COMPARATIVE EVALUATION OF SEALING ABILITY AND OSTEOGENIC POTENTIAL OF CONVENTIONAL GLASS IONOMER CEMENT AND GLASS IONOMER CEMENT MODIFIED WITH CHITOSAN & BIOACTIVE GLASS

- AN IN VITRO STUDY

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CERTIFICATE

This is to certify that **Dr. SUDHARSHANA RANJANI.M,** Post Graduate student (2010 - 2013) in the Department of Conservative Dentistry and Endodontics, has done this dissertation titled "**Comparative Evaluation of Sealing Ability and Osteogenic Potential of Conventional Glass Ionomer Cement and Glass Ionomer Cement modified with Chitosan & Bioactive Glass – An in Vitro Study"** under my direct guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University, Guindy, Chennai – 32** for **M.D.S.** in Conservative Dentistry and Endodontics (Branch IV) Degree Examination.

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DECLARATION

TITLE OF DISSERTATION	**Comparative Evaluation of Sealing Ability and Osteogenic Potential of Conventional Glass Ionomer Cement and Glass Ionomer Cement modified with Chitosan & Bioactive Glass – An in Vitro Study**
PLACE OF THE STUDY	**Tamil Nadu Government Dental College & Hospital, Chennai – 3.**
DURATION OF THE COURSE	**3 YEARS**
NAME OF THE GUIDE	**DR. M. KAVITHA**
HEAD OF THE DEPARTMENT	**DR. M. KAVITHA**

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And

Mrs. Dr. M. Kavitha aged 42 years working as **Professor & HOD** in Department of Conservtive Dentistry & Endodontics at the college, having residence address at 69/4, Mettu street, Ayanavaram, Chennai – 23 (herein after referred to as the 'Principal Investigator')

And

Mrs. Dr. Sudharshana Ranjani.M aged 28 years currently studying as **Post Graduate student** in Department of Conservtive Dentistry & Endodontics, Tamilnadu Government Dental College and Hospital, Chennai - 3 (herein after referred to as the 'PG student and co- investigator').

Whereas the PG student as part of his curriculum undertakes to research on **"COMPARATIVE EVALUATION OF SEALING ABILITY AND OSTEOGENIC POTENTIAL OF CONVENTIONAL GLASS IONOMER CEMENT AND GLASS IONOMER CEMENT MODIFIED WITH CHITOSAN & BIOACTIVE GLASS"** for which purpose the Principal Investigator shall act as principal investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co-investigator

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College represented by its **Principal** **PG Student**

Witnesses **Student Guide**

1.

2.

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Abstract

Aim

The aim of this study was to utilize polarized light microscope for the assessment of the efficacy of CPP-ACP, NovaMin and Amine Fluoride pastes on remineralization of enamel over time.

Materials and Methods

40 teeth were used for the study and were divided into 4 groups with 10 teeth each. Remineralization was done by application of CPP-ACP, NovaMin and Amine fluoride in the first 3 group's respectively following demineralization, while the 4th group (control) received only demineralization. Teeth were sectioned under hard tissue microtome and viewed under polarized light microscope for maximum depth of demineralization.

Results

Statistical analysis was done using student's t-test. Mean value of demineralization were 78.06µm for CPP-ACP, 156.82µm for Novamin, 109.80µm for Amine fluoride and 328.32µm for control group. Although the values were best in CPP-ACP group, there was no statistical significance difference between CPP-ACP and Amine fluoride, and both were better than Novamin.

Conclusion

The study concluded that CCP-ACP and Amine fluoride were better in remineralization followed by NovaMin.

Keywords

Demineralization, Remineralization, CPP-ACP, NovaMin, Amine fluoride, Polarized light microscopy.

INTRODUCTION

The goal of endodontic therapy is to obtain a fluid tight seal apically and coronally. When healing is not achieved after nonsurgical endodontic therapy and when retreatment is not possible or has failed, the surgical approach is indicated.

The aim of periradicular surgery is to remove the etiologic factor, prevent recontamination of the periradicular tissues thereby providing an environment conducive to the regeneration of the periodontium that is, healing and regeneration of the alveolar bone, periodontal ligament and cementum.

This procedure includes exposure of the involved apex, resection of apical end of root, preparation of class I cavity and insertion of a root end filling material[81]. Management of the resected root end during periradicular surgery is critical to a successful outcome. The portion of root apex that is inaccessible to instrumentation and as a consequence, cannot be cleaned, shaped or filled, or is associated with extraradicular infection that is unresponsive to non-surgical treatment, is removed. A filling material is then placed into a prepared root-end cavity as a 'physical seal' to prevent the passage of microorganisms or their products from the root canal system into the adjacent periradicular tissues. The placement of a root-end filling is one of the key steps in managing the root end.

According to ***Gartner and Dorn***, **Kim et al.** and **Chong** an ideal root-end filling material should adhere or bond to tooth tissue and seal the root end three dimensionally, not promote, and preferably inhibit, the growth of pathogenic microorganisms, be dimensionally stable and unaffected by moisture in either the set or unset state, be well

tolerated by periradicular tissues with no inflammatory reactions, stimulate the regeneration of normal periodontium, be non toxic both locally and systemically, not corrode or be electrochemically active, not stain the tooth or the periradicular tissues, be easily distinguishable on radiographs, have a long shelf life and be easy to handle[10].

Metals such as gold-foil, silver posts, titanium screws, tin posts, amalgam (with and without bonding agent) and gallium alloy are some of the solid, commonly used retro-filling materials. Cements and sealers such as ZnOE Cement, IRM, Super EBA, Cavit, Zinc Poly carboxylate, Zinc Phosphate and Glass Ionomer Cements, Mineral Trioxide Aggregate, Calcium Phosphate Cement and Bone Cement have also been employed for retro-fillings. Other commonly used materials are composite resin and gutta-percha. The less commonly used materials are laser, ceramic inlay, teflon, mixture of powdered dentin & sulfathiazole and cynoacrylates[87]. Unfortunately, the ideal retrograde filling material is yet to be found.

Since the introduction of first Glass-Ionomer Cement (GIC) in the late 1960's by Alan Wilson and Brian Kent, a large number of GIC compositions have been investigated, and modifications have been made.

GICs are clinically attractive dental materials and have certain unique properties that make them useful as restorative and adhesive materials. This includes adhesion to moist tooth structure and base metals, anticariogenic property due to the release of fluoride, thermal compatibility with tooth enamel, biocompatibility and low toxicity[83]. Glass Ionomer exerts antibacterial activity resulting from fluoride release, low pH levels when setting, and by the presence of cations such as strontium and zinc[11]. Apart from the

well consolidated application in dentistry, these materials, due to their good biocompatibility, have been used also for fixation of cochlear implants and as artificial bone substitutes for cranial-facial reconstruction[40,69,101].

Chitosan (CH), a crystalline polysaccharide is a partially deacetylated derivative of chitin. It is the primary structural polymer in arthropod exoskeletons. It is a weak base, having at least one primary amino group and two free hydroxyl groups. Chitosan is normally insoluble in aqueous solutions above pH 7. However, in dilute acids (pH < 6), the free amino groups are protonated and the molecule becomes soluble. The high charge density in solution allows chitosan to form insoluble ionic complexes with a wide variety of water soluble polyanionic species[80]. Chitosan is biocompatible, hemostatic, accelerates the formation of osteoblasts, and has antibacterial and antifungal properties[1,68].

Bioactive Glass(BAG), a silica based melt-derived glass developed by ***Larry Hench et al. in 1971***, which are generally composed of SiO_2, CaO, P_2O_5 and Na_2O. This active biomaterial has antimicrobial and anti-inflammatory effects, it has the ability to bond to soft and hard tissues; moreover, when implanted in the bone, it displays osteoconductive properties which may assist the repair of bony defects[53].

Denise F. S. Petri et al.[24] found that the addition of 10 v/v % of Chitosan led to a significant increase in the flexural resistance. Chitosan contents higher than 25 v/v % (50 v/v% & 100 v/v %) led to poor performance. The amount of fluoride ions released from Chitosan, especially from those with 10 v/v % of Chitosan modified GIC was much larger than that released from commercial GIC.

Helena Yli-Urpo et al.[36]determined compressive strength, Young's modulus of elasticity, and Vickers' surface hardness of conventional cure and resin-modified glass ionomer cements after the addition of bioactive glass (BAG) 10-30 wt% and concluded that the addition of BAG to GIC compromises the mechanical properties of the materials to some extent.

Bioactive Glass and Chitosan is mixed with GIC with an intention that they could be applied as root end fillings where their bioactivity could be beneficial and high compressive strength is not necessarily needed.

The purpose of this study is to determine the sealing ability and osteogenic potential of Glass Ionomer Cement on addition of Chitosan(10v/v% and 50 v/v%) and Bioactive glass(10wt% and 30 wt %) so that the combination is best used as a root end filling material.

AIM AND OBJECTIVES

Aim

To evaluate the sealing ability and osteogenic potential of Glass Ionomer Cement (Type II) containing Chitosan and Bioactive glass.

Objectives

1. To evaluate the sealing ability of Glass ionomer cement containing 10 v/v % of Chitosan, 50 v/v % of Chitosan, Glass ionomer cement containing 10wt% of Bioactive glass, 30 wt % of Bioactive glass compared with conventional Glass ionomer cement (Type II) by dye penetration method using confocal laser scanning microscopy.

2. To evaluate the osteogenic potential of Glass ionomer cement containing 10 v/v % of chitosan, 50 v/v % of Chitosan, Glass ionomer cement containing 10wt% of Bioactive glass, 30 wt % of Bioactive glass compared with conventional Glass ionomer cement (Type II) using SaOS-2 cells after culturing and assessing
   - Cell Proliferation using MTT assay after 24 hrs, 48 hrs and 72 hrs.
   - Cell Differentiation by Alkaline Phosphatase activity after 7, 14 and 21 days.

REVIEW OF LITERATURE

SEALING ABILITY

GLASS IONOMER CEMENT

Van Riessen AW et al. [86] (1990) conducted a literature study , based on the assumption that glass ionomer cement will provide a better sealing and will cause less tissue reaction and suggested glass ionomer cement to be preferred to amalgam when it comes to apical sealing properties and tissue reaction. In terms of usability, resorption, hardness and costs, no significant differences were found. They concluded that glass ionomer cement is an equal or perhaps even better alternative for retrograde amalgam.

Roth S [71] (1991) investigated the use of various glass ionomer cements for retrograde root filling from the point of view of sealing qualities, ion release and ease of application. The sealing qualities of the material were tested by dye penetration and microscopic and SEM examination. They concluded that glass ionomer cement is possibly a clinical alternative for the sealing of retrograde cavities; however, the silver-reinforced materials may cause tissue irritation from release of silver ions and their corrosion products.

Chong BS et al.[17] (1991) studied the adaptation and sealing ability of a light-cured glass ionomer cement, conventional glass ionomer cement and amalgam when used as a retrograde root filling using a confocal optical microscope with and without a fluorescent dye and concluded that the sealing ability of the light-cured glass ionomer cement was significantly better than that of amalgam ($P < 0.001$). The dye penetration around the light-cured glass ionomer cement and the conventional glass ionomer cement

was not significantly different ($P > 0.05$). However, the sealing ability of the conventional glass ionomer cement was significantly better than that of amalgam ($P < 0.05$).

Chong BS et al.[18] (1993) investigated the adaptation and sealing ability of a light-cured ionomer cement, without a retrograde cavity and compared with the material used in a retrograde cavity, and with a conventional glass ionomer cement using a confocal optical microscope with a fluorescent dye. They found in the group where the light-cured glass ionomer cement was used in a retrograde cavity, the material was often well adapted to one cavity wall, but gaps were found on the opposite wall. The light-cured and conventional glass ionomer cement retrograde root seals were well adapted to the root face, regardless of the thickness of material used and concluded that the thinly applied (approximately 1 mm) light-cured glass ionomer cement retrograde root seals permitted the least leakage.

Jesslén P et al.[39] (1995) analysed total of 67 teeth in 64 patients treated with apicectomy and retrograde fillings in a comparative clinical study. They were randomized to receive fillings of amalgam or glass ionomer cement. Healing was evaluated clinically and radiographically after 1 and 5 years. Evaluation showed no difference in healing capacity between the two materials and concluded that glass ionomer cement is a valid alternative to amalgam as an apical sealant after apicectomy with equally good long-term clinical results.

Greer BD et al.[31] (2001) evaluated the apical sealing ability of two compomers (Dyract and Geristore), IRM, and Super-EBA using fluid filtration device. The integrity of the seal was evaluated for 5 min at 1, 7, 30, and 180 days and concluded that the new

compomers, Dyract and Geristore are equal or superior to IRM and equivalent to Super-EBA in their ability to reduce apical leakage when used as retrofilling materials.

