EFFECT OF CHITOSAN NANOPARTICLES ON THE FLUORIDE RELEASE FROM FOUR GLASS IONOMER CEMENTS AND ITS INFLUENCE ON THE ANTIBACTERIAL PROPERTY OF HIGH STRENGTH GLASS IONOMER CEMENT-AN INVITRO STUDY

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CERTIFICATE

This is to certify that this dissertation titled **"EFFECT OF CHITOSAN NANOPARTICLES ON THE FLUORIDE RELEASE FROM FOUR GLASS IONOMER CEMENTS AND ITS INFLUENCE ON THE ANTIBACTERIAL PROPERTY OF HIGH STRENGTH GLASS IONOMER CEMENT-AN INVITRO STUDY"** is a bonafide record work done by **Dr. C.NISHANTHINE** under our guidance during her postgraduate study period between 2010 - 2013.

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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Introduction

INTRODUCTION

Dental caries is the localized destruction of susceptible dental hard tissue by acidic by-products from bacterial fermentation of dietary carbohydrates. Thus, it is a bacterial driven, generally chronic, site-specific, multifactorial, dynamic disease process that results from the imbalance in the physiologic equilibrium between the tooth mineral and the plaque fluid; that is, when the pH drop results in net mineral loss over time. The infectious disease process can be arrested at any point in time (DCNA 2010).[19]

Literature review suggested that mortality rate of normal restoration is anywhere between 10 to 20 years. The replacement of a restoration is undesirable, as it may result in longer repetitive restorative cycles where the restoration is replaced by progressively larger restorations. This may adversely affect the outcome of the restorative procedure. Sixty per cent of restorative practice comprised of the replacement of existing restorations. The prime reason for restorations failure is recurrent or secondary caries and marginal defects.[10] The effective means of preventing secondary caries formation should begin at the time of placement of restoration itself. This means can be attained by providing proper dental hygiene instruction, prescribing fluoride containing mouth rinses, gels,

toothpastes and fluoride-releasing restorative material. In addition to above mentioned means salivary cariogenic microorganism assessment, salivary flow rate determination and complete medical evaluation are mandatory.[26]

Fluoride is potent anticariogenic agent. The mechanisms of action of anticariogenicity are multifold. It exhibits its anticariogenic effects by reducing demineralization, the enhancing remineralization, interfering in pellicle and plaque formation and inhibits the microbial growth and its metabolism (Fejerskov et al 1996).[54] In present day the market is flooded with several fluoride-containing dental restoratives like glass-ionomers, resin modified glass-ionomer cements, polyacid-modified composites (compomers), composites (Hicks et al 2003) and stannous fluoride containing amalgams (Tveit et al 1981). But the fluoride releasing ability of these products varies depending upon to their setting mechanisms and matrix formation. However, the antibacterial and cariostatic properties of restoratives are closely linked with the amount of fluoride released.[55]

Glass ionomer cement act as reservoir of fluoride and other ions in the oral cavity, which is useful against dental caries. It creates a mechanical barrier that protects the tooth surface from bacteria and it assist in preserving affected dentine at the base of restoration, by

delivering fluoride and other apatite-forming ions, and it can provide long-lasting seal under the most challenging clinical circumstances. It is used as a restorative material, liner, base, luting cement, fissure sealant and as a surface protectant.[42]

Chitosan is the second most abundant natural biopolymer which is next to cellulose. It is a linear polysaccharide composed of β-1, 4- linked D-glucosamine derived from the deacetylation of chitin.[49] It is found as the main part in the shells of crustaceans such as crabs and shrimp, the cuticles of insects, and the cell walls of fungi. It is a natural substance that has been used in studies on prevention of dental caries as it provides bactericidal and/or bacteriostatic characteristics (Hayashi et al 2007). The antimicrobial properties of chitosan are investigated in food packaging, textile, cosmetic industries, in medicine and in dentistry.[52] An antimicrobial mechanism has been proposed in which the interaction of positively charged chitosan oligomers with negatively charged microbial cell membranes causes the leakage of intracellular contents and damage on bacterial cells. A study by Tarsi et al suggested that chitosan nanoparticles prevent S. mutans adsorption to hydroxyapatite beads thereby preventing the colonization of bacteria on the tooth surface.[34] Chitosan supplementation in dental products such as chitosan

chewing gum (Hayashi et al 2007) and chitosan conjugated chlorhexidine mouthwash (Decker et al 2005) have shown satisfactory results in oral bacteria adhesion and inhibition experiments. Verkaik et al (2011) in an invitro study showed that natural antimicrobials in herbal and chitosan based tooth pastes can be equally effective as chlorhexidine, not only with respect to immediate but also delayed bacterial killing as a result of substantivity of antimicrobials in oral biofilms.[52] Arnaud et al studied the effect of chitosan on dental enamel de-remineralization and suggested that it may act as a barrier against acid penetration and inhibits enamel demineralization.[3] Linden et al prepared polymeric hydrogels based on poly acrylic acid and metal salts and chitosan, which ultimately led to narrowing of lumen spaces of dental hard tissues. Pawlowska 1997 observed that chitosan nanoparticles modified dental primers applied in rat dental pulp caused slight, reversible pathological changes in the pulp, Another study by Petri et al investigated the effect of different concentrations of chitosan nanoparticles on the flexural strength and fluoride ion release from glass ionomer cement, the results suggested that at lowest concentration chitosan modified glass ionomer liquid had improved the flexural strength and catalyzed fluoride release.[45] Chitosan microparticles have previously been investigated for local delivery to

the oral mucosa for therapeutics purpose similar to tetracycline, chlorhexidine and triclosan to evaluate its mucoadhesive potential. These studies used various manufacturing techniques such as inotropic gelation and emulsion polymerization protocols for the production of drug loaded chitosan microparticles.[29]

The aim of this in vitro study was to evaluate the effect of addition of chitosan nanoparticles on the fluoride release from four glass ionomer cements and the evaluation of antibacterial property of chitosan modified high strength posterior glass ionomer cement (GC gold label HS posterior extra).

The objectives of this study are to:

1. Investigate the effect of chitosan nanoparticles on release of fluoride from four glass ionomer cements.

2. Compare the amount of fluoride released among four types of glass ionomer cements (Type II universal restorative, Type II light cure universal restorative, GC Fuji VII (pink), GC HS posterior extra) with and without chitosan nanoparticles.

3. Demonstrate the antibacterial property of high strength posterior extra glass ionomer cement with and without chitosan nanoparticles.

Review of Literature

REVIEW OF LITERATURE

DeSchepper et al (1991)[15] compared the amount and pattern of fluoride release from 11 commercially available glass ionomer cements in artificial saliva over a period of 84 days. The fluoride ion concentration in artificial saliva was assessed by fluoride ion selective electrode and an ion analyzer. The result of this study showed that miracle mix released more amount of fluoride followed by Fuji II cement. All the materials released greatest proportion of fluoride in first 24 hours after mixing.

Hattab et al (1991)[24] evaluated in vivo release of fluoride from glass ionomer cement during an eight day period. In this study maxillary acrylic resin appliance, carrying four glass ionomer cement specimens were worn by four subjects at night. The concentration of fluoride in saliva was assessed by fluoride ion selective electrode and ion analyzer. The result of this study showed that the amount of fluoride ion released from all subjects was significantly more and the release of fluoride was nearly constant during the test period.

Mitra et al (1991)[38] evaluated the amount of fluoride ion release, the effect of curing time and mechanical property on the

amount of fluoride release from Vitrebond light cure glass ionomer liner or base over a period of 740 days. In this study the amount of fluoride release was calculated by fluoride ion selective electrode, incorporation of fluoride ion into the dentine was determined by secondary ion mass spectrometry and the mechanical property was determined by instron universal testing machine. The result of this study showed that the rate of release of fluoride ion was independent of the curing time. There was no change in mechanical properties of cured cement, thus indicating that long term fluoride release did not adversely affect the strength of the material.

Kupietzky et al (1994)[31] evaluated in vivo determination of placing a sealant over a glass ionomer restoration modifies its fluoride release. In this study topical application of sodium fluoride for 4 mins over all the glass ionomer restoration was done for 4 mins. The fluoride release was measured using fluoride ion selective electrode and an ion analyzer. The result of this study showed that there is no significant difference in pattern and quantity of fluoride release but there was significant reduction in fluoride release occurred when the restoration were covered with a sealant.

Pzerrin et al (1994)[44] compared the amount of fluoride release from three glass ionomer cement and cermet cement and studied the influence of dispensing system or powder liquid ratios effect on the fluoride release using an HPLC ion exchange chromatography coupled with a computer analyzer. The result of this study showed that the fluoride release was greater on 1st day, decreased sharply the 2nd day and gradually diminished. After 1 year, all specimens released daily fluoride concentration above 0.5 ppm reaching as much as 7 ppm. The low powder liquid ratios always lead to more fluoride release than high ratios.

Araujo et al (1996)[2] evaluated the amount of fluoride released from fluoride containing material over a period of 28 days. Six disc samples were prepared from each material and materials were randomly divided into six groups. Group1: Chelon Fil, Group2: Chelon Silver, Group3: Vari Glass, Group4: Dyract, Group5: Vitremer, Group6: Vitremer Scotch bond multipurpose, Group7: Fuji II LC. The fluoride release was measured at different time intervals at 1-7 days, 14, 28 days using fluoride ion selective electrode and fluoride ion analyzer. The results showed that Chelon fill released significantly more fluoride for first 7 days than all other materials, followed by Fuji

II LC. At days 14 & 28 Chelon fill, Dyract, Fuji II LC, released similar amount of fluoride that was significantly greater than other products. The fluoride release for all materials at days 1 and 2 was significantly greater than the rest of the time intervals. The amount of fluoride released from all the materials decreased from day 1 to day 28.

Fraga et al (1996)[21] evaluated the antibacterial effects of photo-cured glass ionomer liners and dentin bonding agents during setting. In this study two dentin bonding agents (Optibond and Syntac), an enamel bonding agent (Heliobond), and two photo-cured glass ionomer cements (Vitremer and Variglass VLC) and their inhibitory effects on bacterial growth during setting were examined. Cultures of eight bacterial species were used to test these materials, except for Vitremer glass ionomer cement, which was tested against only five species. The photo-cured glass ionomer cements and the Syntac dentin bonding system with glutaraldehyde demonstrated a significant inhibitory effect on the growth of several bacteria. Meanwhile, Variglass VLC glass ionomer cement did not exhibit this effect on Lactobacillus casei and Streptococcus sorbinus. Optibond light-cured dentin bonding agent, with fluoride and fillers in its composition and Heliobond (negative control group) did not demonstrate any inhibitory effect.

Musa et al (1996)[40] evaluated the fluoride release and compressive strength of one conventional and four resin modified glass ionomer cement with respect to time. In this study the fluoride release was measured using fluoride ion selective electrode and an ion analyzer, compressive strength was assessed using universal testing machine. The result of this study showed that one resin modified GIC (photacFil) released more fluoride than all other materials while Vitremer, Fuji II LC and chemfil suspension release similar amounts. Variglass had very much smaller elution of fluoride ion and the compressive strength of these materials was not affected with time.

