release of Ciprofloxacin in all 3 groups at the end of 24hrs, but at the end of 7 days, showed an increase in release from group I, group II, group III as 0.46 mg, 1.34 mg and 1.38 mg respectively

Release of Minocycline was also seen in the same pattern as Metronidazole at the end of 24hrs release, where it release increased from group I ,group II, group III as 0.052 mg, 0.081 mg and 0.137 mg. At the end of 7 days release in group I and II, showed an increase as 0.139 mg and 0.246 mg, in group III there was a drop in the release to 0.132 mg.

These results were in favour with Palmer et al that in all cases where antimicrobial agents incorporated, only a relatively small amount of the total added material was released. In the case of highest incorporated content of antimicrobial agent, significantly greater percentage of the antimicrobial agent was released.[33]

Total number of 5 specimens were considered from group I, group II and group III, which weighed 200 mg each. The antibiotic drug release evaluated was for total of 1gm from each experimental group.

Clearly, if the release phenomenon is to be utilized effectively, a detailed understanding of the mechanisms of the of incorporation of the organic species within the cement matrix and their release from it is required.[33]

However it could be suggested that the low solubility of drug in water, which might be exacerbated by the drug forming insoluble salts with the silicate and phosphate components of the glass inomer in combination with small surface area of the sample studied. It was further suggested that, due to its low solubility, drugs incorporated in the powder form might be present within the cement matrix as encapsulated particles which only becomes exposed as the glass ionomer deteriorates.[33]

It may be that two release processes occurring simultaneously: initial wash out before the glass ionomer has fully set, together with a slower diffusion controlled release. In the second phase of release where overall only a small portion of the included drug was released, the levelling off may be due to the exhaustion of the free drug within the cement i.e. the drug was not physically or chemically bond to the cement matrix. Alternatively it may be due to the saturation of the solution in which the cement was immersed.[33]

In the present study it was noticed that Metronidazole was released to the maximum levels at 24 hours and 7 days time period followed by lesser release of Minocycline. The least release being that of Ciprofloxacin at 7 days and no release at 24 hours.

One explanation of the release observed in the present study is that all three drugs available for release has been released into the solution and that the residual drug remains either chemically bond or physically bound in the cement. An equilibrium may have been established between the drugs and

the other ions released into the solution and the remaining drug in the cement.[33]

After the cement sets, it is not known whether the drugs are released from the surface alone or also from the deeper sections of the cement. If the drug released from the surface, then an enhance antibacterial effect could be expected in time due to the erosion exposing a new surface to release the drug.[21]

Further research need to be done on the mixture of newer antibiotics with broader spectrum as well as the ratio to be added with GIC without jeopardizing the mechanical properties of glass ionomer cements.

COMPRESSIVE STRENGTH EVALUATRION:

The results of the present study showed increasing the concentration of the antibiotics mixture had increasing adverse effects on the physical properties of the mixture.[60,33] At the end of 24hrs the compressive strength evaluated, showed no statistical difference between the control group (104.9 Mpa) and group I (103.6 Mpa). Whereas group II (49.7

Mpa) and group III (39.0 Mpa) demonstrated statistical difference when compared to the control group.

Similarly, at the end of 7 days samples there was no statistical difference between the control group (111.3 Mpa) and group I (109.8 Mpa). Whereas group II (80.9 Mpa) and group III (52.3 Mpa) demonstrated statistical difference when compared to the control group.

Control group and group I showed more values than group II and group III at the end of 24 hrs and 7 days.

These results are in favour with the study by Palmer G. et al in his study observed that, as the percentage of antibacterial agents incorporation was increased in the conventional GIC, so was a decrease in compressive strength at 1 hour and 24 hours maturation time. This suggests that at lower concentrations of antibacterial agents incorporation, the cement strengths on maturation, whereas above a certain concentration the cements weakens during the maturation phase.[33]

The reason for the experimental groups to have lower values compared to control group could possibly be due to the interaction of the antibiotics reacting to the glass particles and the liquid thereby leaving a number of unreacted particles in the cement structure.[60]

The reason for the 7 days sample to have higher values than 24 hours samples, could be explained by Kleverlann et al that the cross linking reaction is a continuous process evident by the increase in mechanical properties of the cement with time.[24]

Silva et al in his study using Fuji IX stated that GIC material showed an improvement in the mechanical properties as a function of time can be verified, reflecting the continuity of the setting reaction. One possible elucidation may be related to the smaller mean particle size of Fuji IX, resulting in greater surface area for the polymeric acid and glass interaction, leading to faster maturation.[43]

The powdered antibiotics particles, which are added to the GIC, easily absorb water, leading to decrease in compressive strength.[60]

Considering the results of the present study, it could be said that as the concentration of the drug incorporation is increased there is a drastic effect on the physical properties of the cement.

SHEAR BOND STRENGTH EVALUATION TO DENTIN :

The capacity of Glass inomer cement to bond chemically to enamel and dentine is very important[60]. Studies testing the shear bond strength of Glass ionomer cement to dentin have been reported to be ranging from 1.32 Mpa to 4.10 Mpa.

The results of the present study showed increasing the concentration of the antibiotics had adverse effects on the bond strength of the mixture similar to compressive strength evaluation. [60,33]

The group I (3.79 Mpa) produced bonding strength similar to those of the control group (4.5 Mpa). Subsequently, significant reduction in the bonding strength to dentine was observed with group II (2.77 Mpa) and III (2.13 Mpa).