Scheerer SQ et al.[75] (2001) used Prevotella nigrescens to evaluate the sealing ability of Geristore, Super-EBA, and ProRoot when used as root-end filling materials. Results after 47 days indicated there were no significant differences between the three root-end filling materials against penetration of Prevotella nigrescens.

De Bruyne MA et al.[23] (2004) reviewed the basic properties of GICs, such as adhesion, antimicrobial effects and biocompatibility, particularly as they relate to use in endodontics and concluded that in spite of the critical handling characteristics and the inconclusive findings regarding sealing ability and antimicrobial activity, there is substantial evidence to confirm their satisfactory clinical performance. Both soft tissue and bone compatibility make them suitable for use during endodontic surgery.

Economides N et al.[26] (2004) examined microleakage of two root-end-filling materials with and without the use of bonding agents using a fluid transport model at 24 h, 1 month, and 2 months interval under a low pressure of 0.1 atmosphere. At all experimental times, glass-ionomer groups showed significantly less microleakage than resins groups. Between Admira and Admira Bond groups, significantly less leakage was observed in the root sections with Admira Bond at 24 h.

CHITOSAN

Mattioli Belmonte M et al.[52] (1999) found the chemical association of chitosan with inorganic salts, such as calcium phosphate and presented the physical, chemical and

crystallographic characterization of newly-developed cements made of 1) calcium-phosphate and a chitosan gel obtained by acetic acid treatment, and 2) calcium phosphate and a chitosan gel obtained by ascorbic acid treatment. Both cements are self-hardening at room temperature and concluded that the cements are promising for application in endodontics and restorative dentistry.

Shin SY et al.[78] (2005) evaluated the biocompatibility of chitosan nanofiber membranes and examined the effect of the chitosan nanofiber membranes on bone regeneration in rabbit calvarial defects.They confirmed that the biocompatibility of the chitosan nanofiber membrane with enhanced bone regeneration and no evidence of an inflammatory reaction. This experiment showed that chitosan nanofiber membrane may be useful as a tool for guided bone regeneration.

Wang X et al.[91] (2006) studied the hemostatic capability, adhesion ability and biocompatibility of chitosan sponges and compared with commercial collagen sponges and concluded that the chitosan sponge was degraded much slower than the collagen sponge, while tissue responses for the chitosan sponges were much greater than for the collagen sponges.

Hayashi Y et al.[34] (2007) evaluated whether chewing gum containing chitosan, can effectively suppress the growth of oral bacteria. The amount of oral bacteria was found to significantly decreased in the chitosan group and concluded that a supplementation of chitosan to gum is an effective method for controlling the number of cariogenic bacteria in situations where it is difficult to brush one's teeth.

Denise F.S. Petri et al.[24] (2007) determined the effect of chitosan on the flexural strength and on the release of fluoride ions from glass ionomer restoratives. The study used 10 specimens of commercial GIC (Vidrion, SS White) and chitosan modified GIC (0.0044, 0.012, 0.025 and 0.045 wt% chitosan) for the flexural strength and 10 specimens for the fluoride release. They concluded that the addition of 0.0044 wt% chitosan led to a significant increase in the flexural resistance and contents higher than 0.022 wt% led to a poor performance and in the presence of chitosan, the release of fluoride ions from glass ionomer restoratives was catalyzed.

Kim IY et al.[43] (2008) suggested that chitosan and its derivatives are promising candidates as a supporting material for tissue engineering applications owing to their porous structure, gel forming properties, ease of chemical modification, high affinity to in vivo macromolecules and demonstrated the uses of various types of chitosan derivatives in various tissue engineering applications namely, skin, bone, cartilage, liver, nerve and blood vessel.

Shin et al.[78] (2009) evaluated the effect of hydroxyapatite (HA)-chitosan (CS) membrane on bone regeneration in the rat calvarial defect. Surgical implantation of the HA - CS membrane resulted in enhanced local bone formation at both 2 and 8 weeks compared to the control group and suggested that HA-CS membrane would be an effective biomaterial for regeneration of periodontal bone.

Arnaud TM et al.[3](2010) evaluated the in vitro effect of chitosan treatment on enamel de-remineralization behaviour upon a pH cycling assay using Vickers microhardness tester and concluded that Chitosan interferes with the process of

demineralization of the tooth enamel inhibiting the release of phosphorus and the demineralization is influenced by the concentration and exposure time of the biopolymer to the enamel and suggested that chitosan may act as a barrier against acid penetration, contributing to its demineralization inhibition.

Uysal T et al.[84] (2011)tested the hypothesis that there is no significant difference between the chitosan-containing and conventional nonfluoridated dentifrices in inhibition of enamel demineralization around orthodontic brackets and found that Chitosan-containing dentifrice showed lower demineralization than the control and they concluded that Chitosan-containing dentifrice may reduce the enamel decalcification in patients with poor oral hygiene.

BIOACTIVE GLASS

Matsuya S et al.[51] (1999) prepared a new glass ionomer cement using bioactive glass and investigated its setting process using Fourier Transform Infra Red Spectroscopy (FT-IR) and Mass Spectrometry Nuclear Magnetic Reasonance (MAS NMR) analyses and suggested that Calcium was released from the glass powder to form carboxylate salt and degree of polymerization in the silicate network increased. The setting mechanism of the cement was found to be essentially the same as in conventional glass ionomer cement.

Ana ID et al.[2] (2003) studied the effects of added bioactive glass on the basic setting properties of a commercially available resin-modified glass ionomer cement with respect to setting time, mechanical strength, and setting mechanism. It was found to be clinically acceptable whether the setting time was extended or shortened depending on the type of bioactive glass added. The compressive strength of the set cement containing

the bioactive glass decreased and was much higher when compared with the conventional type glass ionomer cement containing bioactive glass. The Fourier-transform infrared and MAS-NMR spectroscopies revealed that the extent of the acid-base reaction was larger in the cements containing bioactive glass than in the commercial resin-modified glass ionomer cement because of its high basicity in the bioactive glass.

Helena Yli-Urpo et al.[35] (2005) studied Conventional cure and resin-modified light-curing GIC by adding 10-30 wt% bioactive glass (BAG) using Scanning Electron Microscope (SEM), Energy-dispersive X-ray Spectroscopy (EDS) and visual analysis to examine the bioactivity and the ability to mineralize dentin in intact beagle dog teeth. The restorations were followed clinically for 1, 3 or 6 weeks. Resin-modified GIC containing BAG showed uniform Calcium Phosphate surface formation on the restorations and concluded that resin-modified GIC containing BAG have good potential in clinical applications where enhanced mineralization is expected.

Helena Yli-Urpo et al.[36](2005) determined compressive strength, Young's modulus of elasticity, and Vickers' surface hardness of conventional cure and resin-modified glass ionomer cements after the addition of bioactive glass (BAG) added in 10-30 wt% . They found that addition of BAG to GIC compromises the mechanical properties of the materials to some extent and concluded that their clinical use ought to be restricted to applications where their bioactivity can be beneficial, such as root surface fillings and liners in dentistry, and where high compressive strength is not necessarily needed.

T. Waltimo et al.[89] (2007) tested the hypothesis that nanometric bioactive glass releases more alkaline species, and consequently displays a stronger antimicrobial effect, than the currently applied micron-sized material. The shift from micron- to nano-sized treatment materials afforded a ten-fold increase in silica release and solution pH elevation by more than three units. Furthermore, the killing efficacy was substantially higher with the new material against all tested strains.

Choi JY et al.[16] (2008) examined the setting time, diametral tensile strength, and in vitro bioactivity of the GIC–Sol gel (SG) derived Glass with a bioactive composition added in 10 and 30 wt %. The setting time of the GIC–SG cements increased with increasing amount of SG. However, the addition of SG did not significantly alter the diametral tensile strength of the GIC. GIC–SG induced the precipitation of an apatite bone-mineral phase on the surface after immersion in a simulated body fluid (SBF), showing in vitro bone bioactivity and confirmed that the GIC– SG samples produced higher cell viability than the GIC sample with cell culturing for up to 7 days.

Xie D et al.[96] (2008) developed a novel bioactive resin-modified glass-ionomer cement system with therapeutic function to dentin capping mineralization. In the system, the newly synthesized star-shape poly acrylic acid was formulated with water, Fuji II LC filler, and bioactive glass to form resin-modified glass-ionomer cement. Compressive strength (CS) and the effect of aging in simulated body fluid (SBF) on CS and microhardness of the cements was investigated. The results showed that the system not only provided strengths comparable to original commercial Fuji II LC cement but also allowed the cement to help mineralize the dentin in the presence of SBF and concluded

that this bioactive glass-ionomer cement system has direct therapeutic impact on dental restorations that require root surface fillings.

Mousavinasab SM et al.[57] (2011) compared the flexural strengths (FS) of a resin-modified glass-ionomer containing bioactive glass (RMGIBAG) with that of a commonly used resin-modified glass-ionomer (RMGI) using three-point bending test at a crosshead speed of 0.5 mm/min. and concluded that adding 20 wt% of BAG to the RMGI powder decreases FS of the material significantly, while it is still clinically acceptable considering the flexural strength values reported for clinically used GIs and RMGIs.

Huang X et al.[38] (2012) analysed the antimicrobial activity and physicochemical properties of glass ionomer cement and resin-modified glass ionomer cement incorporated with chlorhexidine and bioactive glass. They concluded that glass ionomer cements incorporated with chlorhexidine can maintain its mechanical properties as well as reduce early S. mutans biofilm formation. Controlled release/sustained release technology may be required to optimize the antibacterial activity of glass ionomer cements incorporated with bioactive glass.

OSTEOCONDUCTIVE PROPERTY

GLASS IONOMER CEMENT

Zetterqvist L et al.[99] (1987) investigated the tissue reaction following the use of glass-ionomer and amalgam, as retrograde filling materials using 8 monkeys. After apicectomy of the upper central incisors, amalgam and glass-ionomer cement was used at random as retrograde filling material. 2 animals at a time were sacrificed after 2 weeks, 1, 3 and 6 months. Irrespective of the length of time, the tissue reactions were similar for the 2 materials. After 3 and 6 months, there was complete healing with no inflammatory reaction and a mature alveolar bone surrounding the apicectomized roots.

Callis P D and Santini A[12] (1987) compared tissue healing after apicectomy and filling of ferret lower canines with glass ionomer (Ketacfil) or gutta-percha/sealer (Tubliseal). Both materials provoked an inflammatory response after 7 days, but the response to glass ionomer was less severe. The response after 28 days was different. Mild inflammation related to the gutta-percha was still present, but no inflammation was found in relation to the glass ionomer.

I.M. Brook et al.[8] (1991) studied the in vitro response of osteoblast and periosteal cells to the component and composite forms of three different glass-ionomer (polyalkenoic) cements, comparing them to densely sintered hydroxyapatite and tricalcium phosphate ceramics. Qualitative analysis by scanning and transmission electron microscopy revealed that osteoblasts colonized all the solid test materials, although there was a less favourable response to materials with a rough surface topography and to unset and fluoride-containing glasses. A collagen-containing

extracellular matrix was elaborated on to the ceramics and set glass-ionomer cements, except for one (AquaCem).

Meyer U et al.[54] (1993) studied the in vitro behaviour of cells on the ionomeric bone cement (IC). The cells produced bone matrix proteins (osteocalcin, bone sialoprotein II) and were osteoblast-like. The osteoblast-like cells colonized the substrate in monolayers and produced an extracellular matrix as seen by light and scanning electron microscopy. Morphological comparison between cells growing on the ionomeric bone cement and cortical bone revealed no significant difference in phenotypic expression.

Oliva A et al.[61] (1996) compared the response of cultured human osteoblastic cells to a number of commercial glass ionomer cements. The GICs tested were: Ketac-Fil Aplicap, lonocem lonocap 1.0, GC Fuji II, GC Fuji II LC and Vitremer 3M. The results obtained indicated that four of the five glass ionomer cements tested are biocompatible, showing vital cells adhering to the materials, proliferating and expressing the biochemical markers of osteoblastic phenotype, whereas Vitremer 3M exhibited a great cytotoxicity toward the cells.

L.G. Brentegani et al.[7] (1997) implanted type III glass-ionomer cement (Vidrion F), into rat dental alveolus immediately after tooth extraction and its biocompatibility was analysed in terms of incorporation into alveolar bone in the wound healing process. Quantitative data confirmed progressive new bone formation in parallel with a decrease in the percentage fraction of connective tissue in the trial areas around the implants. The

results revealed that the tested material is biologically compatible, being progressively incorporated into alveolar bone in the wound healing process.