Thevadass et al (1996)[50] evaluated the method for enhancing the fluoride releases of glass ionomer cement. In this study water activated glass ionomer cement was mixed with NaF solution of different concentration and effect of different mixing solutions on the working time, setting time and compressive strength was also determined. The result of this study showed that cement mixed with the 4% solution of NaF released significantly more fluoride than the water mixed control but there was no significant difference in the compressive strength. All the materials became progressively stronger on storage, mixing the cement with a 4% NaF increased the initial

fluoride release of the glass ionomer without seriously affecting other physical properties.

Aboush et al (1998)[1] evaluated the amount of fluoride release from light activated glass ionomer cement, a conventional glass ionomer cement, a compomer and a fluoridated composite using fluoride ion selective electrode and an ion analyzer. The result of this study showed that the pattern of fluoride release from light activated glass ionomer was similar to that of the conventional glass ionomer cement. The light activated glass ionomer released significantly more fluoride than the conventional glass ionomer cement. The composite and compomer released significantly less fluoride than any glass ionomer cements tested.

Preston A.J.et al (1999)[47] compared the amount of fluoride release from two glass ionomer cements, a resin modified glass ionomer cement, a compomer and a fluoride containing composite. Disc shaped samples were stored in deionized water or artificial saliva. The amount of fluoride release was assessed using fluoride ion selective electrode over a period of 64 days. The results showed that the fluoride release rate for all the materials tested in both solutions

decreased dramatically after 24 hours. The release rate in artificial saliva was significantly less than in deionized water.

Yap et al (1999)[58] compared the amount of fluoride, the pattern of fluoride release and the antibacterial property of fluoride releasing materials (i) composites (Tetric, experimental X), (ii) compomers (Dyract, Compoglass) and a resin modified glass ionomer cement (Fuji II LC). Conventional glass ionomer cement (Fuji II cap) was used as a control. In this study five samples of each restorative material were evaluated for daily fluoride release over a period of 35 days by means of ion chromatography. The result of this study showed that the amount of fluoride release, ranking from least to greatest over 35 days was as follow Tetric < Experimental X < Dyract < Fuji II LC < Compo glass < Fuji II cap. Antibacterial testing was conducted using the agar diffusion inhibitory test against Lactobacillus casei, Streptococcus mutans and Streptococcus sorbinus. Five samples of each restorative material were assessed at baseline (1 hour after mixing / light polymerization) and weekly intervals upto 35 days. The result of this antibacterial testing showed that none of restorative materials affected the growth of lactobacillus casei, streptococcus mutans, streptococcus

sorbinus, there was no correlation noted between fluoride release potential and antibacterial properties.

Francci et al (1999)[22] evaluated the amount of fluoride release from several adhesive restorative materials and its effect on dentine resistance to demineralization and on bacterial metabolism in modified in vitro system. Fluoride release was measured by using fluoride ion selective electrode and an ion analyzer. The result of this study showed that fluoride released from Fuji IX GP and Fuji II LC was significantly greater than from other materials, in restored dentine specimens increased resistance to demineralization from lactic acid challenge was directly related to fluoride release.

Yip et al (2000)[61] compared the amount of fluoride ion release from a freshly mixed polyacid modified resin composite or compomer (Dyract) and three resin modified glass ionomer cements (Fuji II LC, PhotacFil, Vitremer) and to compare the use of 3 units for measuring fluoride release. In this study five specimens of each material were prepared; the specimens were immersed in deionized water and stored at 37◦C. The levels of fluoride were analyzed at day 1, 7 and 30 and subsequently for every 28 days for 253 days. The fluoride measurement was carried out using fluoride ion selective electrode and

the fluoride ion release was measured in ppm, mg/sq cm, mg/cu mm. The result of this study showed that resin modified glass ionomer cements showed high initial release values, Dyract released less amount of fluoride ion release than other resin modified GIC. The amount of fluoride ion release measured at any time interval varied with the units of measurement chosen but the pattern of release remained the same.

Lee et al (2000)[32] evaluated fluoride ion release, diffusion process and fluoride diffusivity from GC Fuji lining LC glass ionomer cement, in this study fluoride ion concentration was measured using fluoride ion selective electrode and an ion analyzer. The result of this study showed that fluoride release was greater in ground set cement than in control samples of unmixed powder.

Yamamoto et al (2001)[60] evaluated fluoride uptake of human teeth using fluoride releasing restorative material invivo and invitro conditions. In this study class V cavities were prepared in second premolars and restored with fluoride releasing resin. The fluoride uptake around the cavity wall on the cut surfaces was measured using an electron probe micro analyzer wavelength dispersive X-ray method. The result of this study showed that the teeth in invivo and invitro

condition showed similar degree of fluoride uptake from the fluoride releasing material.

Hattab et al (2001)[25] evaluated the invitro effect of fluoride release from conventional and metal reinforced glass ionomers. In this study the following criteria was evaluated.

1. The release of F in deionized water compared with artificial saliva.
2. The effect of various surface coating on F release.
3. The uptake of released F by hydroxyapatite.
4. The expression of the release data in mathematical model.
5. The F content in the powder and set material.
6. Surface morphology of varnished and resin coated specimens.

The material evaluated in this study was (i) glass ionomer KetacFil (ii) Fuji II (iii) Ketac –Silver. The release of fluoride for 28 days and the concentration of F were measured with F ion specific electrode. The result of this study showed a strong initial release of F which decreased with time. The fluoride release from Ketacfil and Fuji II was comparable in both pattern and magnitude. They released

four times more F than Ketacfil and Fuji II. In the entire specimen the release of fluoride is artificial saliva was less than in deionized water surface coating the specimen significantly reduced the fluoride release. All the fluoride released is aqueous solution was taken up by the hydroxyl apatite with Fuji II ranking the highest in increasing hydroxyapatite fluoride concentration. The F concentration in set material was more in Ketacfil and Fuji II than in Ketac- silver. Micro morphological examination revealed remnants of surface coating on all specimens after 14 days storage in artificial saliva.

Attar et al (2002)[5] investigated the fluoride release, the uptake and subsequent release from two composite resin, two poly acid modified resin composites and conventional glass ionomer cement. The fluoride recharge was done using 1000ppm of sodium fluoride and amount of fluoride release was measured using fluoride ion selective electrode at different intervals for 60 days. The result of this study revealed that all the fluoride containing materials released greatest amount of fluoride during first day.

Asmussen et al (2002)[4] evaluated the long term fluoride release from glass ionomer cement, a compomer and from experimental resin composites. Five discs of each specimen were stored in distilled water

at room temperature. The amount of fluoride release was evaluated by fluoride ion selective electrode and ion analyzer over a period of 3 years. The result of this study showed that glass ionomer cement released more amount of fluoride than any other cement.

Miranda et al (2002)[37] evaluated the fluoride release from three restorative material: Vitremer (3M), Heliomolar and Z100 using an adhesive application (Scotch bond multipurpose). In this study 10 discs of each material were prepared. Five were covered with the adhesive and five were not covered with adhesive. The disc was immersed in artificial saliva which was changed daily. Fluoride release was measured at days 1, 5, 10, 15 and 20 using fluoride ion selective electrode combined with ion analyzer. The results showed that the use of dental adhesive significantly decreased the fluoride release.

Itota et al (2003)[27] evaluated the effect of fluoride releasing adhesives on inhibition of secondary caries in outer and wall lesions. Two commercial fluoride releasing and a commercial adhesive without fluoride release were tested in this study. Class V cavities were prepared on extracted human premolars and restored with various materials. The restored teeth were incubated in bacterial medium containing sucrose with Streptococcus mutans for 14 days and the

lesions were analyzed using microradiographs. The results indicate that fluoride releasing adhesives are effective in the prevention of wall lesions little effect in outer lesion inhibition. Hence it was concluded that the combined restoration using a fluoride-releasing adhesive and fluoride releasing restorative material should be selected to inhibit secondary caries.

Attar et al (2003)[6] evaluated the amount of fluoride release and uptake characteristics of four flowable resin composites (Heliomolar Flow, Tetric Flow, Wave, Prema Flo), one flowable compomer (Dyract flow), one conventional glass ionomer cement mixed with two different powder liquid ratios (Chemflex syringeable and chemflex condensable), one packable resin composite (Surefil), one ion releasing composite (Ariston pH) and one resin modified glass ionomer cement (Vitremer). Seven discs of each material were prepared, each disc was immersed in 3.5ml of deionized water within a plastic vial and stored at 37◦C and the release of fluoride was measured for 30 days. The samples were recharged with 2 ml of 1.23% acidulated phosphate fluoride gel for four minutes. Then all the samples were analyzed for additional 10 days. The fluoride release was measured with fluoride ion selective electrode and an ion analyzer. The result of this study

showed that all the material tested released highest amount of fluoride after 1st day but gradually diminished with time Ariston pH released the highest amount of fluoride followed by chemflex condensable.

Hicks et al (2003)[26] reviewed the fluoride releasing restorative materials and secondary caries. It was concluded that fluoride releasing dental materials had improved resistance against primary and secondary caries in coronal and root surfaces. Plaque and saliva fluoride levels are elevated to a level that facilitates remineralization. In addition fluoride released to dental plaque adversely affects the growth of Lactobacillus and Streptococcus mutans by interfering with bacterial enzymes. Fluoride recharging of these dental materials is readily achieved with fluoridated tooth paste, fluoride mouth rinse and other sources of topical fluoride. This allows fluoride releasing dental materials to act as intraoral fluoride reservoirs.

Xu et al (2003)[55] evaluated the compressive strength using universal instron testing machine, the amount of fluoride release using fluoride ion selective analyzer and fluoride recharge was done using 2% NaF for 15 commercial fluoride releasing restorative materials. The results showed a negative linear correlation between compressive strength and fluoride release, that is the restorative materials with high

fluoride release have lower mechanical properties and the material with high fluoride releasing ability have high recharge capability.

Dionysopoulos et al (2003)[16] evaluated the fluoride release and recharge of a conventional glass ionomer, a resin modified glass ionomer and two compomers. All the prepared samples were immersed in deionized water and the amount of fluoride release was measured using fluoride ion selective electrode at every 2 days till 22 days. The result of this study shows that greatest amount of fluoride release during the first day followed by sharp decline then slower decline and the fluoride recharge with 0.2% NaF was effective. Hence it was concluded that, from clinical point of view all the fluoride releasing material can act as intra oral device for controlled release of fluoride at risk sites of recurrent caries.