The lower bonding strength results from the interference in the polar and ionic attraction between the carboxylate and the inorganic ions with the dentine.[60,33]

In an earlier study conducted by Takahashi et al where Chlorhexidine was incorporated in various concentrations into Fuji IX showed that significant reduction in bond strength was observed as the concentrations increases.[46]

A possible reason for the decrease in mechanical properties can attribute to drugs which hamper the reaction of polyacrylic acid and glass because setting time also extended by the addition of drugs. Mixing ratio of powder and liquid affects mechanical properties of glass ionomer cements, therefore slight modification in powder / liquid ratios by adding drug to the powder may also contribute to influence

on mechanical strength. Bond strength reduced if more than 0.75 % of drug was incorporated into the cement.[46]

Dentin conditioners are considered a useful step in the Glass inomer cement restorative process, as they have shown to increase the bond strength of the material to dentine surface. Polyacrylic acid has been used as a dentine conditioner for the cement, as it creates a clean surface by removing the smear layer and surface contaminants without opening the dentine tubules too widely.[5] It acts as a weak etching agent.[1] However according to Lucas et al in his study stated that Poly acrylic acid in the liquid component of a GIC is capable of decalcifying the dentine surface even after being mixed with the glass ionomer powder so there is no need to pre – treat the dentin with decalcifying agents before a GIC is applied.[28]

Glass inomer cement always contains numerous air inclusions that can act as stress points, thus giving rise to the increased likelihood of cohesive failure within the cement which was seen as the most common form of failure.[9] It was reported most failures occurred cohesively in the cement

mass, which seems to be atypical finding for Glass inomer cements.[46] This was in accordance to other studies by Lucas et al[28] and Carvalho et al[10] implying that the interfacial strength of the cement tooth bond is higher than the inherent strength of the material.[28] Hence the control group showed higher bond strength value compared to the experimental groups.

On the contrary Czarnecka et al stated in his study, that failure of bonded glass ionomer has been found to be a mixture of adhesive and cohesive.[11]

Another important reason to be considered is the interface of the glass ionomer bonded to dentin which is the intermediate layer. For Fuji IX a very distinct zone hyberd layer could be detected on the SEM microscope. This is a hyber layer of approximate 6 micrometer was reported by Burrow et al which was believed to be a region that had been demineralized by the dentin conditioner but had not been well penetrated by the Fuji IX. This zone may be a weak region in the GIC bond and because of this conditioning should be used

as a means to clean the tooth surface but not necessarily to demineralize the underlying dentine.[9]

Czarnecka et al compared the shear bond strength of Fuji IX to sound and prepared carious dentine and stated that, in sclerotic dentine blocking of the tubules would adversely affect the tag formation, which would also contribute to the reduction in bond strength.[11] Therefore in the present study the shear bond strength tested was not performed for the carious dentine.

The direction of the dentinal tubule also play a vital role in the bonding of the material which was described by Carvalho et al, that the bond strength is greater when the tubules are parallel to the bonding interface than when the tubules are cut perpendicular, it may be suggested that, in clinical situations the bond strength is much greater on the cavity walls than on the floor.[61]

The results of the present study indicates that incorporation of higher concentration of the antibiotic drug to Fugi IX compromises the bonding strength to dentin im accord to the reports of previous studie.[46,33]

SUMMARY

This study evaluated the antibacterial effect; physical properties and bonding strength of conventional glass ionomer cement containing antibiotic mixture.

The three antibiotic mixture used in this study were Metronidazole, Ciprofloxacin and Minocycline. They are mixed with the glass ionomer cement powder to obtain concentration ratios of 0.75%w/w, 1.5% w/w and 3.0% w/w respectively. The antibacterial activity of each cement ratio was evaluated against Streptococcus mutans and Lactobacillus casei using agar diffusion test.

The antibiotic drug release from drug mixture was analyzed at the end of 24 hrs and 7 days by High Performance Liquid Chromatography (HPLC).

The Compressive strength at the end of 24 hrs and 7days and the Bond strength was measured and compared with the control group using Instron Universal Testing Machine.

Statistical analysis was made by analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT).

The experimental groups showed significantly greater inhibition zones in comparison with the control group. The bacterial inhibition zone for both the selected bacteria for the groups 1.5%w/w and 3.0%w/w were almost the same in set an unset speciments.

Drugs released from the drug mixture also showed increased drug release with increase in concentration. 0.75%w/w and 1.5%w/w showed sustained release at the end of 24hrs and 7 days. Whereas 3.0%w/w a drop in release was observed at the end of 7 days.

At 3.0%w/w the properties of the glass ionomer cement was compromised. The compressive strength was less for 3.0%w/w when compared to the control group, 0.75% and 1.5% groups at the end of 24 hrs and 7 days

Altering the composition of the glass ionomer cements also results in change in their Bond strength. At a high concentration of 3.0%w/w showed the bond strength was

decreased when compared with the control group and 0.75% w/w and 1.5% w/w at the end of 24 hrs.

Therefore addition of 0.75% w/w antibiotic mixture was optimal in achieving effective antibacterial properties, sustained drug release and to have an appropriate physical properties and bonding strength.

CONCLUSION

The results of this In vitro study confirmed the following findings and substantiated few past findings

1. Glass ionomer containing antibiotic mixture have added advantage of being more effective in inhibiting bacteria associated with dental caries than pure glass ionomer.
2. There was a sustained amount of drug released observed both at the end of 24hrs and 7 days, for all the groups.
3. The antibiotic mixture at a concentration ratio of 0.75% w/w resulted in achieving favorable physical properties like better.compressive strength at the end of 7 days, and bonding strength.

However, the long – term pharmacological and clinical effectiveness of Glass Ionomer Cement containing Antibiotics should be investigated in future studies.

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