Nikola Buric et al.[59] (2003) reported the results of experimental use of glass-ionomer microimplants(GIMIs) in the augmentation of the maxillary alveolar ridge in dogs. Histological examination showed that the glass-ionomer microimplants were extremely osteoconductive and inert materials. Stimulation of growth of new bone tissue in contact with the glassionomer microimplants was evident. No inflammatory cells were detected on or adjacent to the GIMIs.

Carlos Alberto de Souza Costa et al.[13] (2003) evaluated the cytotoxic effects of five glass-ionomer cements (GICs) on an odontoblast cell line (MDPC-23). Disks of every material were prepared and divided into Group 1: Vitrebond, Group 2: Vitremer, Group 3: Fuji II LC, Group 4: Fuji IX GP, Group 5: Ketac-Molar, Group 6: Z-100 (positive control). In groups 1, 2, 3, 4, and 5, the experimental GICs reduced the cell metabolism by 79%, 84%, 54%, 40%, and 42.5%, respectively. Despite the fact that all experimental materials were cytotoxic to the MDPC-23 cells, the GICs were the least cytotoxic. On the other hand, the RMGICs caused the highest cytophatic effects.

Pedro P.C. Souza et al.[64] (2006) evaluated the effects of current resin-modified glass-ionomer cements (RMGICs) applied on culture of cells or implanted into subcutaneous tissue of rats. Rely X Luting Cement (RL), Vitremer (VM), and Vitrebond (VB) were placed into wells with 1.1 mL of culture medium (DMEM), and incubated for 24, 48 and 72 hrs. The extracts from every sample were applied on the MDPC-23 cells. The experimental materials were implanted into the dorsal subcutaneous tissue of rats.

At 7, 30, and 90 days the animals were killed and the biopsies were processed for histological evaluation. The extracts obtained at 24h were less cytotoxic than 48 and 72h incubation. VB showed the highest cytotoxic effect while there was no statistical difference in the cytotoxic effect of VM and RL for the 24-hour period. All RMGICs elicited a moderate to intense inflammatory reaction at 7 days which decreased over time. At 90-day evaluation connective healing occurred for most of samples.

H.J. Chang et al.[14] (2009) demonstrated concentration of collagen integrated into glass ionomer may improve both biocompatibility and the mechanical properties of the material. The glass-ionomer/collagen hybrids presented enhanced compressive strength when integrated with 0.01% collagen, while higher concentrations of collagen compromised their mechanical property. In summary, collagen improved both the mechanical and biocompatible properties of glass ionomers.

Delia S Brauer et al.[22] (2011) created zinc-containing Glass Polyalkeonate Cements and characterized their mechanical properties and biocompatibility. Zinc-containing cements showed adhesion to bone close to 1 MPa, which was significantly greater than that of zinc-free cements (<0.05 MPa) and other currently approved biological adhesives. Results showed that although low levels of zinc may be beneficial to cells, zinc concentrations of 400 μM Zn^{2+} or more resulted in cell death.

CHITOSAN

Ashkan Lahiji et al.[4] (2000) tested the hypothesis that chitosan promotes the survival and function of osteoblasts and chondrocytes. Chitosan was coated onto plastic coverslips that had been fitted into 24 well plates. Human osteoblasts and articular

chondrocytes were coated on either uncoated or chitosan coated cover slips at 1x 10^5 cells per well.Cultures were incubated at 37^0C, 5% CO_2 for a period of 7 days. Cell viability was assessed using a fluorescent molecular probe.Greater than 90% of human osteoblasts and chondrocytes propagated on chitosan remained viable. Reverse transcriptase polymerase chain reaction and immunochemistry revealed that human osteoblasts propagated on chitosan films continued to express collagen type I whereas chondrocytes expressed collagen type II and aggrecan. They concluded that chitosan may have potential use as a tissue engineering tool for the repair of osseous and chondral defects.

Zhang Y et al.[100] (2003) studied the response of Human osteoblast-like MG63 cells cultured on the composite scaffolds fabricated with macroporous calcium phosphate–chitosan . Cell morphology, total protein content, and expression of classic markers for osteoblast differentiation were characterized. They concluded that the hydroxyapatite–matrix composite scaffolds might enhance the phenotype expression of MG63 cells, in comparison with chitosan–matrix scaffolds. Soluble calcium phosphate glasses should be added to the scaffolds to prevent chitosan from fast degradation that may affect the differentiation of osteoblast cells.

Kadriye Tuzlakoglu et al.[42] (2004) reported on the production of chitosan fibers and 3-D fiber meshes for the use as tissue engineering scaffolds. After 14 d of immersion in simulated body fluid (SBF), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), and inductively coupled plasma emission (ICP) spectroscopy analysis showed that a bioactive Ca-P layer was formed on the surface of the fibers, meaning that they exhibit a bioactive behavior .By means of using short-term MEM

extraction test, both fibers and scaffolds were found to be non-cytotoxic to fibroblasts. Furthermore, osteoblasts directly cultured over chitosan fiber mesh scaffolds presented good morphology and no inhibition of cell proliferation could be observed.

Morales JG et al.[55] (2009) tested the hypotheses that addition of chitosan particles to the media of human bone marrow stromal cell (BMSC) cultures stimulates osteogenesis by promoting osteoblastic differentiation and by favoring the release of angiogenic factors in vitro. They demonstrated that chitosan particles alone are not sufficient to promote osteoblast differentiation of BMSCs in vitro, and suggest that chitosan promotes osteogenesis in vivo through indirect mechanisms and showed that continuous addition of dexamethasone promotes osteoblastic differentiation in vitro partly by inhibiting gelatinase activity and by suppressing inflammatory cytokines which result in increased cell attachment and cell cycle exit.

Nitra Rakkiettiwong et al.[60] (2011) investigated the effect of BIO-GIC with added TGF-beta1 on pulp cells. BIO-GIC was prepared from GIC (conventional type) incorporated with 15% of chitosan and 10% of BSA. TGF- beta1 (100 ng) was added in BIO-GIC+TGF-beta1 and GIC+TGF-beta1 groups during each disk specimen (10 mm diameter, 1 mm high) preparation. The effect of each specimen on pulp cells was investigated by using the Transwell plate technique. Cell proliferation was determined by MTT assay at 2 time periods (each period lasting 3 days). Pulp cell differentiation was examined by alkaline phosphatase activity and also by cell mineralization, which was measured by calculating the area of mineralization with von Kossa staining.They concluded that BIO-GIC could retain the effect of TGF-beta1.

Mathews S et al.[50] (2011) evaluated effects of chitosan-coated tissue culture plates at different coating densities on adhesion and osteoblast differentiation processes of human mesenchymal stem cells (hMSCs), isolated from adult bone marrow using alkaline phosphatase assay, demonstration of presence of calcium and real time PCR.This study demonstrated for the first time that chitosan enhanced mineralization by upregulating the associated genes.

Chen Y et al.[15] (2012)evaluated the in vitro cell biocompatibility of an in situ forming composite consisting of chitosan (CS), nano-hydroxyapatite and collagen (nHAC), which has a complex hierarchical structure similar to natural bone using MC3T3-E1 mouse calvarial preosteoblasts . Cytotoxicity, cell proliferation, and cell expression of osteogenic markers such as alkaline phosphatase (ALP), type 1 collagen (COL-1), RUNX-2, and osteocalcin (OCN) were examined by biochemical assay and reverse transcription polymerase chain reaction and concluded that CS/nHAC scaffolds were superior to chitosan-only scaffolds in facilitating osteoblast mineralization to be used in bone tissue engineering

BIOACTIVE GLASS

Ugo E.Pazzaglia et al.[82] (1989) gave the first report of manufacture and osteoconductivity of silicate based bioactive glass fibres.

Xynos I D et al.[97] (2000) investigated the concept of using bioactive substrates as templates for in vitro synthesis of bone tissue for transplantation by assessing the osteogenic potential of a melt-derived bioactive glass ceramic (Bioglass 45S5) in vitro.

Bioactive glass ceramic and bioinert (plastic) substrates were seeded with human primary osteoblasts and evaluated after 2, 6, and 12 days. The study showed that Bioglass 45S5 has the ability to stimulate the growth and osteogenic differentiation of human primary osteoblasts.

Effah Kaufmann EA et al.[27] (2000) studied osteoblast response to porous bioactive glass substrates following the expression of the classical markers for osteoblast differentiation like alkaline phosphatase (AP) activity, as well as the expression of mRNA for collagen type I (Coll-1), osteonectin (OSN), osteopontin (OPN), osteocalcin (OCN), and bone sialoprotein (BSP). The results confirmed that porous bioactive glass substrates are capable of supporting the in vitro growth and maturation of osteoblast-like cells. At a porosity of 42% and an average pore size of 80 microns, the substrates promote the expression and maintenance of the osteoblastic phenotype.

Loty C et al. [49] (2001) investigated the behavior of fetal rat osteoblasts cultured on bioactive glasses with 55 wt% silica content (55S) and on a bioinert glass (60S) used either in the form of granules or in the form of disks. Cytoenzymatic localization of alkaline phosphatase (ALP) and immunolabeling with bone sialoprotein antibody revealed a positive staining for the bone nodules formed in cultures on 55S. The interfacial analysis showed a firm bone bonding to the 55S surface through an intervening apatite layer, confirmed by the X-ray mappings. All these results indicated the importance of the surface composition in supporting differentiation of osteogenic cells and the subsequent apposition of bone matrix allowing a strong bond of the bioactive materials to bone.

S. Hattar et al.[33] (2005) examined the effects of Bioactive glasses on the proliferation and differentiation of the mouse preosteoblastic cell line MC3T3-E1. Cells were cultured up to 28 days in contact with three types of granules: Bioglass 45S5 granules (BG), 45S5 granules coated with enamel matrix proteins (Emdogain), and a less reactive glass used as a control (60S). Findings indicated that Bioglass alone or combined with Emdogain, have the ability to support the growth of osteoblast-like cells in vitro and to promote osteoblast differentiation by stimulating the expression of major phenotypic markers. In addition the bioactive granules coated with Emdogain revealed significantly higher protein production than the bioactive granules alone at day 20.

Venu G. Varanasi et al.[88] (2011) studied enhanced collagen type 1 and osteocalcin expression in human periodontal ligament fibroblasts (hPDLF) when exposed to bioactive glass conditioned media that subsequently may promote early mineralized tissue development. Differentiating hPDLF cultures showed enhanced expression of collagen type 1 (Col1α1, Col1α2), osteocalcin, and alkaline phosphatase gene expression. The results indicated the osteogenic potential of bioactive coating glass in periodontal bone defect filling applications.

MATERIALS AND METHODS

The following armamentaria and materials were used in this study.

Preparation of material specimen (Fig.26)

Glass Beaker 100ml

Distilled water

Digital weighing Balance

Cement mixing pad

Mixing spatula

Plastic filling instrument

Teflon mould 2 mm x 5 mm

For sealing ability (Fig. 3-5 & 17)

60 Maxillary central incisors

Airotor hand piece (NSK)

Micro motor straight handpiece (NSK)

Diamond disc

3% sodium hypochlorite (CE Prime Dent Products)

17% EDTA

Normal saline

Disposable syringe and needle (25 Gauge)

Endobloc (Dentsply)

701 Plain fissure bur

K files 15-80 (Dentsply)

Barbed broaches 15-40 (Dentsply)

Finger spreaders 15-40 (Dentsply)

Finger pluggers 15-40(Dentsply)

Paper points (Dentsply)

Zincoxide eugenol (Prevest Denpro)

Glass Slab

Stainless steel Spatula

Plastic filling instrument

Dental composite Kit(Tetric N Ceram, Ivoclar)

Nail varnish

Ultrasonic unit (ProUltra Peizon Booster)

Ultrasonic retro tips (Satelec) – (S12-90ND)

Rhodamine B dye

LSM 510 Meta Confocal Laser Scanning Microscope (Carl Zeiss)

Auto polymerizing acrylic resin

Hard tissue Microtome (Leica SP 1600)

For Cell culture studies (Fig.18-25)

24 well, 6 well microtiter plates

SaOS-2 cell line (NCCS, Pune)

Trypsin EDTA solution

Dulbecco's Modified Eagle's Medium (DMEM)

Foetal bovine serum (FBS)

Ascorbic acid

β-Glycerophosphate

CO_2 Incubator (Galaxy 170 S, New Brunswick)

MTT dye agent

Dimethyl sulphoxide (DMSO)

p- Nitrophenyl phosphate

Sodium hydroxide

ELISA reader

AutoAnalyser

Laminar air flow chamber

Inverted phase contrast microscope

MATERIALS USED IN THIS STUDY (Fig.1)

Glass Ionomer Cement (Fuji II, GC Corporation. Tokyo, Japan)

Chitosan (Panvo Organics, Tamilnadu, India)

Bioactive glass (Perioglass, Novabone products. FL, USA)

METHODOLOGY

Preparation of Chitosan modified GIC

1.8ml of Glacial acetic acid is made upto 100 ml with distilled water in a 100 ml standard flask. 20 mg of chitosan was weighed and dissolved in 0.3N acetic acid, and made upto 100 ml with the same acetic acid in a 100ml standard flask to get 0.2 mg/ml chitosan solution. 0.1ml of 0.2mg / ml of chitosan solution is added to 0.9 ml of conventional glass ionomer cement liquid to get 10 v/v% chitosan modified glass ionomer cement.100 mg of chitosan was weighed and dissolved in 0.3 N acetic acid, and made upto 100 ml with the same acetic acid in a 100 ml standard flask to get 1mg/ml chitosan solution. 0.5 ml of 1 mg/ml of chitosan solution is added to 0.5 ml of conventional glass ionomer liquid to get 50 v/v% chitosan modified glass ionomer cement.