Yaman et al (2004)[56] compared the in vitro caries inhibition of various resin based materials. In this study class V cavities were prepared in 25 freshly extracted human premolars which were then restored with GIC (ChemFil II), compomer (CompoglassF, Dyract AP) and composite resin (Tetric Ceram, Z100). The teeth were submerged in an acid gel (10 % methyl cellulose, 0.1m lactic acid) for 6 weeks. Each specimen were sectioned and left in water for 24 hours then

examined under polarized light microscope. The lesion consists of outer surface lesion and the cavity wall lesion. There was no significant difference in the body depth of outer lesion and in depth of wall lesion among the teeth restored with CompoglassF, Dyract AP and Chemfil. There was a significant difference between those restored with Z100 and Tetric Ceram. The length of the wall lesion for the teeth restored with Chemfil II was significantly smaller than that in remaining groups. The length of the wall lesion for the teeth restored with Z100 and Tetric flow was significantly higher than in remaining groups. The results suggest that composite materials and compomer provide less caries inhibition than glass ionomer cements.

Barata et al (2004)[7] evaluated the influence of different storage solution on fluoride release from glass ionomer cements. It was concluded from this review that either distilled water or deionized water do not represent the conditions in the oral aqueous environment. Different media tested include artificial saliva, acidic solution and artificial saliva supplemented with esterase and it was observed that more fluoride leached into acidic medium followed by distilled water and artificial saliva.

Lobo et al (2005)[33] evaluated the fluoride releasing capacity and cariostatic effect provided by sealants. In this study occlusal fissures with area measuring 12mm^2 were delimited in 48 extracted molars, randomly divided into four groups, group 1: no sealing, group 2: sealing with resin modified glass ionomer, group 3: sealing with a fluoride releasing composite sealant, group 4: sealing with a non-fluoridated composite sealant. A 4mm^2 window was outlined on the buccal enamel for analysis of fluoride uptake. Following treatment, group 2, 3 and 4 were subjected to 5 days of pH cycling, while group 1 was kept in a moist environment at 37◦C. Fluoride uptake was assessed by dental biopsy, and the amount of fluoride release to the pH cycling solutions was determined by ion analysis. Cariostatic effect of enamel demineralization around the sealant was evaluated by cross sectional microhardness analysis. The result of this study showed that group 2 released high amount of fluoride and high uptake of fluoride by enamel and lower level of demineralization than group 3 and group 4.

Burke et al (2006)[10] reviewed the clinical benefits of fluoride release from fluoride containing restorative materials. In this review it was concluded that the long term measurable release of fluoride can be observed from certain restorative material, in-vitro, particularly glass

ionomer cement, resin modified glass ionomer cement, fluoridated cements, fluoridated dental amalgam and certain fissure sealants. In general the rate of fluoride release is not constant but exhibits a rapid initial rate which decreases with time. Fluoride releasing materials may feature greater clinically longevity, reduced incidence of marginal failure, an elevated concentration of fluoride in plaque. In addition, this material may perform better in caries inhibition in artificial caries model studies than non – fluoridated materials.

Garcez et al (2007)[23] evaluated the amount of fluoride release of Dyract, Ariston pH, Definite Tetric Ceram and Vitremer, Z100 as positive and negative controls in two storage protocols: deionized water and pH cycling solutions for 15 days. Eight discs of each material were prepared and suspended individually in 4 ml of each solution, which were changed daily. Fluoride release was analyzed using fluoride ion selective electrode and an ion analyzer at 1, 7 and 15 days. The result of this showed that all materials released more fluoride in pH cycling solutions, except for Ariston pH which maintained a constant release during the experiment. The highest fluoride release was noted in positive control, Vitremer in pH cycling solution and by Ariston pH in deionized water.

Petri et al (2007)[45] investigated the effect of chitosan (CH) on the flexural strength and fluoride release from glass ionomer cement in this study different concentration of CH in GIC liquid had been investigated. The concentration used were 0 v/v% control, 10 v/v% , 25 v/v%, 50 v/v% and 100v/v% and the result of the study showed that concentration of 10v/v% chitosan modified GIC liquid had improved the flexural strength and catalyzed fluoride release.

Wiegand et al (2007)[54] reviewed the fluoride release, recharge capabilities and antibacterial properties of fluoride releasing dental restorations and discussed the current status concerning the prevention or inhibition of caries development and progression. In this review original scientific papers and reviews listed in PubMed were included. Conclusion of this review is that the potential to release fluoride from fluoride containing dental materials varies between different materials and different brands. The optimal fluoride release from restoration is related to their matrices, setting mechanism, fluoride content and several other environmental conditions.

Mousavinasab et al (2009)[39] measured the amount of fluoride release from fluoride containing material (i) 4 types of GIC (Fuji IX, Fuji IX extra, Fuji VII, Fuji II LC) (ii) a compomers (iii) a giomer

using fluoride ion specific electrode and ion analyzer at different intervals 1-7 days, 14th day and at 21st day. The result of this study shows that Fuji VII released more fluoride at 1-7 days followed by Fuji IX extra, Fuji II LC, Fuji IX, compomer and giomer and this remained the same at 14th and 21st days.

Arnaud et al (2010)[3] evaluated the in vitro effect of chitosan treatment on enamel de-remineralization. In this study, to evaluate the microhardness and loss of phosphorous, different groups of human tooth samples were exposed to de remineralising solution of controlled pH. The result of this study showed that chitosan interfered with the process of demineralization of tooth enamel and inhibit the release of phosphorous. In addition optical coherence tomography imaging (OCT) was done to measure the depth of penetration of chitosan into enamel. The result of the OCT image showed that chitosan penetrated into tooth samples upto the level of DEJ, Thus chitosan may act as a mechanical barrier for acid penetration, contributing to its demineralization inhibition.

Kiran et al (2010)[30] evaluated and compared the amount and pattern of fluoride release from three types of glass ionomer cements GC Fuji II, GC Fuji VII and GC Fuji IX in water (pH 7) and lactic acid

(pH 5.2) for a period of 28 days at five intervals using fluoride ion selective electrode. The result of this study showed that the pattern of fluoride release from all the restorative materials was similar. An "initial fluoride burst" was seen for first few days after being placed in the storage solutions.

Chavez de Paz et al (2011)[13] evaluated the antimicrobial effect of nanoparticle complexes from chitosans of various molecular weights and degrees of deacetylation. In this study antimicrobial effect was assessed by live or dead Bac Light technique in conjugation with confocal scanning laser microscopy and image analysis. The result of this study showed that nanoparticle complexes prepared from chitosans with high MW showed low antimicrobial effect, whereas those prepared from low MW chitosan showed high antimicrobial effect.

Ferreira et al (2011)[18] evaluated the in vitro antibacterial effect of GIC against Streptococcus mutans, S.oralis, S.salivarius and Streptococcus species by measuring the diameter of growth inhibition halos in mueller hinton agar plates and the result of this study shows that GIC promoted growth inhibition of cariogenic bacteria.

Carvalho et al (2011)[12] reviewed chitosan as an oral antimicrobial agent. This review showed that chitosan has excellent biocompatibility, almost no toxicity to human beings and animals, high bio activity, biodegradability, reactivity of the deacetylated amino group, selective permeability, poly electrolyte action, antimicrobial activity, ability to form gel and film, chelation ability and absorptive capacity.

Neilands et al (2011)[41] evaluated the effect of chitosan nanoparticles on the acid tolerance response of adhered Streptococcus mutans. In this study acid tolerance response was induced by exposing Streptococcus mutans to pH 5.5 for 2 hours and confirmed by exposing the acid adapted cells to pH 3.5 for 30 mins, the cell viability was assessed by live/dead technique. The result of this study showed that chitosan nanoparticles tested had the ability to hinder acid tolerance response induction in adhered streptococcus mutans.

Paschoal et al (2011)[43] compared the fluoride release pattern of a nanofilled resin modified GIC (Ketac N100 – KN) and a nano filled resin composite. Six discs of each material were prepared and immersed into 4ml of deionized water for 15 days. Fluoride release was measured on each day using fluoride ion selective electrode.

In order to analyze the difference among materials and the influence of time in the daily fluoride release, statistical analysis was performed. The result of this study showed significant difference between the daily fluoride release overtime upto third day only for GIC materials. Thus it indicates that the fluoride release profile of nanofilled resin modified GIC is comparable to the resin composite.

Uysal et al (2011)[51] tested the null hypothesis that there is no significant difference between the chitosan containing and non-fluoridated dentifrice in inhibition of enamel demineralization around orthodontic brackets. In this study 16 orthodontic patients who were scheduled to have extraction of four first premolars were divided into two groups (i) experimental group patient were instructed to use chitosan containing dentifrice (ii) control group patient were instructed to use non-fluoridated dentifrices. After 60 days, the teeth were extracted and longitudinally sectioned. The demineralization was assessed by cross sectional microhardness. The result of this study showed that chitosan containing dentifrice showed lower demineralization than the control group.

Wang et al (2011)[53] studied the recent advances of chitosan nanoparticles as drug carrier because of their good biocompatibility,

biodegradability and can be really modified. This review states that as a new drug delivery system, they have attracted increasing attention for their wide application in for example loading protein drugs, gene drugs and anti-cancer chemical drugs, and via various routes of administration including oral, nasal, intravenous and ocular.

Martinez-Mier et al (2011)[36] analyzed different techniques to develop standardized method for fluoride release in biological and non-biological samples for dental research. This study was undertaken in three phases: phase1: comparison of currently used techniques such as direct method and micro diffusion method, phase2: comparative tests conducted to resolve identified differences, phase3: develop universal gold standard test. The result of this study showed that standardization of direct and diffusion technique will benefit all studies requiring the use of standard fluoride solution and those specially dealing with fluoride ingestion and toxicity and the determination of fluoride concentration in different matrices.

Dastjerdie et al (2012)[14] compared the antimicrobial properties of glass ionomer cement with zinc phosphate cement. In this study these brands of GIC Resilience, Band-Tite, Ariadent and three brands of zinc phosphate cement. Harvard, Hoffman's, Ariadent were

selected. The antibacterial property of these cements was evaluated against Streptococcus mutans and Candida albicans after 2 days and 7 days of incubation. The result of this study showed that the antibacterial activity of all the glass ionomer cements was more than that of zinc phosphate cement.

Elaska et al (2012)[17] evaluated the antibacterial property of adhesive resin incorporating chitosan and its adhesive characteristics. In this study different concentration of chitosan solution to single bond adhesive resin was used and the antibacterial property was assessed using direct contact test against Streptococcus mutans. The result of this study showed that the adhesive resin containing 0.12%w/w chitosan has promising antibacterial property and it does not adversely affect the adhesive properties.

Keegan et al (2012)[29] assessed the controlled delivery of fluoride from chitosan microparticles prepared by spray drying technique. In this study chitosan microparticles were manufactured from dispersion containing 1.0% & 2% w/v chitosan and 0.20% or 0.04% w/v NaF. The fluoride loading and release were determined by fluoride ion selective electrode. The result obtained, the isolated chitosan / fluoride microparticles have potential utility as vehicles to

enhance fluoride retention and promote its controlled fluoride delivery in the oral cavity from a variety of oral care formats.