Preparation of Bioactive glass modified GIC

Glass ionomer cement containing Bioactive Glass was prepared by addition of 10 wt % and 30 wt% of Bioactive Glass to the Glass ionomer powder.

The experimental groups considered were (Fig.2)

Group I - Conventional Glass ionomer cement

Group II - Glass ionomer cement containing 10 v/v% Chitosan

Group III - Glass ionomer cement containing 50 v/v% Chitosan

Group IV - Glass ionomer cement containing 10 wt% Bioactive Glass

Group V - Glass ionomer cement containing 30 wt% Bioactive Glass

EXPERIMENTAL MATERIALS

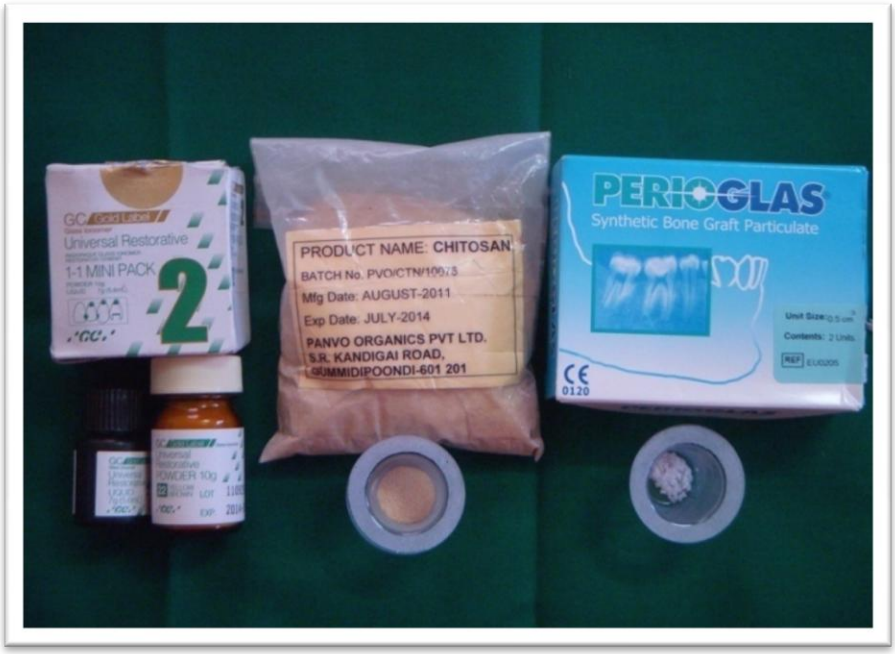


Fig.1 GIC, Chitosan, Bioactive Glass

Fig.2 GIC, GIC+Chitosan (10v/v% & 50 v/v%), GIC+Bioactive glass(10 wt% & 30wt%)

SEALING ABILITY

Sixty freshly extracted human maxillary central incisors with completely formed apices and straight canals were collected and stored in normal saline until use. Teeth with calcified canals, tortuous canals and root caries were excluded. The teeth were cleaned ultrasonically and sectioned at Cemento-enamel junction with a diamond disk, standardizing the root lengths to approximately 16 mm (Fig.6). The pulp tissue was extirpated with a barbed broach. K- File # 15 was used to confirm canal patency. The working length was determined by subtracting 0.5 mm from the length at which # 15 K file appeared at the apical foramen and confirmed with the help of radiographs.

Canals were cleaned and shaped using step back technique. 3% sodium hypochlorite and 17% EDTA were used as irrigants. All the canals were enlarged upto No. 50 K- file (master apical file) at the apical foramen. The specimens were stored in normal saline until obturation. Canals were dried using absorbent paper points and master cone selection was confirmed with radiographs (Fig.7). Canals were obturated with gutta percha by lateral compaction technique. Radiographs were taken to confirm the quality of obturation (Fig.8) and the access cavities were sealed with composite resin restorative material after 24 hours. The teeth were then stored in saline for 1 week.

Apical root resections were performed on 55 roots by removing 3 mm of each apex at 90 degrees to the long axis of the tooth with a # 701 fissure bur in a high-speed handpiece with water coolant (Fig.9). The 3 mm deep retrograde cavity was prepared with an ultrasonic tip, powered by an ultrasonic unit (Fig.10). The cavities were irrigated with saline and dried (Fig. 11,12).

50 specimens were randomly divided into 5 groups of 10 specimens each. The experimental materials were manipulated according to the manufacturer's instructions and the the test materials were condensed into the cavities using P-40 Plugger (Fig.13, 14). The specimens were stored in moist cotton at room temperature. They were coated with three coats of nail varnish except at the apical 1 mm of the resected root, and then were allowed to dry (Fig. 15).

Five instrumented roots with retro-preparations received no retrograde filling, and these were used as positive controls. Another five roots were instrumented and obturated with gutta-percha and sealer without retro preparation; their entire root surfaces were covered with two coats of nail polish and were used as negative controls.

All the specimens were suspended in 0.5% Rhodamine B dye for 24 hours (Fig.16). Following this, the roots were rinsed for 1 hr under tap water. The teeth were mounted in acrylic blocks and split longitudinally with a hard tissue microtome using a water coolant (Fig.17). The specimens were examined under confocal laser scanning microscope at 10X magnification (Fig. 5) and microleakage associated with different root end filling materials were evaluated in millimeters.

PROCEDURAL FLOWCHART FOR ASSESSING SEALING ABILITY

Sixty freshly extracted human maxillary central incisors with completely formed apices and straight canals were collected and stored in normal saline

↓

The teeth were cleaned ultrasonically and sectioned at Cemento-enamel junction with a diamond disk standardizing the root lengths to approximately 16 mm.

↓

The pulp tissue was extirpated with a barbed broach.

↓

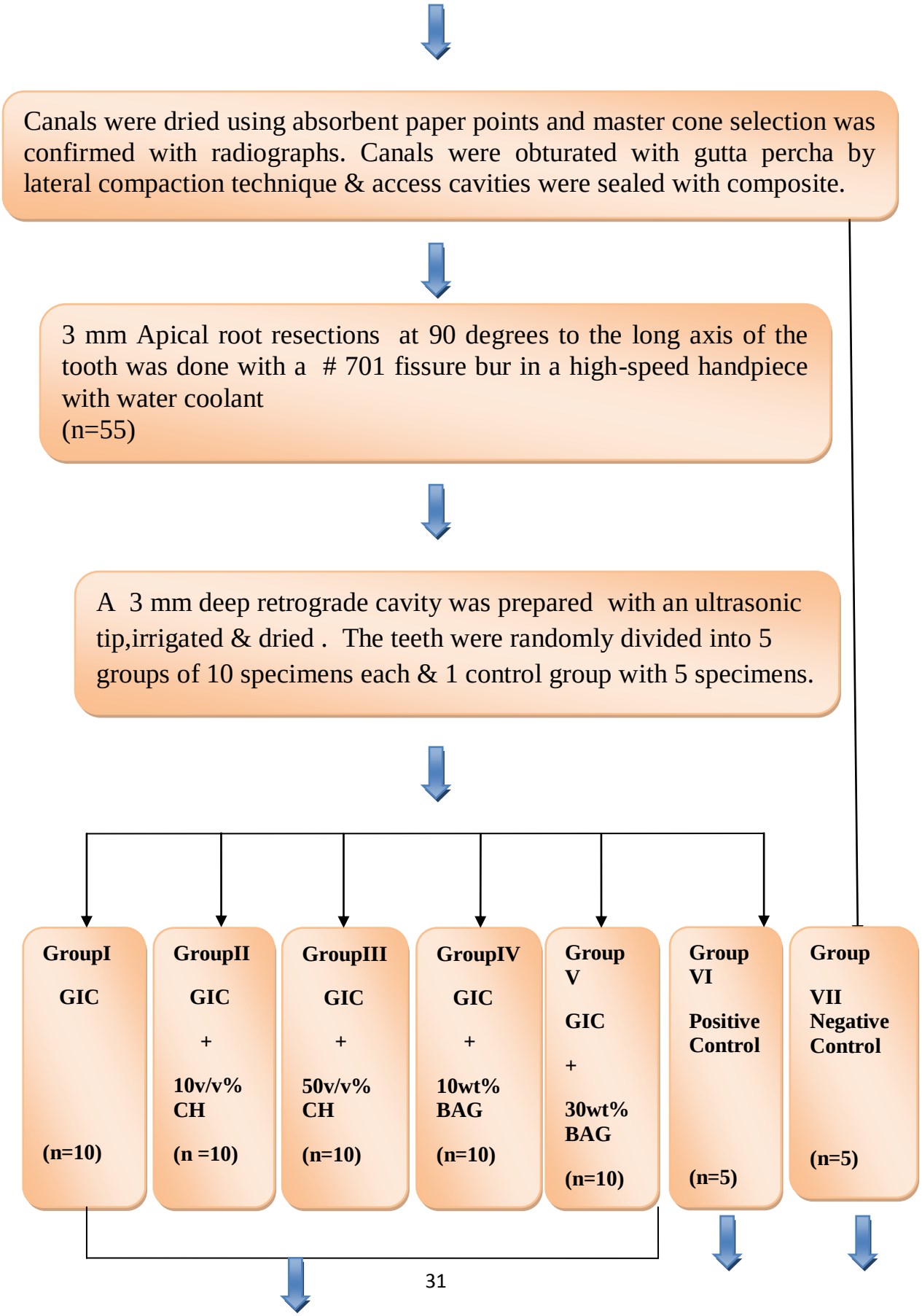
Canal patency & working length was determined using Kfiles & confirmed by radiographs.

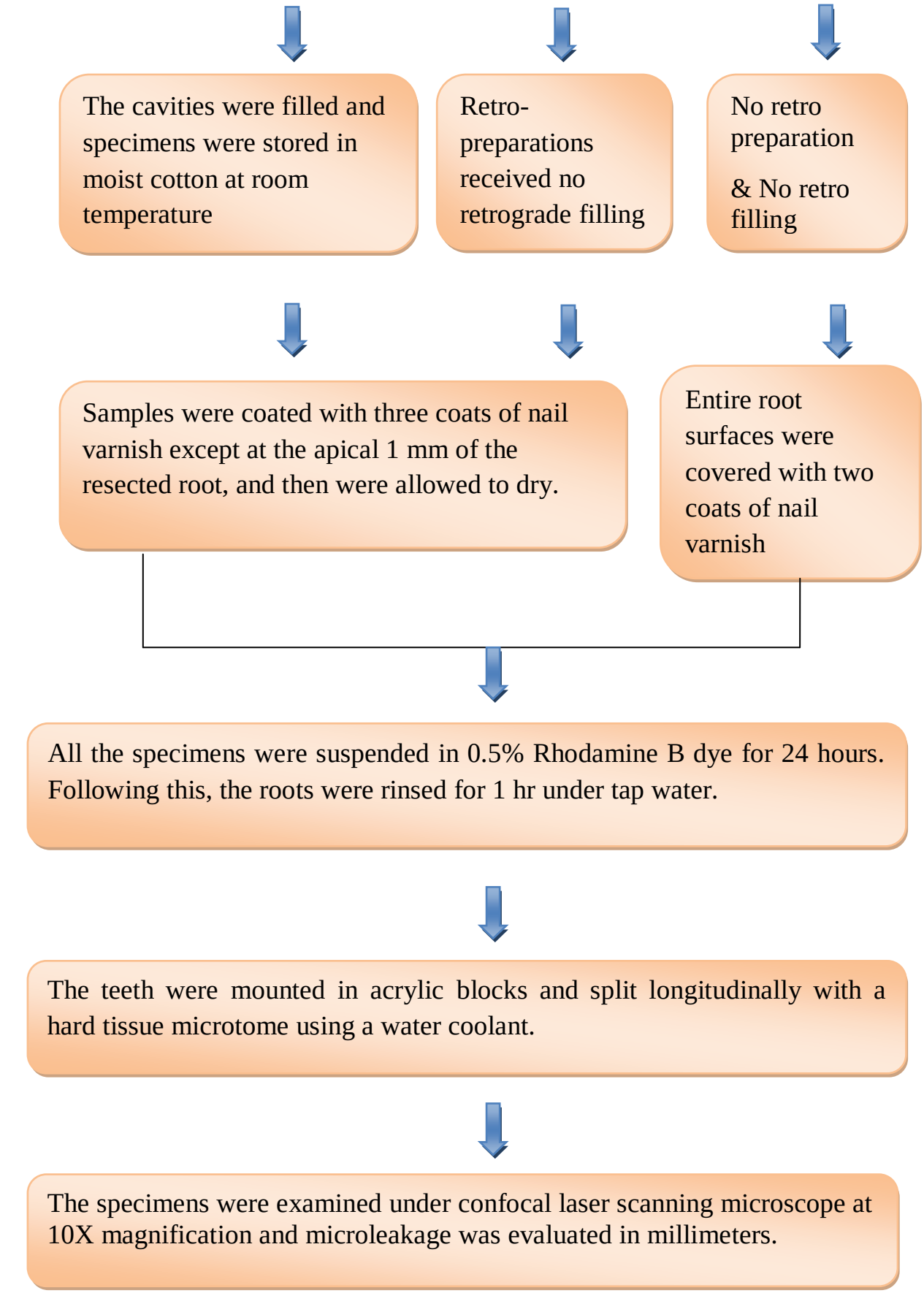
↓

Canals were enlarged upto No. 50 K- file using step back technique & 3% sodium hypochlorite and 17% EDTA as irrigants.