Mahapoka et al (2012)[34] developed a resin based sealant containing chitosan whiskers for use as a pit and fissure sealer. In this study chitosan whiskers were synthesized and then characterized using Fourier transform infrared spectrometry and transmission electron microscopy. The whiskers were then incorporated into dimethacrylate monomer at various ratios by weight and subsequently analyzed for their antibacterial and physical properties. The result of this study showed that dimethacrylate based sealant containing chitosan whiskers had a greater antibacterial activity and they were comparable with antimicrobial commercial resin sealants. The inclusion of the whiskers did not reduce the curing depth or degree of double bond conversion and the reduction in hardness was minimal.

Materials and Method

MATERIALS AND METHODS

MATERIALS:

1. Type II universal restorative- ISO 9917:2003(E)
2. Type II light cure universal restorative -ISO 9917-2:1998(E)
3. GC Fuji VII (pink) - ISO 9917-1991(E)
4. GC HS posterior extra- ISO 9917-1: 2007(E)
5. Deionized water
6. TISAB II (Total Ionic Strength Adjustor)
7. Bacterial strains (Streptococcus mutans, Streptococcus salivarius and Lactobacillus casei)
8. Brain heart infusion agar
9. Blood agar plates

ARMAMENTARIUM:

1. Teflon moulds (10x2mm)
2. Waxed dental floss
3. Microscopic slides

4. Pipette
5. Vernier caliper
6. Tweezer
7. Plastic vials & holder
8. Conical flask
9. Cement spatula
10. Petri dish
11. Gloves
12. Mouth mask
13. Inoculation loop
14. Test tubes

SPECIAL EQUIPMENTS

1. Incubator
2. UV light sterilizer
3. Curing unit
4. Fluoride ion selective electrode
5. Ion analyzer (Orion-Expandable ion analyzer EA 940)

METHODOLOGY:

STANDARD PREPARATION OF CHITOSAN SOLUTION:

1.8ml of glacial acetic acid is made up to 100ml with distilled water in 100ml standard flask. 20mg of chitosan (CH) nanoparticles (Sigma –Aldrich chemicals) was weighed and dissolved in 0.3N acetic acid and made up to 100ml with the same acetic acid in a 100ml standard flask to get 0.2mg/ml chitosan solution.

STANDARD PREPARATION OF CHITOSAN MODIFIED GLASS IONOMER: LIQUID

0.1ml of prepared 0.2mg /ml of chitosan solution is added to 0.9ml of glass ionomer liquid to attain a concentration of 10v/v% of chitosan modified glass ionomer liquid.

STUDY DESIGN:

This study comprised of evaluation of two properties of glass ionomer cements:

(i) Fluoride release from four types of glass ionomer cements with and without chitosan.

(ii) Antibacterial property of Type IX HS posterior extra glass ionomer cement with and without chitosan against Streptococcus mutans, Streptococcus salivarius and Lactobacillus casei.

GROUPING OF SAMPLES:

Four types of glass ionomer cements (Type II universal restorative, Type II light cure universal restorative, GC Fuji VII (pink), GC HS posterior extra) were tested to determine the amount fluoride release from the samples immersed in deionized water at different time intervals.

The glass ionomer cements used are as follows.

GROUP I A	TYPE II GIC
GROUP I B	TYPE II CH MODIFIED GIC
GROUP II A	TYPE II LC GIC
GROUP II B	TYPE II LC CH MODIFIED GIC
GROUP III A	TYPE VII GIC
GROUP III B	TYPE VII CH MODIFIED GIC
GROUP IV A	TYPE IX HS POSTERIOR EXTRA GIC
GROUP IV B	TYPE IX HS POSTERIOR EXTRA CH MODIFIED GIC

SAMPLE PREPARATION FOR FLUORIDE RELEASE:

Six samples of each group were prepared using disposable teflon moulds of 10mm internal diameter and 2mm thickness.

In groups I A, II A, III A, IV A the powder and liquid ratio of glass ionomer cement were proportioned according to manufacturer's instruction using scoops. The proportioned powder and liquid were hand mixed using plastic spatula and mixing pad. In groups I B, II B, III B, IV B the powder liquid ratio of glass ionomer cement were proportioned according to manufacturer's instruction with the liquid component as chitosan modified glass ionomer liquid. The proportioned powder and liquid were hand mixed using plastic spatula and mixing pad.

The hand mixed glass ionomer cements were loaded into disposable teflon moulds. Microscopic slides were pressed against teflon moulds on both sides to remove the cement excess in all the sample preparations. Waxed dental floss was incorporated into the cement during setting to facilitate suspension of the samples in testing medium (deionized water).

The samples in group I A, I B, III A, III B, IV A and IV B were allowed to set at room temperature for 10 minutes. The samples in groups II A and II B were light cured using dental curing light source for 20 seconds on each side from the top and bottom surfaces of samples. The discs were then removed from the disposable teflon moulds and suspended inside a plastic vial containing 4ml of deionized water.

The plastic vials containing the samples were incubated at 37◦C. The deionized water was changed after 1 day, 7 days, 14 days, 21 days and 28 days intervals. After buffering the solution with the equal volumes of TISAB II (total ionic strength adjustor, pH 5.0 used to provide constant background ionic strength, decomplex fluoride and adjust solution pH). Fluoride release was measured with a fluoride ion selective electrode and an ion analyzer which was previously calibrated using standard fluoride solutions containing 0.20, 1.00, 2.00, 10.00, 20.00, 100 ppm of F respectively. The concentration of the quantity of fluoride released in different time periods was evaluated and the amount of fluoride released was recorded in Parts Per Million during each interval and statistically analyzed.

ANTIBACTERIAL PROPERTY ASSESSMNET:

The antibacterial activity of group IV A and IV B was evaluated against Streptococcus mutans, Streptococcus salivarius and Lactobacillus casei.

MICROORGANISMS:

The bacterial strains used are Streptococcus mutans (MTCC NO:497) Streptococcus salivarius (MTCC NO: 10306) and Lactobacillus casei (MTCC NO: 2696). All are human isolates and were obtained from IMTECH Chandigarh. These strains were reactivated in brain heart infusion culture media. After 24 hours incubation period in bacteriological incubator at 37˚C, 100 µl of the strains inoculum were plated out by using disposable straps on blood agar culture media.

SAMPLE PREPARATION FOR ANTIBACTERIAL ACTIVITY:

The specimen discs were prepared as described for fluoride release. Microscopic slides were pressed against teflon moulds on both sides to remove the cement excess and to assure a flat contact

surface in all the sample preparations. The samples were allowed to set at room temperature for 10 minutes. After separation from moulds, the specimen discs were transferred on to the bacterial plates with microorganisms. Six discs of each group were tested for each microorganism. Moulds, glass slides and other necessary items were disinfected with methylated spirit and subjected to 15 minutes of ultra violet light sterilization between uses. The prepared discs were placed on the plates. The plates with Streptococci were incubated with additional Co_2 and those with lactobacillus were incubated for 48 hours at 37°C. The diameter of zones of inhibition produced around the specimens was measured at three different points. The size of inhibition zone was calculated through subtracting the diameter of specimens using vernier calipers and statistically analyzed. All the procedures were conducted using aseptic techniques and carried out in an ultra violet light sterilized biohazard hood.