↓

The specimens were stored in normal saline until obturation.


Canals were dried using absorbent paper points and master cone selection was confirmed with radiographs. Canals were obturated with gutta percha by lateral compaction technique & access cavities were sealed with composite.
3 mm Apical root resections at 90 degrees to the long axis of the tooth was done with a # 701 fissure bur in a high-speed handpiece with water coolant
(n=55)
A 3 mm deep retrograde cavity was prepared with an ultrasonic tip,irrigated & dried . The teeth were randomly divided into 5 groups of 10 specimens each & 1 control group with 5 specimens.
GroupI GIC (n=10)
GroupII GIC + 10v/v% CH (n =10)
GroupIII GIC + 50v/v% CH (n=10)
GroupIV GIC + 10wt% BAG (n=10)
Group V GIC + 30wt% BAG (n=10)
Group VI Positive Control (n=5)
Group VII Negative Control (n=5)


The cavities were filled and specimens were stored in moist cotton at room temperature
Retro-preparations received no retrograde filling
No retro preparation
& No retro filling
Samples were coated with three coats of nail varnish except at the apical 1 mm of the resected root, and then were allowed to dry.
Entire root surfaces were covered with two coats of nail varnish
All the specimens were suspended in 0.5% Rhodamine B dye for 24 hours. Following this, the roots were rinsed for 1 hr under tap water.
The teeth were mounted in acrylic blocks and split longitudinally with a hard tissue microtome using a water coolant.
The specimens were examined under confocal laser scanning microscope at 10X magnification and microleakage was evaluated in millimeters.

ARMAMENTARIA FOR SEALING ABILITY

Fig. 3 Airotor hand piece, Micro motor straight handpiece , Diamond disc, 3% sodium hypochlorite, 17% EDTA, Normal saline, Endobloc , # 701 Plain fissure bur, K files 15-80 ,Barbed broaches 15-40 ,Finger spreaders 15-40, Finger pluggers 15-40, Zincoxide eugenol, Plastic filling instrument, Dental composite Kit, Nail varnish, Rhodamine B dye

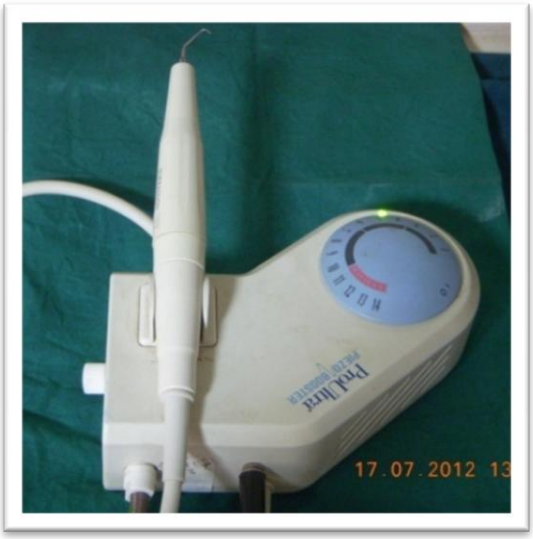


Fig.4 Ultrasonic Instrument

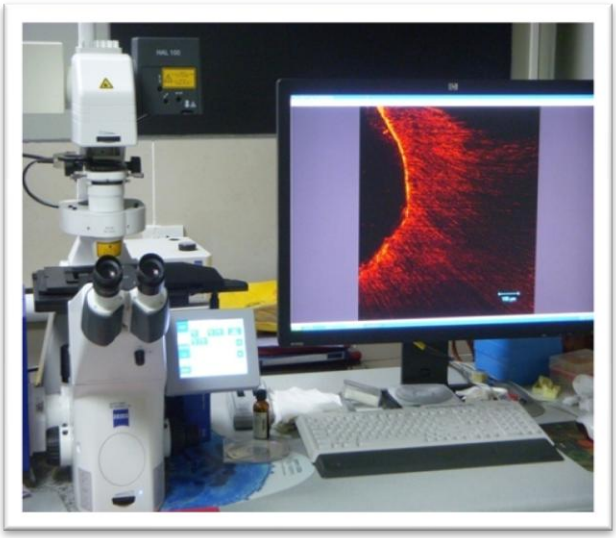


Fig.5 Confocal Laser Scanning Microscopy

METHODOLOGY FOR SEALING ABILITY-DYE PENETRATION TEST USING CONFOCAL LASER SCANNING MICROSCOPY

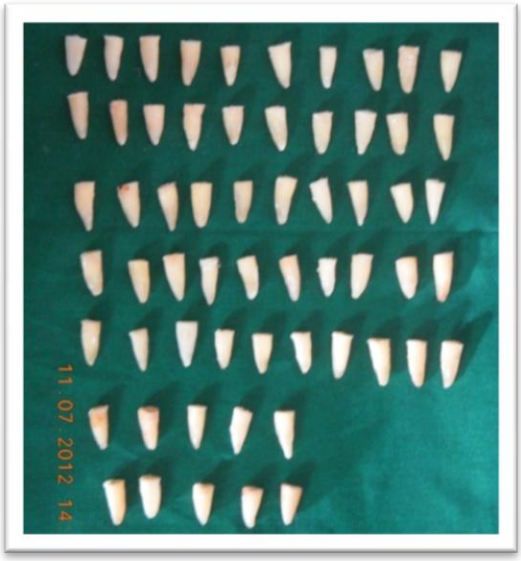
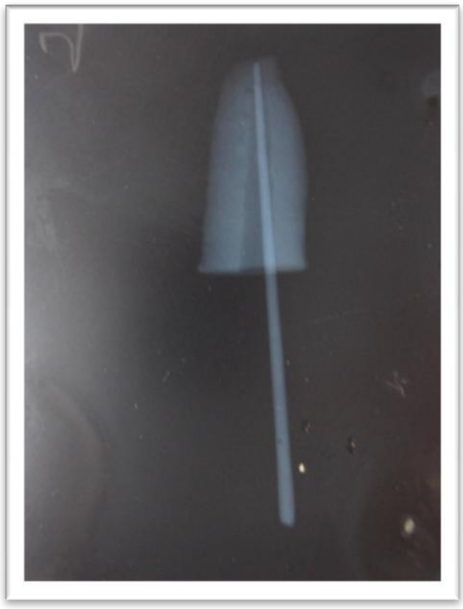


Fig.6 Specimens of Maxillary central incisors



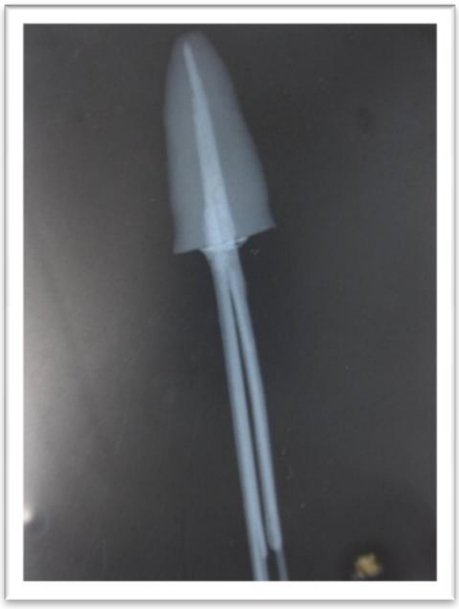


Fig.7,8 Radiographic picture of master cone verification and obturation

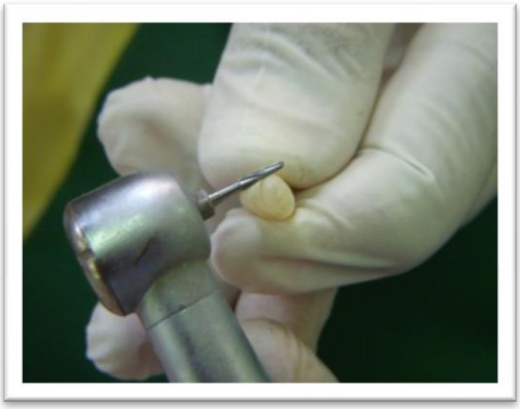


Fig.9 Root end resection

Fig.10 Root end cavity preparation

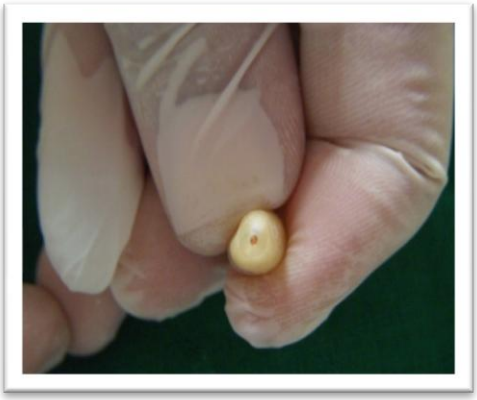


Fig.11 Root end Cavity

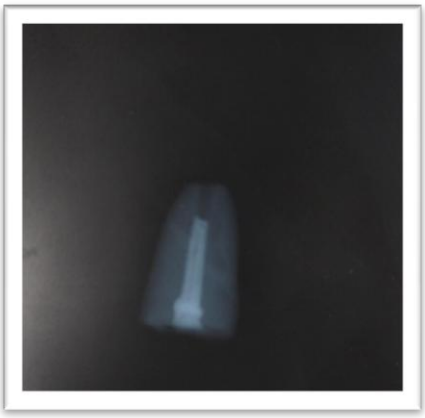


Fig.12 Radiographic picture of root end cavity

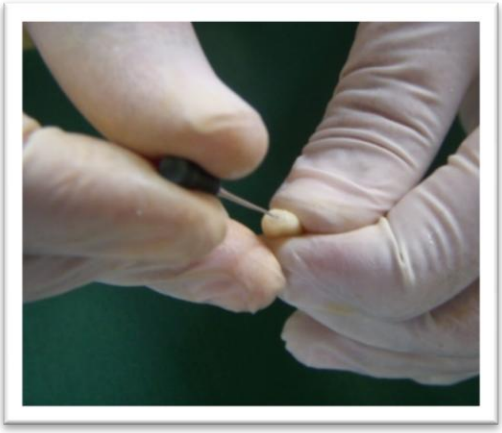


Fig.13 Root end filling

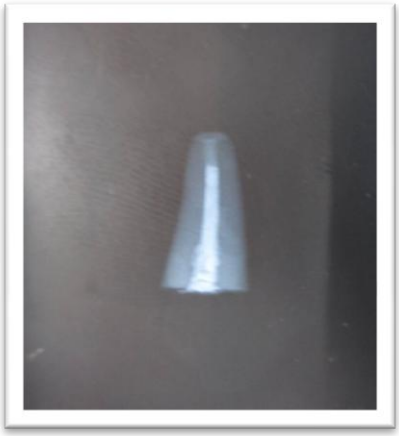


Fig.14 Radiographic picture of root end filling

Fig. 15 Specimens coated with nail varnish

Fig.16 Specimens stored in Rhodamine B dye

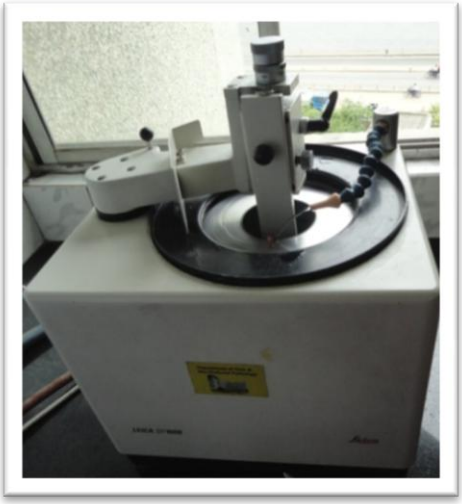


Fig.17 Specimen sectioned with microtome

OSTEOGENIC POTENTIAL

Culture reagents

1. **Dulbecco's Modified Eagle's Medium (DMEM) (pH 7.4):** 10 g of DMEM was dissolved in 800 ml of sterile distilled water. To this solution, 32.5 ml of 7.5% sodium bicarbonate solution was added followed by addition of 10 ml penicillin/streptomycin-amphotericin B solution. The pH was adjusted to 7.4. The final volume was made up to 1 litre with distilled water. Then the medium was sterile filtered (0.22 µm pore size) and stored at 4°C.
2. **DMEM with 10% FBS:** 10 ml of FBS was made up to100 ml using sterile DMEM. It was stored in a sterile container in cool and aseptic condition.
3. **Osteogenic medium (OM):** Osteogenic medium was prepared by supplementing DMEM with 10% CSS, 10 mM ß-glycerophosphate and 50 µg/ml ascorbic acid.
4. **Phosphate buffered saline (PBS) (pH 7.4)**
5. **Trypsin-EDTA solution**