METHODOLOGY FLOWCHART

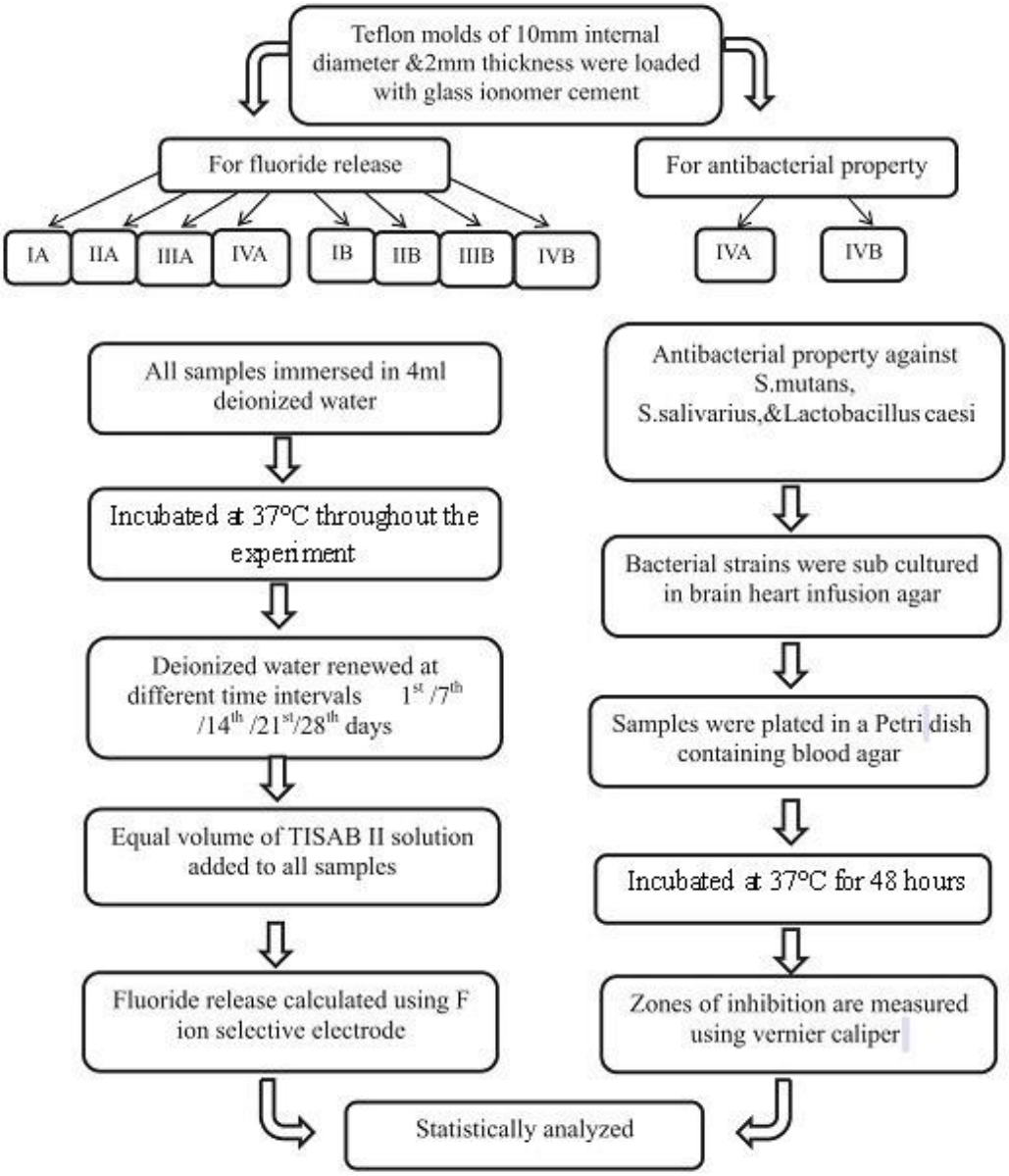


Fig.1: Materials for Fluoride Release Analysis

Fig.2: Weighing Balance

Fig.3: Prepared Chitosan Solution

Fig.4: Sample Preparation

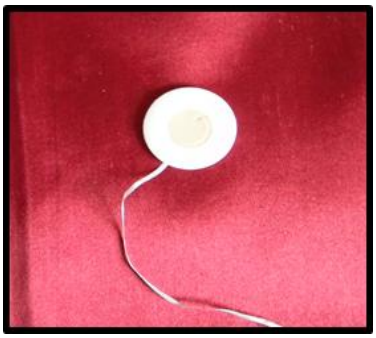


Fig.5: GIC Disc in Teflon Mould

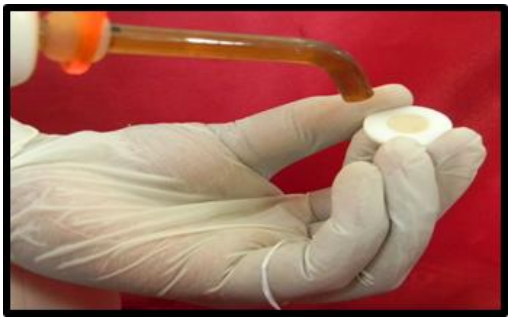


Fig.6: Curing of Sample

Fig.7: Samples Immersed in Deionized Water

Fig.8: Samples Stored at 37°C in Incubator

Fig.9: Ion Analyzer

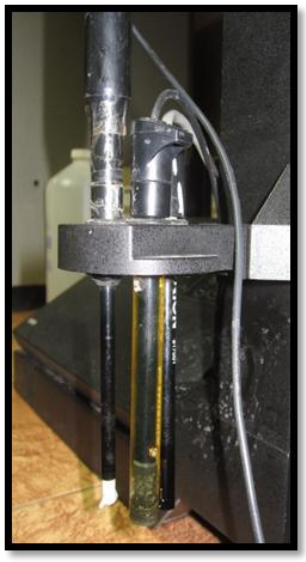


Fig.10: F Ion Selective Meter, Magnetic Stirrer &pH Electrode

Fig.11: Materials for Antibacterial Activity Assessment

Fig.12: Bacterial Colony

Fig.13: Inoculation

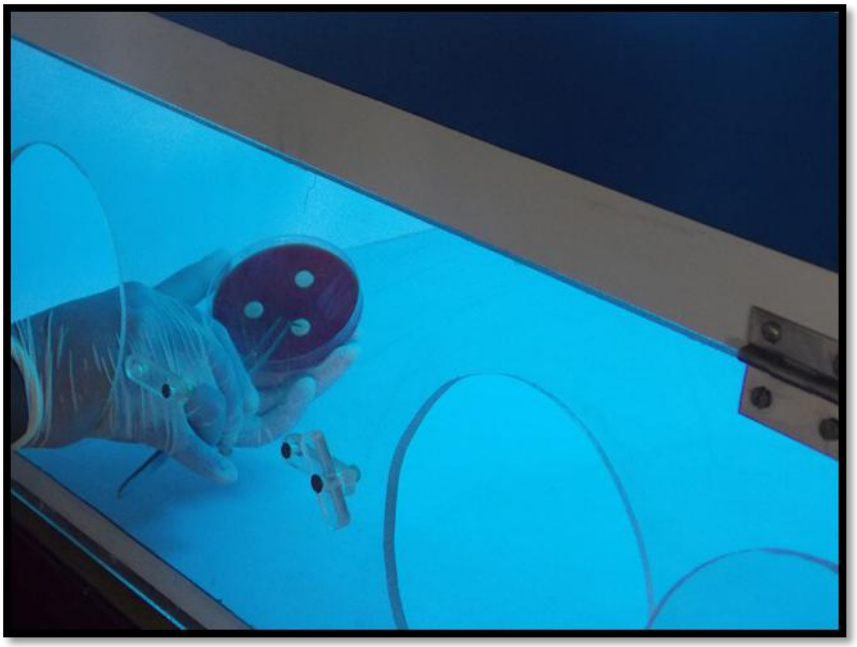


Fig.14: Samples Embedded on Blood Agar Plate

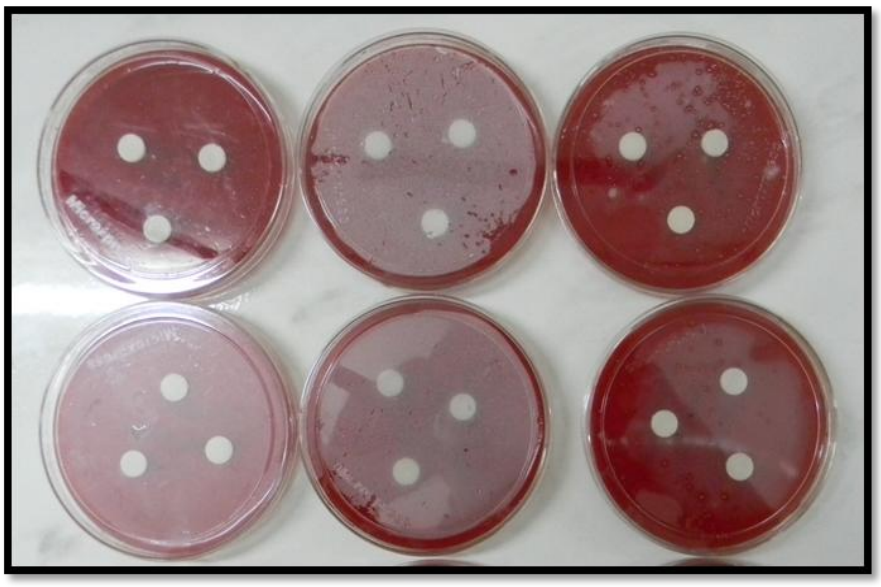


Fig.15: GIC Discs in Blood Agar Plates

Fig.16: Samples Incubated

Fig.17: Zone of Inhibition for S.Mutans

Fig 18: Zone of Inhibition for S.Salivarius

Fig.19: Zone of Inhibition for Lactobacillus Casei

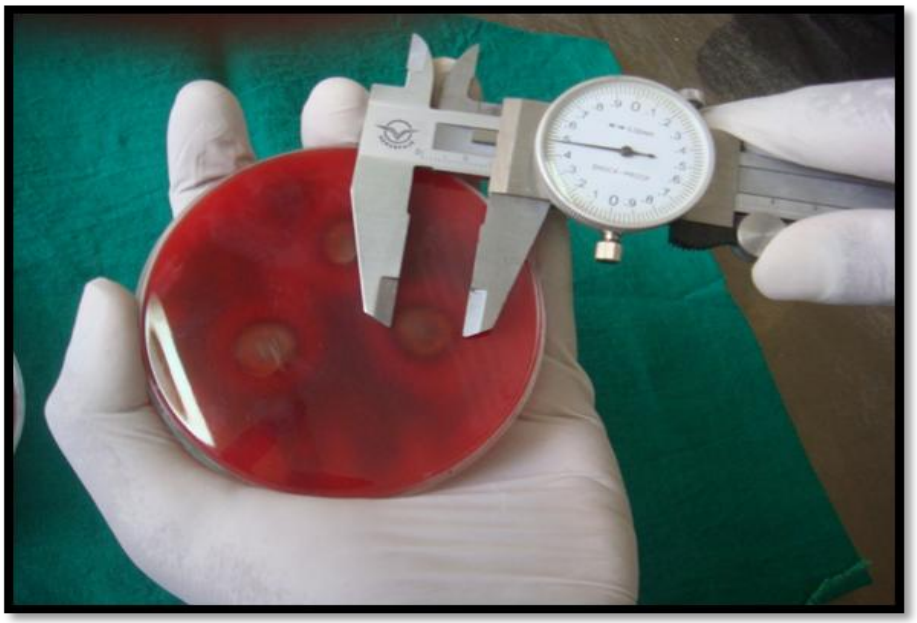


Fig.20: Measuring Zone of Inhibition using Vernier Caliper

TABLE-1: COMPARISON OF FLUORIDE RELEASE BETWEEN GROUPS I A & I B

GROUPS	(Mean ±SD) ppm					P VALUE BETWEEN DAYS
	1 Day	7 Days	14 Days	21 Days	28 Days	
I A	30.83±1.17^{e}	20.33 ±1.03^{d}	17.67 ±0.82^{c}	14.67 ±1.37^{b}	10.67±0.52^{a}	<0.001***
I B	71.67± 7.89^{d}	49.50 ±3.51^{c}	36.50± 3.51 ^{b}	31.33±2.73^{ab}	25.50 ±1.97^{a}	<0.001***

TABLE-2: COMPARISON OF FLUORIDE RELEASE BETWEEN GROUPS II A & II B

GROUPS	(Mean ±SD) ppm					P VALUE BETWEEN DAYS
	1 Day	7 Days	14 Days	21 Days	28 Days	
II A	17.00± 2.37^{d}	12.33± 0.52^{c}	11.33±0.52^{bc}	10.17±0.99^{ab}	8.60± 0.46^{a}	<0.001***
II B	69.17± 4.67^{e}	55.50± 3.45^{d}	38.17 ±2.71^{c}	31.38 ±0.98^{b}	23.33±0.82 ^{a}	<0.001***

NOTE:

1) *** denotes significance at 0.001 level

2) Different alphabets between days denote significance at 1% level using tukey HSD test.

TABLE-3: COMPARISON OF FLUORIDE RELEASE BETWEEN

GROUPS III A & III B

GROUPS	(Mean ±SD) ppm					P VALUE BETWEEN DAYS
	1 Day	7 Days	14 Days	21 Days	28 Days	
III A	55.83±4.75^{e}	34.33± 2.88^{d}	22.50± 1.64^{c}	22.67 ±1.37^{bc}	16.50±1.22^{a}	<0.001***
III B	70.17 ±6.94^{e}	53.00 ±4.15^{d}	39.83± 3.37^{c}	34.33 ±0.82^{b}	26.33±2.66^{a}	<0.001***

TABLE-4: COMPARISON OF FLUORIDE RELEASE BETWEEN

GROUPS IV A & I V B

GROUPS	(Mean ±SD) ppm					P VALUE BETWEEN DAYS
	1 Day	7 Days	14 Days	21 Days	28 Days	
IV A	117.67± 35.22^{e}	49.17± 4.92 ^{d}	42.33 ±2.25^{cd}	30.33 ±4.23^{ab}	20.00 ±0.89^{a}	<0.001***
IV B	155.00 ±23.45^{e}	170.00 ±10.95^{d}	126.67 ±13.66 ^{c}	98.83 ±8.82^{b}	78.00 ±12.20^{a}	<0.001***

NOTE:

1) *** denotes significance at 0.001 level

2) Different alphabets between days denote significance at 1% level using tukey HSD test.

TABLE-5: OVER ALL COMPARISON OF GICs

GROUPS	(Mean ±SD) ppm				
	1 Day	7 Days	14 Days	21 Days	28 Days
I A	30.83±1.17[ab]	20.33 ±1.03 [b]	17.67 ±0.82 [b]	14.67 ±1.37[b]	10.67±0.52[b]
II A	17.00± 2.37 [a]	12.33± 0.52 [a]	11.33± 0.52[a]	10.17± 0.99[a]	8.60± 0.46[a]
III A	55.83±4.75[b]	34.33± 2.88 [c]	22.50± 1.64 [c]	22.67 ±1.37 [c]	16.50± 1.22 [c]
IV A	117.67± 35.22[c]	49.17± 4.92 [d]	42.33 ±2.25 [d]	30.33 ±4.23[d]	20.00 ±0.89 [d]
P VALUE BETWEEN GROUPS	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

TABLE-6: OVER ALL COMPARISON OF GICs WITH CHITOSAN

GROUPS	(Mean ±SD) ppm				
	1 Day	7 Days	14 Days	21 Days	28 Days
I B	71.67± 7.89[a]	49.50 ±3.51[a]	36.50± 3.51[a]	31.33± 2.73[a]	25.50 ±1.97[a]
II B	69.17± 4.67[a]	55.50± 3.45 [a]	38.17 ±2.71[a]	31.38 ±0.98[a]	23.33 ±0.82 [a]
III B	70.17 ±6.94 [a]	53.00 ±4.15 [a]	39.83± 3.37 [a]	34.33 ±0.82 [a]	26.33 ±2.66 [a]
IV B	155.00 ±23.45[b]	170.00 ±10.95[b]	126.67 ±13.66[b]	98.83 ±8.82 [b]	78.00 ±12.20 [b]
P VALUE BETWEEN GROUPS	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

NOTE:

1) *** denotes significance at 0.001 level
2) Different alphabets between groups denote significance at 1% level using tukey HSD test.

TABLE-7: ANTIBACTERIAL PROPERTY OF GROUPS IV A & IV B AGAINST THREE MICROORGANISMS

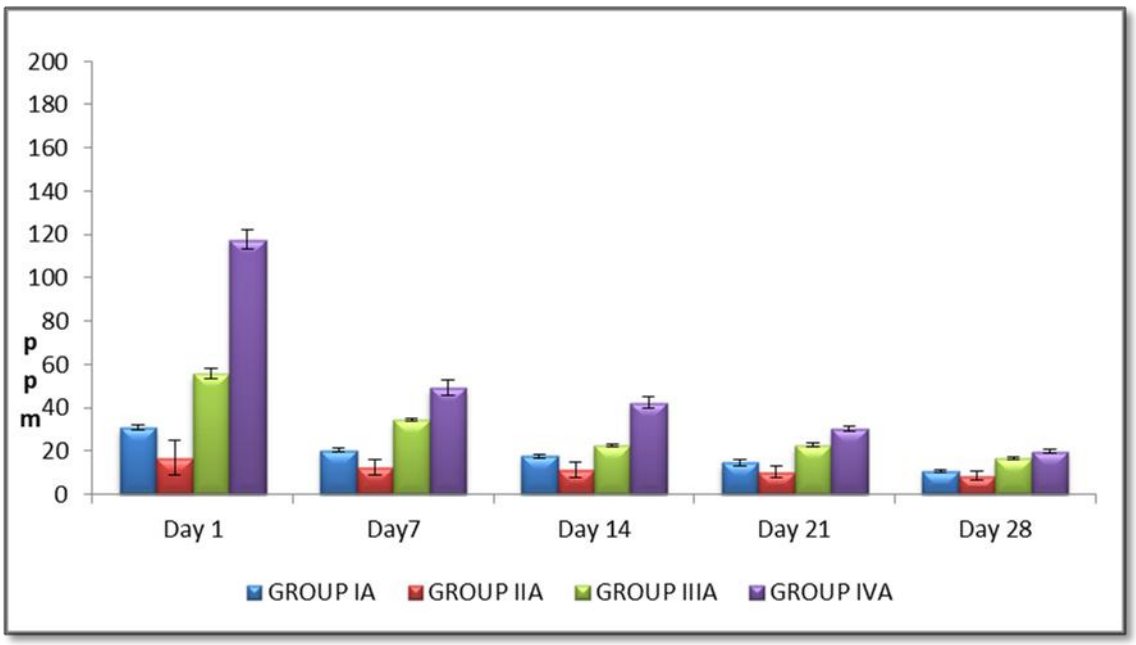
GROUPS	ZONE OF INHIBITION IN (mm)			P VALUE BETWEEEN GROUPS
	S. MUTANS	S.SALIVARIUS	L.CASEI	
IV A	0.3400 ±.05477[a]	0.3500± 0.5477[a]	0.3000 ±0.8944[a]	<0.001***
IV B	3.4000 ±0.21909[a]	3.1500± 0.5477[a]	4.3000 ±0.20000[b]	<0.001***

NOTE:

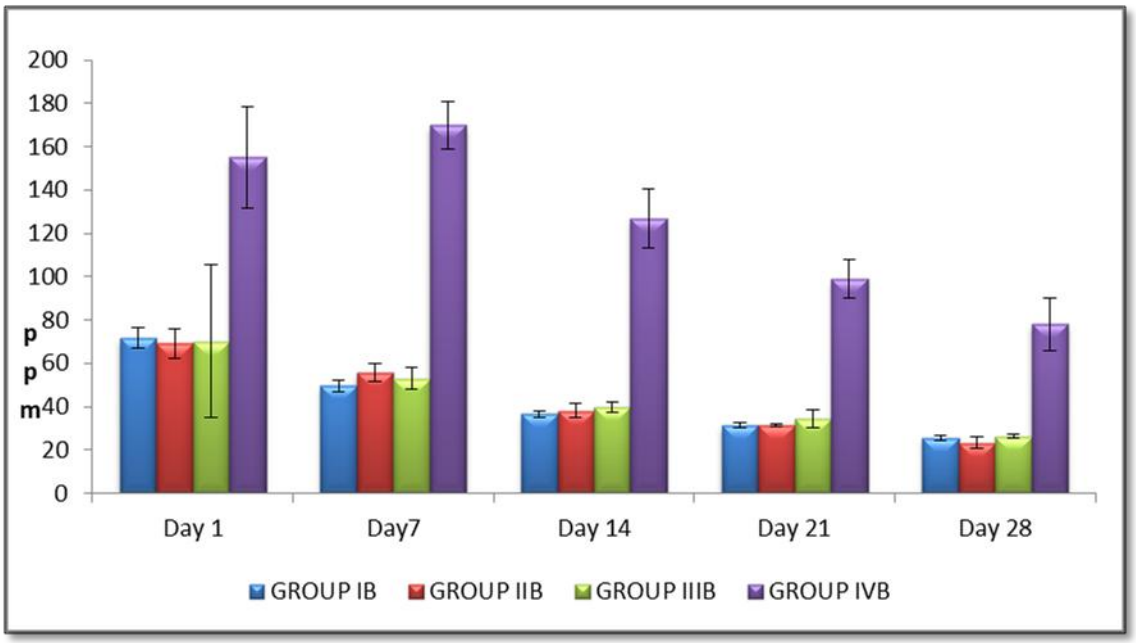
1) *** denotes significance at 0.001 level

2) Different alphabets between groups denote significance at 1% level using tukey HSD test.