Culture of SaOS-2 cells:

SaOS-2 cell line was procured from the National center for cell sciences (NCCS), Pune, India (Fig.24, 25). The cells were grown in culture flasks containing DMEM supplemented with 10% FBS. Upon reaching confluence, as observed in the inverted phase contrast microscope, the cells were detached using trypsin-EDTA solution and used for subculture or treatment.

Passaging the cells:

The medium from the culture flask was aspirated. The flask was rinsed with 2 ml of phosphate buffered saline (PBS) and aspirated quickly. One ml of trypsin- EDTA solution was added to flask and swirled gently to cover the entire area for 10 sec and aspirated quickly. Then the flask was incubated at 37°C for 10 min. The detached cells were then resuspended in 10 ml of 10% FBS – DMEM, gently mixed well by pipetting up and down. From the cell suspension, a drop was placed to the edge of the cover slip of Neubauer haemocytometer and the drop was let to run under the cover slip by capillary action. Then the cells from the E_1, E_2, E_3, E_4 and E_5 squares were counted under the microscope. The number of cells was calculated using the formula:

No. of cells = No. of cells counted × 50,000

= X cells/ml.

Preparation of test samples

Round-shaped samples measuring 2mm thick and 5mm in diameter were prepared (Fig.26, 27).

For MTT Assay-9 samples for every experimental material were prepared.

For Alkaline Phosphatase Assay-9 samples for every experimental material were prepared.

Cell proliferation assessment using MTT assay.

Reagents

1. **MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide):** MTT was dissolved in DMEM at the concentration of 0.5 mg /ml.
2. **Solubilization solution:** Dimethylsulfoxide (DMSO)
3. **Phosphate buffered saline (PBS) (pH 7.4)**

Procedure

The SaOS-2 cells were plated in 24 well plates at a concentration of 3 x 10^4 cells/well (Fig. 28). 24 h after plating, cells were washed twice with 100 µl of phosphate buffered saline and starved by incubating the cells with 0.1% BSA for 12 h at 37^o C in CO_2 incubator. The test samples were rinsed three times with Phosphate buffered saline (PBS) and α-MEM medium & were placed into the wells of 24-well microtiter plates for 24 and 48 and 72 hrs and incubated at 37º C (Fig. 29). At the end of treatment, the medium from control and test material treated cells were discarded and 100 µl of MTT

containing DMEM medium was added to each well. The cells were then incubated for 3 hours.

The MTT containing medium was then discarded and the cells were washed with PBS (200 µl). The crystals were then dissolved by adding 1 ml of DMSO. The colour developed is directly proportional to the number of live cells (Fig. 30). The intensity of purple colour was immediately measured in an ELISA reader at 545 nm (Fig.22).

Cell Differentiation assessment using Alkaline Phosphatase assay

Reagents

1. **Glycine buffer (pH 10.5, 0.1M)**
2. **p-nitrophenyl phosphate (0.4%)**
3. **Sodium hydroxide (1N)**

2×10^4 SaOS-2 cells were seeded on test samples under culture conditions in osteogenic medium & the level of ALP activity was determined at day 7, 14 and 21(Fig. 31). The cells were detached from discs using trypsin/EDTA, and centrifuged for 5 min at 1000 rpm after being washed twice with PBS. Cell lysate was obtained & ALP activity was determined by using p-nitrophenyl phosphate as the substrate (Fig. 32). All experiments were done in triplicate. Upon dephosphorylated by ALP, p-Nitrophenyl phosphate (pNPP) turned yellow and its color change was directly proportional to ALP. The reaction was stopped by the addition of 1 N NaOH to reaction mixture. This colorimetric assay was finished by detecting the absorbance at 405 nm (OD value) using

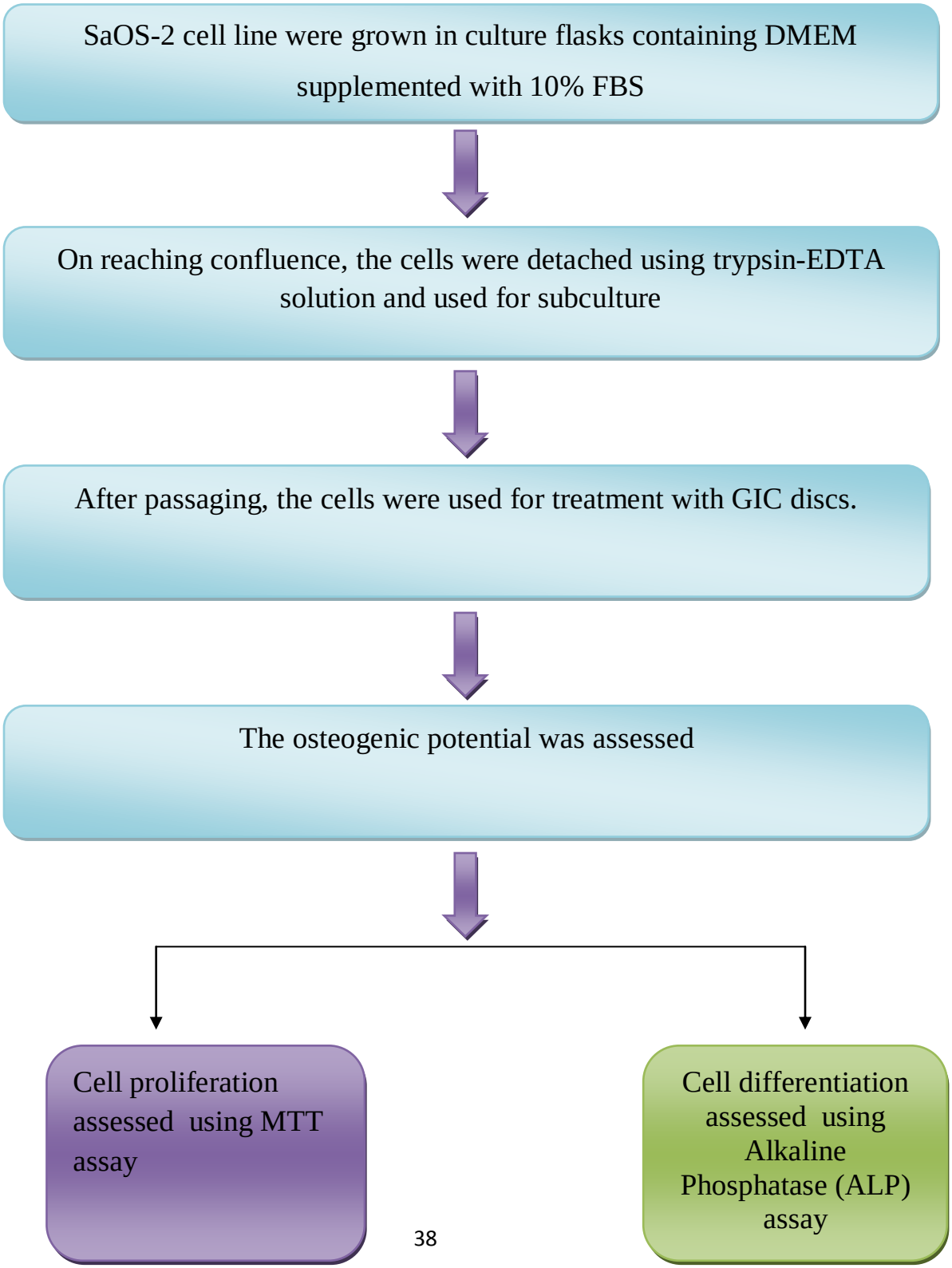
auto analyser (Fig. 23). The alkaline phosphatase activity was expressed as μ moles of ρ-nitrophenol formed per min per microgram of protein.

The alkaline phosphatase activity was calculated using the formula:

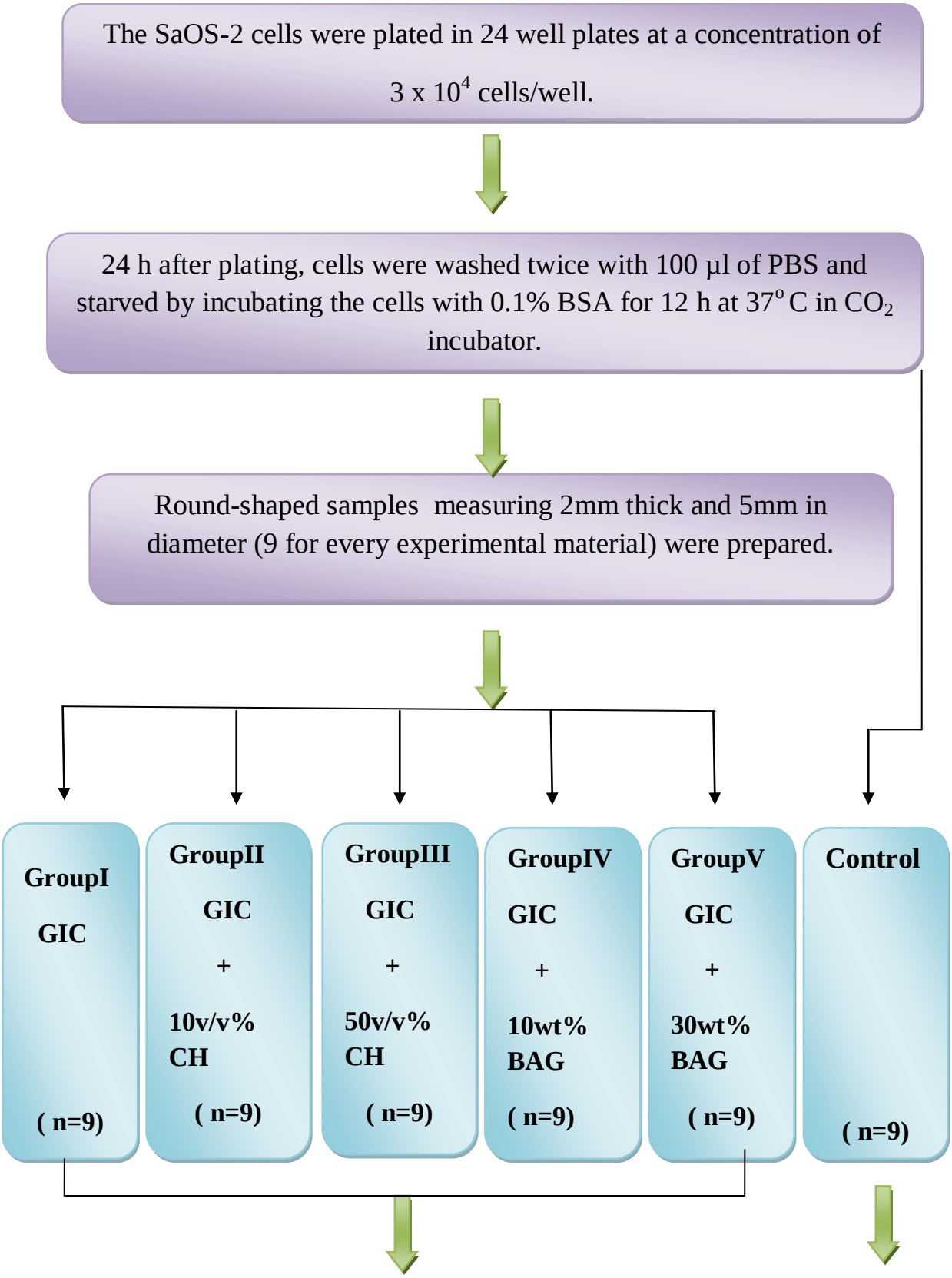
$$\frac{\text{O.D of unknown}}{\text{O.D of known}} \times \text{Standard Concentration} \times \text{Time factor correction} \times \frac{1}{\mu g \text{ protein}}$$

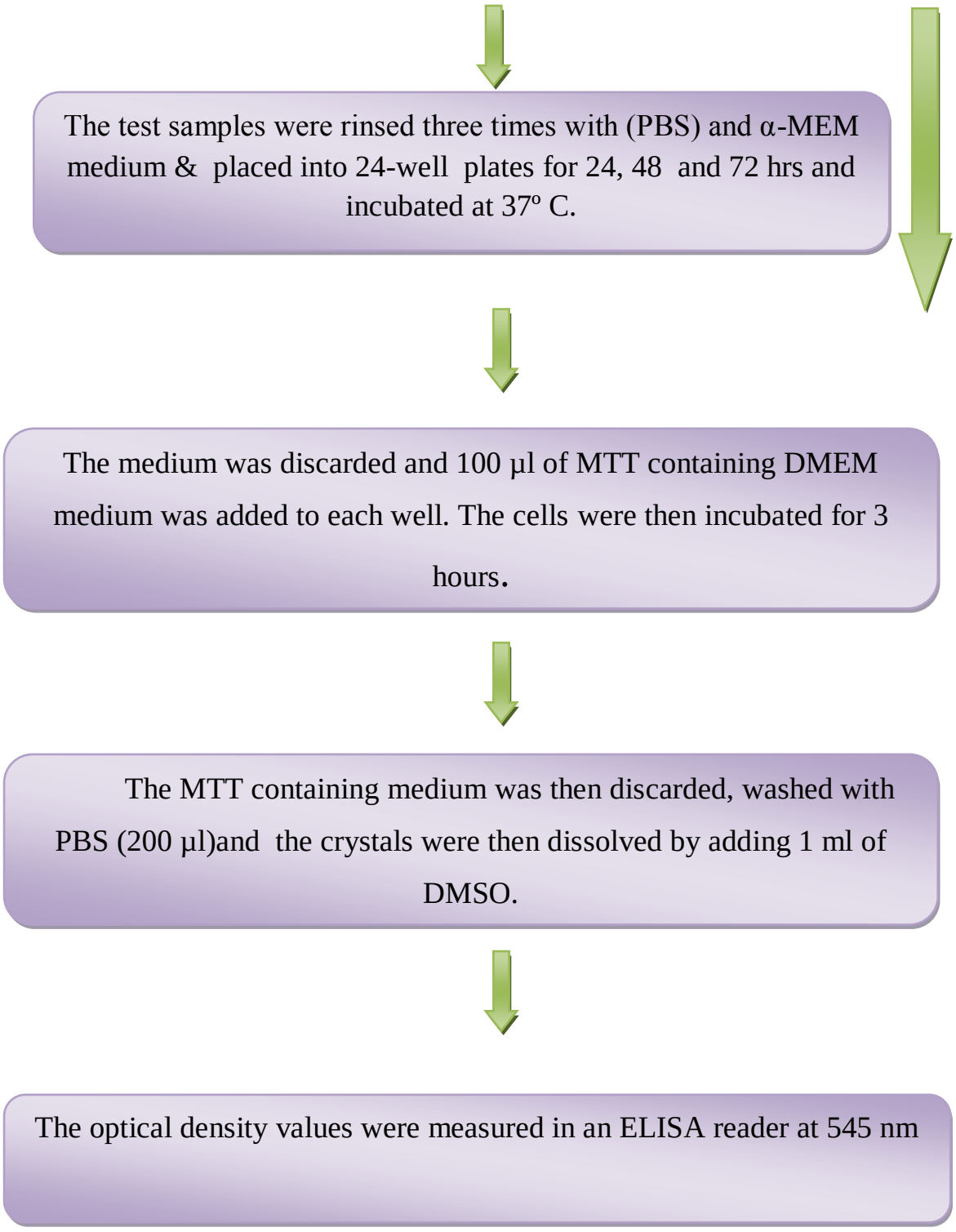
= μ moles of p-nitrophenol formed per min per μg protein.

PROCEDURAL FLOWCHART FOR ASSESSING OSTEOGENIC POTENTIAL



MTT ASSAY


The SaOS-2 cells were plated in 24 well plates at a concentration of 3 x 10^4 cells/well.
24 h after plating, cells were washed twice with 100 µl of PBS and starved by incubating the cells with 0.1% BSA for 12 h at 37° C in CO2 incubator.
Round-shaped samples measuring 2mm thick and 5mm in diameter (9 for every experimental material) were prepared.
GroupI GIC (n=9)
GroupII GIC + 10v/v% CH (n=9)
GroupIII GIC + 50v/v% CH (n=9)
GroupIV GIC + 10wt% BAG (n=9)
GroupV GIC + 30wt% BAG (n=9)
Control (n=9)


The test samples were rinsed three times with (PBS) and α-MEM medium & placed into 24-well plates for 24, 48 and 72 hrs and incubated at 37º C.
The medium was discarded and 100 µl of MTT containing DMEM medium was added to each well. The cells were then incubated for 3 hours.
The MTT containing medium was then discarded, washed with PBS (200 µl)and the crystals were then dissolved by adding 1 ml of DMSO.
The optical density values were measured in an ELISA reader at 545 nm

ALP ASSAY

Test material discs were prepared & divided into 5 groups of 9 wells each as mentioned for MTT assay

↓

2×10^4 SaOS-2 cells were seeded on test material discs under culture conditions in osteogenic medium

↓

The cells were detached from discs using trypsin/EDTA, washed with PBS & centrifuged for 5 min at 1000 rpm

↓

Cell lysate was obtained & ALP activity was determined by using p-nitrophenyl phosphate as the substrate.

↓

The reaction was stopped by the addition of 1 N NaOH to reaction mixture.

↓

The absorbance at 405 nm (OD value) was measured using auto analyser and alkaline phosphatase activity was expressed as µ moles of ρ-nitrophenol / min / µg protein

ARMAMENTARIA FOR OSTEOGENIC POTENTIAL

Fig.18 Dulbecco's Minimal essential medium, Foetal Bovine serum, Micropipette, Trypsin- EDTA

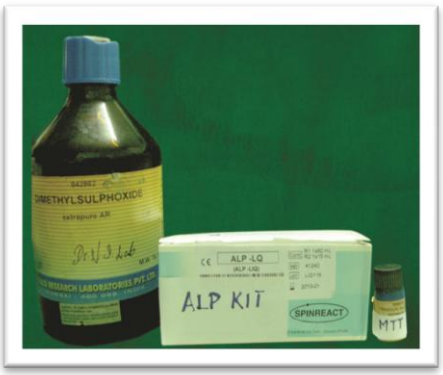


Fig.19 MTT, ALP Kits

Fig.20 Laminar air flow chamber

Fig.21 Inverted phase contrast microscope

Fig.22 ELISA Reader

Fig.23 Autoanalyser

METHODOLOGY FOR OSTEOGENIC POTENTIAL

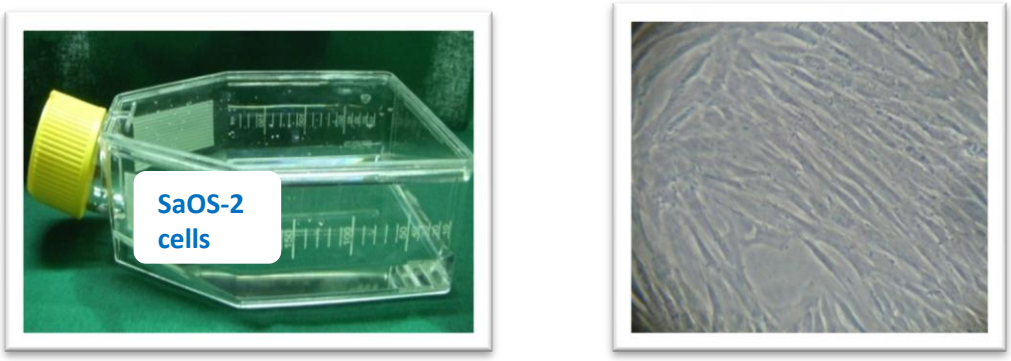


Fig. 24, 25 SaOS-2 Cell Line

Fig.26, 27 Preparation of discs for cell culture

MTT ASSAY

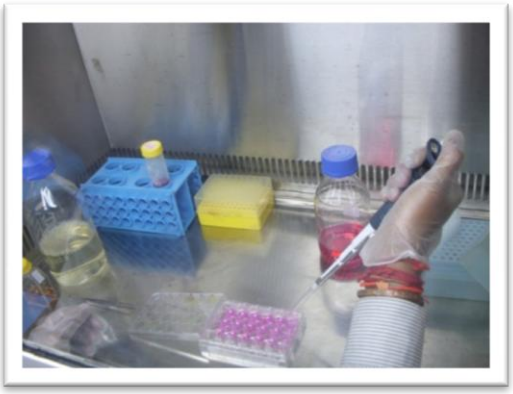


Fig. 28 Samples loaded with cells

Fig.29 Incubated in CO_2 Incubator

Fig.30 Purple formazan formation

ALKALINE PHOSPHATASE ASSAY

Fig.31 Samples loaded with cells in osteogenic medium

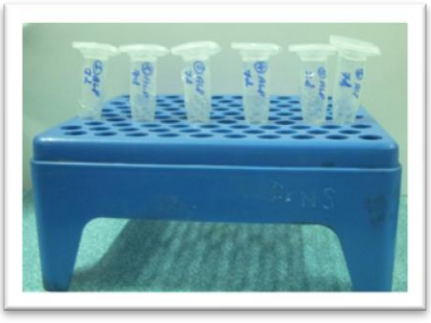


Fig.32 Aliquots separated for ALP Assay

SEALING ABILITY

Table1. Dye Leakage Values in mm.

Sample No.	Group I	Group II	Group III	Group IV	Group V	Group VI Positive control	Group VI I Negative control
1	2.32	1.08	2.89	1.23	0.97	3	0
2	1.95	0.89	3.00	0.92	1.20	3	0
3	2.87	0.98	2.51	0.76	0.82	3	0
4	2.14	0.76	1.92	1.28	1.24	3	0
5	1.74	1.22	2.38	1.00	1.20	3	0
6	1.21	1.34	2.86	1.35	0.77		
7	1.83	0.50	2.67	1.48	1.52		
8	2.35	1.50	2.96	0.72	1.50		
9	1.53	1.13	2.80	1.30	1.29		
10	1.70	1.10	2.92	1.15	1.52		

The dye leakage values were analysed using ONE WAY ANOVA and TUKEY HSD POST HOC multiple comparisons at 0.05 level significance.