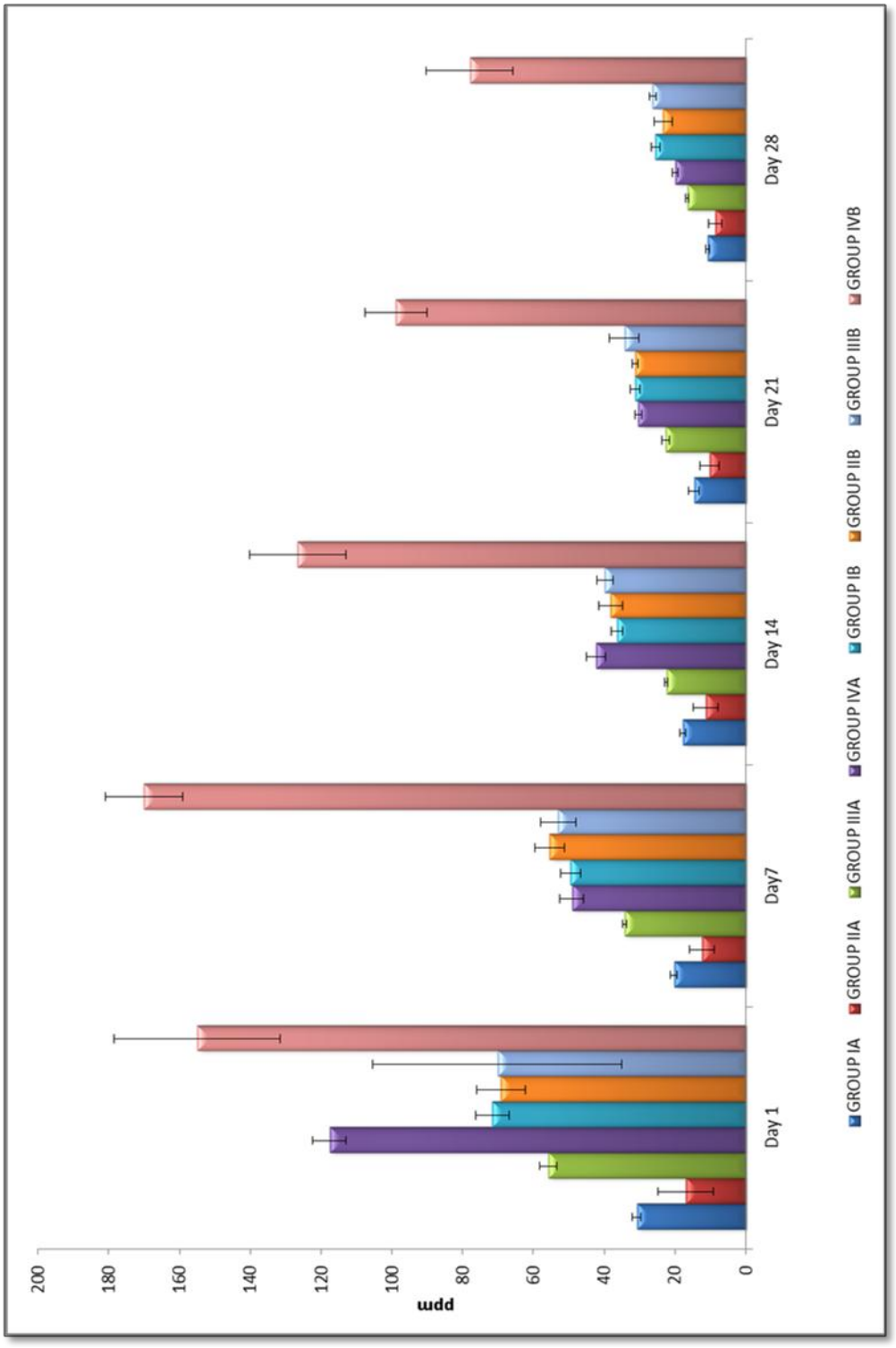
GRAPH-1: COMPARISON OF FLUORIDE RELEASE BETWEEN GROUPS I A, II A, III A, IV A



GRAPH-2: COMPARISON OF FLUORIDE RELEASE BETWEEN GROUPS I B, II B, III B, IV B



GRAPH-3: OVERALL COMPARISON OF FLUORIDE RELEASE



Results

RESULTS

The fluoride ion release profiles on deionized water from group I A (GC Fuji type II GIC), group I B (GC Fuji type II chitosan modified GIC), group II A (GC Fuji type II LC GIC), group II B (GC Fuji type II LC chitosan modified GIC), group III A (GC Fuji type VII GIC), group III B (GC Fuji type VII chitosan modified GIC), group IV A (GC Fuji type IX high strength posterior GIC), group IV B (GC Fuji type IX high strength posterior chitosan modified GIC) for a period of 28 days at five intervals (day 1, day 7, day 14, day 21 and day 28) was recorded. The amount of fluoride released was recorded in Parts Per Million. The antibacterial activity of group IV A and IV B against Streptococcus mutans, Streptococcus salivarius and Lactobacillus casei was evaluated by measuring the zone of inhibition in millimeters at 37°C incubation for 48 hours.

Statistical analysis: The fluoride release between days and between groups was statistically analyzed using ANOVA followed by tukey HSD (Post HOC) and independent samples comparison was analyzed using ‘t’ test. The antibacterial activity of group IV A and IV B against Streptococcus mutans, Streptococcus salivarius and

Lactobacillus casei was statistically analyzed using ANOVA followed by tukey HSD (Post HOC).

Table 1 shows the comparison of fluoride release between group I A and group I B. Group I B released more amount of fluoride than group I A at all the time interval which was statistically significant (P<0.001). The amount of fluoride released was highest after day 1 followed by gradual decrease in amount of fluoride released till 28 days in both the groups.

Table 2 shows the comparison of fluoride release between group II A and group II B. Group II B released more amount of fluoride than group II A at all the time interval which was statistically significant (P<0.001). The amount of fluoride released was highest after day 1 followed by gradual decrease in amount of fluoride released till 28 days in both the groups.

Table 3 shows the comparison of fluoride release between group III A and group III B. Group III B released more amount of fluoride than group III A at all the time interval which was statistically significant (P<0.001). The amount of fluoride released

was highest after day 1 followed by gradual decrease in amount of fluoride released till 28 days in both the groups.

Table 4 shows the comparison of fluoride release between group IV A and group IV B. Group IV B released more amount of fluoride than group IV A at all the time interval which was statistically significant (P<0.001). The amount of fluoride released was higher at day 7 than at day 1 which was statistically significant, followed by gradual decrease in amount of fluoride released till 28 days in both the groups.

Table 5 shows the overall comparison of fluoride release between group I A, group II A, group III A and group IV A. The amount of fluoride released was highest for group IV A at all the time intervals followed by group III A, group I A and group II A. The difference was statistically significant (P<0.001).

Table 6 shows the overall comparison of fluoride release between group I B, group II B, group III B and group IV B. The amount of fluoride released was highest for group IV B at all the time intervals followed by all other groups III B, I B and II B. The difference was statistically significant (P<0.001).

Table 7 shows the antibacterial activity of group IV A and group IV B against Streptococcus mutans, Streptococcus salivarius and Lactobacillus casei. The antibacterial activity of group IV B was more for Lactobacillus casei followed by Streptococcus mutans and Streptococcus salivarius. The difference was statistically significant ($P<0.001$).

SUMMARY OF THE RESULTS:

The results suggested that the addition of chitosan to all types of glass ionomer cement increased the release of fluoride from day 1 to day 28. Among the types of glass ionomer cement tested Type IX high strength posterior extra with chitosan released more amount of fluoride at all the time intervals tested. The antibacterial activity of chitosan containing Type IX high strength posterior extra glass ionomer cement was significantly more for Lactobacillus casei. Hence chitosan had influenced the release of fluoride from glass ionomer cements and the antibacterial activity of Type IX high strength posterior extra glass ionomer cement.

Discussion

DISCUSSION

Dental caries can be regarded as one of the most common diseases occurring in humans and is prevalent in most countries. It has a ubiquitous presence among humans irrespective of their race, religion or region. It affects all the age groups there by existing demand for ongoing preventive and restorative care. A breakthrough was the discovery of using fluoride for dental caries prevention. Despite the widespread use of different sources of fluoride, dental caries continuous to be the single most prevalent and costly oral infectious disease (NIH 2001; Marsh 2003; Dye et al 2007).[28]

Various restorative materials such as amalgam,composite resin, gold restorations and glass ionomer cements are available to restore a cavitated tooth. Though many options are available, the main reason for a restoration failure is due to secondary caries in both the primary and permanent dentition. But the failure rate is dependent upon the restorative material that is been used.[26]

Fluoride is known to inhibit demineralization and enhance remineralization; this can be established by fluoride release in small amounts surrounding the teeth. Some of the restorative materials

available today have the ability to release fluoride to the adjacent tooth structure and into the oral environment.[16] Brief reviews of the categories of fluoride releasing materials are in the following order. In 1950's when silicate cement was first introduced, it was well-known as a tooth coloured restorative material. In addition to this, it was noted that secondary caries was significantly reduced. This property was later found to be attributed to fluoride release spawned by the restorative material.[26]

Fluorides are now considered to play a major role in the prevention and control of dental caries. The discovery of fluorides as an anti-cariogenic agent is one of the most important milestone in the history of dentistry. The amount of fluoride release and duration of fluoride release determines the anticariogenic effect of fluoride releasing restoration.[16] The mechanisms by which the fluoride exhibits anti-cariogenic effect are by, interference of pellicle and plaque formation, reduction of demineralization, enhancement of remineralization, the inhibition of microbial growth and metabolism.[54]

Long term release of fluoride and other ions is said to exhibit the antibacterial property of glass ionomer restoratives but the effect can also be related to the composition of the material such as presence or absence of oxides, types of acids present in the composition.[18] Fluoride may be released from dental restorative materials as part of the setting reaction, or it may be added to the formulation with the specific intention of fluoride release. Fluoride containing restorative materials include glass ionomer cement, poly acid modified resin composite (compomers), resin composites, fissure sealants and stannous fluoride containing dental amalgam.

Glass ionomer was invented four decades ago by Wilson and Kent in 1969. These materials form part of contemporary restorative dentistry largely due to their ability to chemically bond to enamel and dentine with insignificant heat formation or shrinkage, biocompatibility with pulp and periodontal tissues and fluoride releasing properties which produce cariostatic and antimicrobial action. They are used today in a variety of clinical situations such as restorative, lining, luting and sealing materials. The ability of set glass ionomer cements to leach fluoride over an extended time period has been reported in a large number of laboratory studies.[42]

Chitosan nanoparticles are drug carriers which have been widely used in medical field. Chitosan is a natural product with replenishing pharmaceutical adjunct and good biocompatibility. They have the advantage of slow or controlled drug release, which improves drug solubility, stability, enhance efficacy and reduce toxicity. Because of their small size they are capable of passing through biological barrier in vivo and delivering drugs to the lesion site to enhance the efficacy. The physical and chemical properties of chitosan depend mainly on its molecular weight and degree of deacetylation. Specific functional groups of chitosan-chintin co polymers and their derivatives have been shown to exhibit many biological phenomena, including antimicrobial activity.[53] The mode of anti-microbial activity is by the interaction of positively charged chitosan oligomers with negatively charged microbial cell membrane causing the leakage of intracellular contents and damages the bacterial cells.[43] Chitosan's antimicrobial spectrum includes variety of microorganisms, algae, fungi and bacteria. The antimicrobial activity is more effective against gram-positive bacteria and yeast. The antimicrobial action of chitosan is influenced by several factors, which include 1. Microbial factors (microbial species, age of the cell);

2. Physical state factors (soluble and solid state); 3. Intrinsic factors of the chitosan (positive charge density, molecular weight, hydrophobic and hydrophilic characteristics, chelation capacity) and 4. Environmental factors (pH, ionic forces, temperature and time).[12] Petri et al investigated the effect of chitosan nanoparticles on the flexural strength and on the fluoride ion release from glass ionomer restoratives. The results confirmed that the addition of 0.0044wt% of CH led to a significant increase in the flexural resistance and the amount of fluoride ions released.[45]

The aim of this in vitro study is to evaluate the effect of chitosan nanoparticles on release of fluoride from four glass ionomer cements (Type II universal restorative, Type II light cure universal restorative, GC Fuji VII (pink), GC HS posterior extra) using fluoride ion selective electrode and its influence on the antibacterial property of high strength posterior extra glass ionomer cement.

In the present study four different types of glass ionomer cements were selected and 0.1ml of prepared 0.2mg /ml of chitosan solution (20mg of chitosan dissolved in 0.3 N acetic acid) is added to 0.9ml of glass ionomer liquid to attain a concentration of 10v/v% of chitosan modified glass ionomer liquid.[45] The addition of chitosan

under acid condition is mandatory to guarantee its solubility. At pH 1.0 protonated chitosan chains are not able to interact with the particle surface or with poly acid chains by electrostatic interactions, because there is little negative charge on them on the other hand, chitosan chains carry many hydroxyl groups and acetamide groups which are able to bind to the particles hydroxyl groups and to polyacrylic acid carboxylic groups by hydrogen bonding. The network formed by chitosan and polyacrylic acid around the inorganic particle might reduce the interfacial tension among the glass ionomer restorative components, improving mechanical performance at this concentration.[45] The powder liquid ratio of glass ionomer cement were proportioned according to manufacturer's instruction with the liquid component as chitosan modified glass ionomer liquid. The proportioned powder and liquid were hand mixed using plastic spatula and mixing pad. The hand mixed glass ionomer cements were loaded into disposable teflon moulds of 10mm internal diameter and 2mm thickness and allowed to set at room temperature for 10 minutes except for light cure GIC which is cured for 20 seconds on both sides.

Then the samples were stored in 4ml of deionized water. Studies by Rezk-Lega et al (1991), Damen et al (1996), El Mallakh &

Sarkar (1990) demonstrated that the amount of fluoride released was more in deionized water than in artificial saliva.[43] However the fluoride release in artificial saliva might produce intraoral conditions than the deionized water which is used in this study, the artificial saliva cannot simulate the clinical conditions because the presence of plaque or pellicle in the oral environment was not taken into considerations.[25,6,7] The artificial saliva which contains calcium and phosphate ions produces higher ionic strength and the formation of CaF surface coating on the specimens could act as diffusion barrier, restricts fluoride release.[8] In the present study the deionized water is used as a medium, as it reflects well the fluoride releasing property without any influence of minerals or organic molecules which might present in the re/demineralizing solutions or artificial saliva.

The analysis for fluoride release was done for 28 days based on the earlier study as it was shown that the fluoride release by glass ionomer cement is almost constant after the 28 days.[59] The deionized water of 4ml was changed after 1day, 7 days, 14 days, 21 days, 28 days interval. At each interval equal volumes of TISAB II which contained 2% CDTA (2-diaminocyclohexane N, N, N'', N'-tetra acetic acid) a metal-chelating agent which de complexes fluoride from

polyvalent cations,[30,24] making fluoride available for measurement in storage medium was added to 4ml of deionized water and this sample is subjected to ion analyzer for fluoride ion detection. The amount of fluoride released was measured in ppm using a fluoride ion selective electrode and an ion analyzer which was previously calibrated using standard fluoride containing solutions.

Fluoride analysis progressed from simple colorimetric analysis, which yielded crude results and experienced interference of other ions present in the samples to more complex methods such as mass spectrometry, gas chromatography, ion chromatography, electro analysis, catalytic-enzymatic and radio analytical methods. At present, gas chromatography, ion chromatography and the fluoride ion selective electrode are the most frequently used techniques for fluoride analysis of samples. In the present study, ion selective electrode based potentiometer methods were selected as the technology for the standardization of fluoride analytical methods since those are most universally used. It was considered that the ion selective electrode based methods are quite easily accessible and have an acceptable lower detection limit.[36]

Ion Selective Electrodes (ISE) is a membrane electrode that consist of a sensing element bonded into epoxy body that reacts selectively to specific ions in the presence of other ions. The basic ISE setup requires a probe, a digital meter and a few additional reagents for controlling the ionic strength and pH of the sample. Ion Selective Electrodes work on the basic principle of the galvanic cell by measuring the electric potential generated across a membrane by selective ions and then comparing it to a reference electrode, thereby determining the net charge. The fluoride ion selective electrode produces a potential across a lanthanum fluoride solid ion exchange phase and these are measured using probes that determine specific ions and gases in the solution. The strength of the charge that is determined is directly proportional to the concentration of the selective ion.[30]

In this study four types of glass ionomer cements Type II universal restorative, Type II light cure universal restorative, GC Fuji VII (pink), GC HS posterior extra with chitosan were tested to determine the amount fluoride release from deionized water. Glass ionomer cements are based on an ion-leachable glass, which releases fluoride in the setting process with polyacid. Advantages of glass

ionomer cements include chemical adhesion to enamel and dentine in the presence of moisture, resistance to microleakage, good marginal integrity, and dimensional stability at high humidity, coefficient of thermal expansion similar to tooth structure, biocompatibility, fluoride release, recharge ability with fluoride, and less shrinkage than resin cements upon setting with no free monomer being released. Chitosan nanoparticles are highly biocompatible, hydrophilic and possess mucoadhesive properties due to molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces. The advantages of adding chitosan to different types of GICs had shown catalytic effect on fluoride release and improve the mechanical properties of glass ionomer cement.[45]

Dental caries results from the interaction of specific bacteria and their metabolic or virulence products with salivary constituents and dietary carbohydrates that occur on the susceptible tooth surfaces. In this complex process, the microorganisms, particularly Streptococcus species, have an important role in its etiology. The microorganisms used in this study are cariogenic in human (Hardie 1992) and thought to indicative of progressive lesion

(Boyar & bowden1985). Bacteria such as Streptococcus, Lactobacillus in plaque result in more acid production at faster rates and enhance demineralization of dental hard tissues. The Streptococcus mutans is a member of the oral microbial community which plays a key role in modulating the transition of the nonpathogenic state to highly cariogenic biofilms.[28]

The most common method for assessing the antibacterial property of dental materials is agar diffusion test (ADT) which is based on placing samples on agar plates seeded with microorganisms and then evaluating antibacterial activity by measuring the inhibition zone around the disc which enables measurement of the activity of soluble ingredients of the tested material in the surrounding medium indicated by an inhibition halo. However limitations such as agar diffusion test is qualitative in nature, has ability to measure only soluble components, inability to distinguish between bacteriostatic and bactericidal effects and difficulties in comparing a large number of samples and in controlling a large number of variables is considered.[21]

Fluoride released which was assessed using fluoride ion selective electrode at 1, 7, 14, 21, 28 days was subjected to ANOVA

and tukey HSD statistical analysis. For all the materials tested, the amount of fluoride released was highest after day 1 followed by gradual decrease in amount of fluoride released till 28 days. This pattern of fluoride release was consistent with the results of earlier studies (Tay&Barden, Crisp, Lewis & Wilson, Verbeecket et al, Araujo et al, Yap et al, Lee et al, Hattab et al, Attar et al, Mousavinasab et al).

Two mechanisms have been proposed by which fluoride may be released from glass-ionomers into an aqueous environment. One mechanism is a short-term reaction, which involves rapid dissolution from outer surface in to solution, whereas the second is more gradual and resulted in the sustained diffusion of ions from the bulk cement. After the initial burst, fluoride release slows down and is followed by a prolonged long-term fluoride release, cumulative amount of fluoride ions released from glass ionomer cements, after a short period of time is diffusion controlled and follows a decreasing gradient.[61]

The fluoride released between four groups I A, II A, III A and IV A was assessed. The amount of fluoride released was highest for group IV A (type IX HS posterior extra) at all the time intervals followed by III A (GC Fuji VII), I A (Type II universal restorative)

and II A (Type II light cure universal restorative). This difference was statistically significant for all the four materials tested (Table 5).

In groups I B, II B, III B and IV B, the addition of chitosan to all four types of glass ionomer cements had increased amount of fluoride release from day 1 to day 28. Among the different groups tested group IV B (type IX HS posterior extra with chitosan) released more amount of fluoride at all the time intervals, followed by III B (GC Fuji VII with chitosan), I B (Type II universal restorative with chitosan), II B (Type II light cure universal restorative with chitosan) and difference was statistically significant (Table 6). The addition of 0.0044wt% of chitosan in the glass ionomer restoration has a catalytic effect on the fluoride release; it makes the diffusion of fluoride ion through the glass ionomer restoratives towards the medium faster. The catalytic effect is due to the formation of polymeric network which binds strongly around the inorganic filler and this effect can be related to the entropic gain associated with the fluoride release. The release of fluoride ions from the inorganic matrix was favored when reinforced complexes (poly acrylic acid adsorbing onto chitosan bound to the GIC particle surface) have been formed. Even in the case of segregation of some chitosan chains, the amount of fluoride

release is faster than GIC without chitosan.[45] Among the various groups of cements tested group IV A and IV B (Type IX HS posterior extra without chitosan and with chitosan) released highest amount of fluoride at all the intervals tested. Filler composition and particle size have significant influence on the fluoride release. Fluoroaluminosilicate glass is the major component of the filler which is the main source of fluoride ion release from the cement. Smaller the filler particle size will have larger surface areas which can increase the fluoride release.[55] Group II A and II B (Type II light cure universal restorative without chitosan and with chitosan) which is resin modified cement released least amount of fluoride. The light cure material takes up water with time and the carboxylic groups of acidic monomer can undergo an acid base reaction with metal ions of glass filler; this in turn leads to the formation of carboxylate salts and the release of fluoride. It seems that this reaction is weak and results in low fluoride release.[5]

The antibacterial activity of group IV A and IV B tested against Streptococcus mutans streptococcus Salivarius and Lactobacillus casei .The antibacterial activity of group IV B (chitosan containing type IX high strength posterior extra) showed increased

zone of inhibition for Lactobacillus casei followed by Streptococcus mutans and Streptococcus salivarius. The fluoride release from the glass ionomer material combined with pH fall around the material caused reduced bacterial growth.[18] Fluoride can inhibit many enzymes involved in bacteria metabolism such as inhibition of the glycolytic enzyme enolase, the proton-extruding ATP-ase; acid phosphatase, pyrophosphatase, peroxidase and catalase. In such a way fluoride inhibits the production of bacterial acids and glucans, especially insoluble glucan produced by Streptococcus mutans. As insoluble glucans are important for virulence of mutans Streptococci, the inhibitory actions of fluoride could significantly affect cariogenicity.

The antibacterial property of chitosan is due to interaction of positively charged chitosan and negatively charged bacterial cell wall .This causes alteration in bacterial cell permeability, leading to leakage of proteinaceous and other intracellular constituents, other mechanism is based on binding of chitosan with microbial DNA, in turn interfering with mRNA and protein synthesis.[12]

Thus, the addition of chitosan nanoparticles to glass ionomer cements had a catalytic effect on the fluoride release and favored the antibacterial effect.

Summary

SUMMARY

The aim of this in vitro study was to evaluate the effect of chitosan nanoparticles on release of fluoride from four glass ionomer cements and its influence on the antibacterial property of high strength posterior glass ionomer cement. Four types of glass ionomer cements with and without chitosan were divided into following groups: Type II universal restorative with and without chitosan (group I A & I B), Type II light cure universal restorative with and without chitosan (group II A & II B), GC Fuji VII (pink) with and without chitosan (group III A & III B), GC HS posterior extra with and without chitosan (group IV A & IV B). Six samples of each group were prepared using teflon moulds of 10mm internal diameter and 2mm thickness and immersed in deionized water. The amount of fluoride release was analyzed after 1day, 7 days, 14 days, 21 days, 28 days interval using fluoride ion selective electrode. The results showed that the addition of chitosan to all types of glass ionomer cement increased the release of fluoride from day 1 to day 28. Among the types of glass ionomer cement tested type IX high strength posterior extra with chitosan released more amount of fluoride at all the time intervals. The antibacterial activity of group IV A and IV B was evaluated against Streptococcus mutans, Streptococcus salivarius and Lactobacillus casei using blood agar diffusion

method by measuring the zone of inhibition. The results showed that the antibacterial activity of group IV B was more against Lactobacillus casei followed by Streptococcus mutans and Streptococcus salivarius.

Conclusion

CONCLUSION

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

1. The addition of chitosan nanoparticles to glass ionomer cements had a catalytic effect on the fluoride release in all the experimental groups tested.

2. Among the four types of glass ionomer cements: Type II universal restorative, Type II light cure universal restorative, GC Fuji VII (pink), Type IX GC HS posterior extra tested for fluoride release, Type IX high strength posterior extra exhibited highest amount of fluoride release and the least amount of fluoride release was observed with Type II light cure glass ionomer cement.

3. The chitosan containing Type IX high strength posterior extra glass ionomer cement had a significant antibacterial activity against Lactobacillus casei, Streptococcus mutans and Streptococcus salivarius.

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