Table 2. ONEWAY ANOVA FOR SEALING ABILITY

	Mean	Std. Deviation	F	Sig.
GROUP I	1.9640	.47404	74.40039	0.000
GROUP II	1.0500	.28721		
GROUP III	2.6910	.33742		
GROUP IV	1.1190	.25766		
GROUP V	1.2030	.27621		
GROUP VI PC	3.0000	.00000		
GROUP VII NC	.0000	.00000		
Total	1.5878	.90016		

Table 3. POST HOC TUKEY HSD TEST FOR SEALING ABILITY

Multiple Comparisons

(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP I	GROUP II	.91400*	.13837	.000	.4900	1.3380
	GROUP III	-.72700*	.13837	.000	-1.1510	-.3030
	GROUP IV	.84500*	.13837	.000	.4210	1.2690
	GROUP V	.76100*	.13837	.000	.3370	1.1850
	GROUP VI PC	-1.03600*	.16947	.000	-1.5553	-.5167
	GROUP VII NC	1.96400*	.16947	.000	1.4447	2.4833
GROUP II	GROUP I	-.91400*	.13837	.000	-1.3380	-.4900
	GROUP III	-1.64100*	.13837	.000	-2.0650	-1.2170
	GROUP IV	-.06900	.13837	.999	-.4930	.3550
	GROUP V	-.15300	.13837	.924	-.5770	.2710
	GROUP VI PC	-1.95000*	.16947	.000	-2.4693	-1.4307
	GROUP VII NC	1.05000*	.16947	.000	.5307	1.5693
GROUP III	GROUP I	.72700*	.13837	.000	.3030	1.1510
	GROUP II	1.64100*	.13837	.000	1.2170	2.0650
	GROUP IV	1.57200*	.13837	.000	1.1480	1.9960
	GROUP V	1.48800*	.13837	.000	1.0640	1.9120
	GROUP VI PC	-.30900	.16947	.539	-.8283	.2103
	GROUP VII NC	2.69100*	.16947	.000	2.1717	3.2103
GROUP IV	GROUP I	-.84500*	.13837	.000	-1.2690	-.4210
	GROUP II	.06900	.13837	.999	-.3550	.4930
	GROUP III	-1.57200*	.13837	.000	-1.9960	-1.1480
	GROUP V	-.08400	.13837	.996	-.5080	.3400
	GROUP VI PC	-1.88100*	.16947	.000	-2.4003	-1.3617
	GROUP VII NC	1.11900*	.16947	.000	.5997	1.6383
GROUP V	GROUP I	-.76100*	.13837	.000	-1.1850	-.3370
	GROUP II	.15300	.13837	.924	-.2710	.5770
	GROUP III	-1.48800*	.13837	.000	-1.9120	-1.0640
	GROUP IV	.08400	.13837	.996	-.3400	.5080
	GROUP VI	-1.79700*	.16947	.000	-2.3163	-1.2777

	PC					
	GROUP VII NC	1.20300*	.16947	.000	.6837	1.7223
GROUP VI PC	GROUP I	1.03600*	.16947	.000	.5167	1.5553
	GROUP II	1.95000*	.16947	.000	1.4307	2.4693
	GROUP III	.30900	.16947	.539	-.2103	.8283
	GROUP IV	1.88100*	.16947	.000	1.3617	2.4003
	GROUP V	1.79700*	.16947	.000	1.2777	2.3163
	GROUP VII NC	3.00000*	.19568	.000	2.4004	3.5996
GROUP VII NC	GROUP I	-1.96400*	.16947	.000	-2.4833	-1.4447
	GROUP II	-1.05000*	.16947	.000	-1.5693	-.5307
	GROUP III	-2.69100*	.16947	.000	-3.2103	-2.1717
	GROUP IV	-1.11900*	.16947	.000	-1.6383	-.5997
	GROUP V	-1.20300*	.16947	.000	-1.7223	-.6837
	GROUP VI PC	-3.00000*	.19568	.000	-3.5996	-2.4004

* The mean difference is significant at the .05 level.

INTERPRETATION OF RESULTS OF SEALING ABILITY

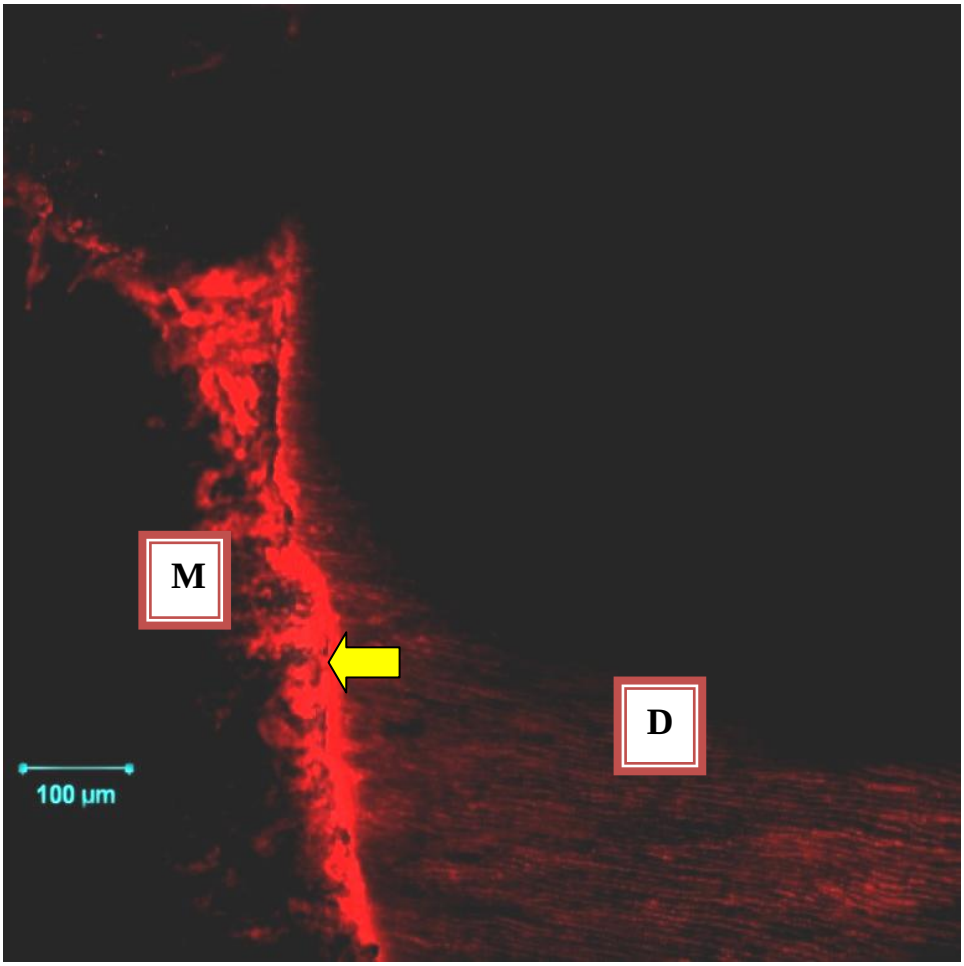
Analysis of mean values of the sealing ability at 0.05 level significance reveals

- Positive control showed maximum leakage & negative control showed no leakage.
- There was significant leakage in all groups when compared with Group VII (negative control)
- Group III significantly showed more leakage than Group I and there was no significant difference in leakage values between Group III & positive control.
- Group II, IV, V showed significantly less leakage values than Group I ($p<0.05$).
- There was no significant difference in leakage values among Group II, IV, and

Positive control ≥ Group III > Group I > Group V ≥ Group IV ≥ Group II > negative control

CONFOCAL IMAGES (10X magnification)

GROUP I- GIC

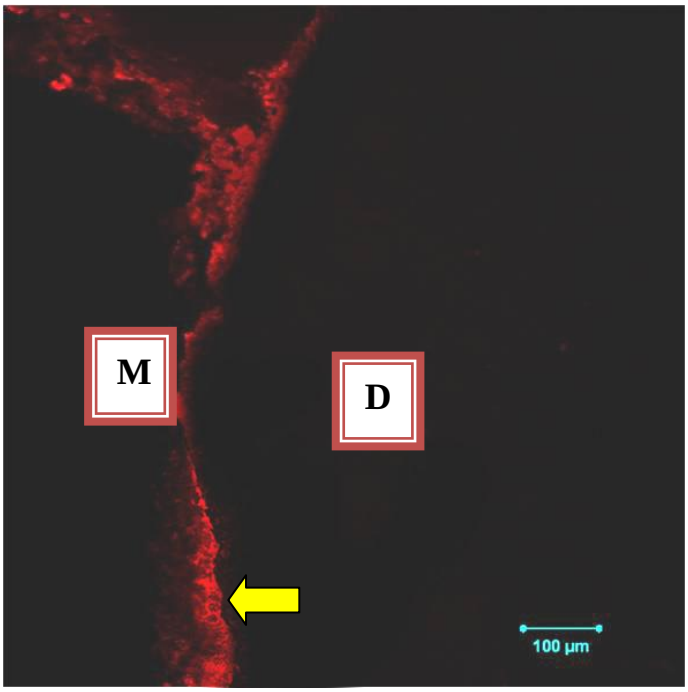


- **DYE LEAKAGE**

M **EXPERIMENTAL MATERIAL**

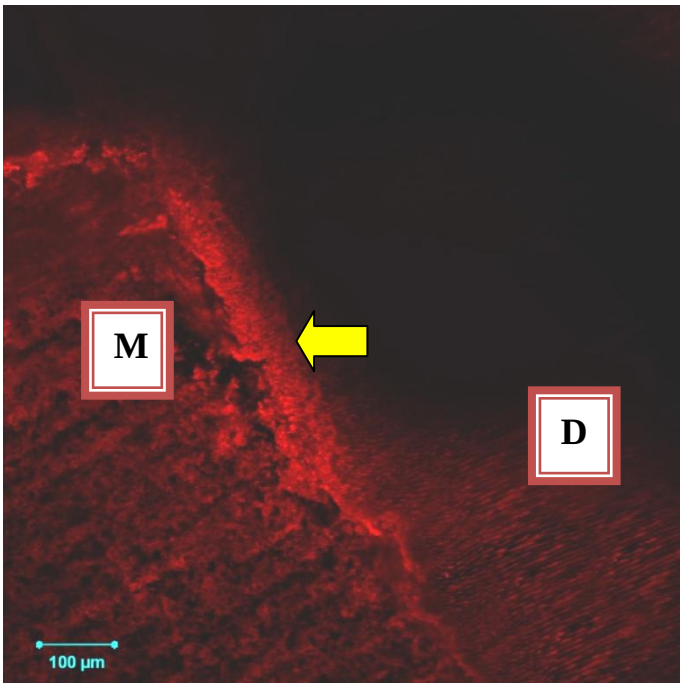
D - **DENTIN**

GROUP II- GIC +10 V/V% CHITOSAN



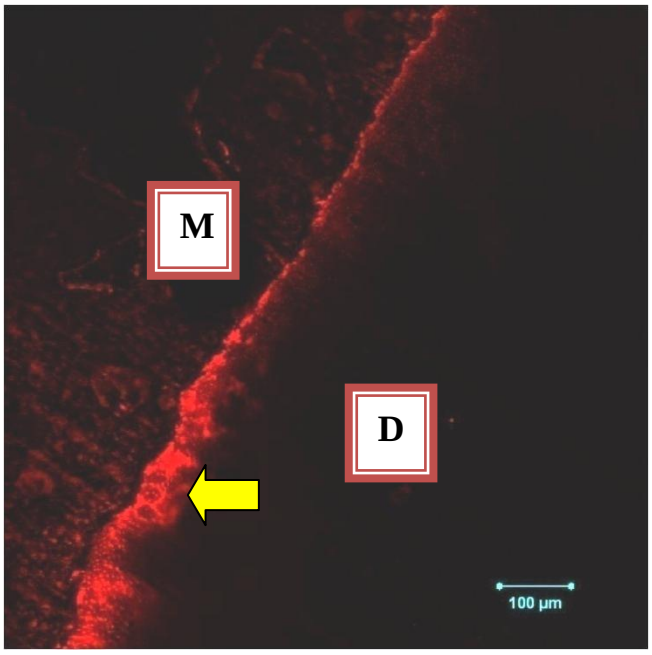
MINIMAL DYE LEAKAGE

GROUP III- GIC +50 V/V% CHITOSAN



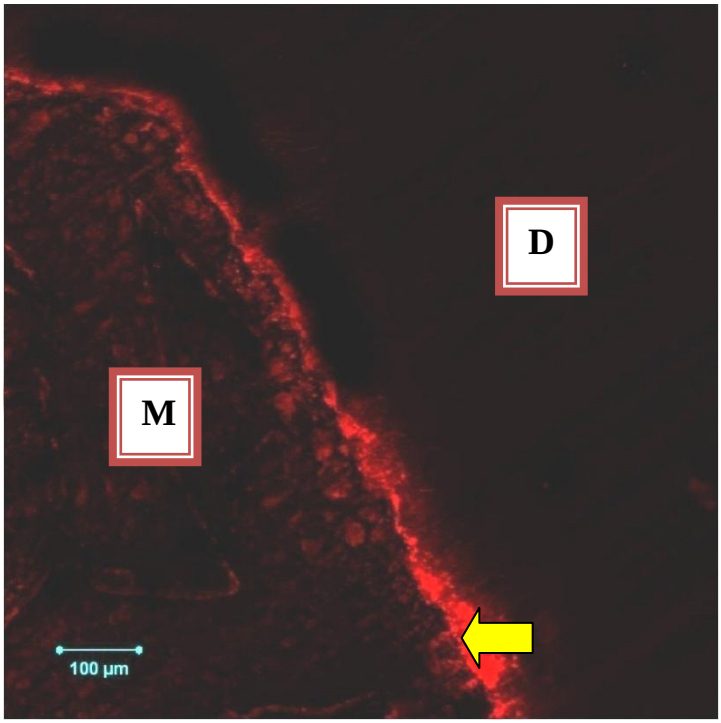
MASSIVE DYE LEAKAGE

GROUP IV- GIC+ 10 wt% BIOACTIVE GLASS



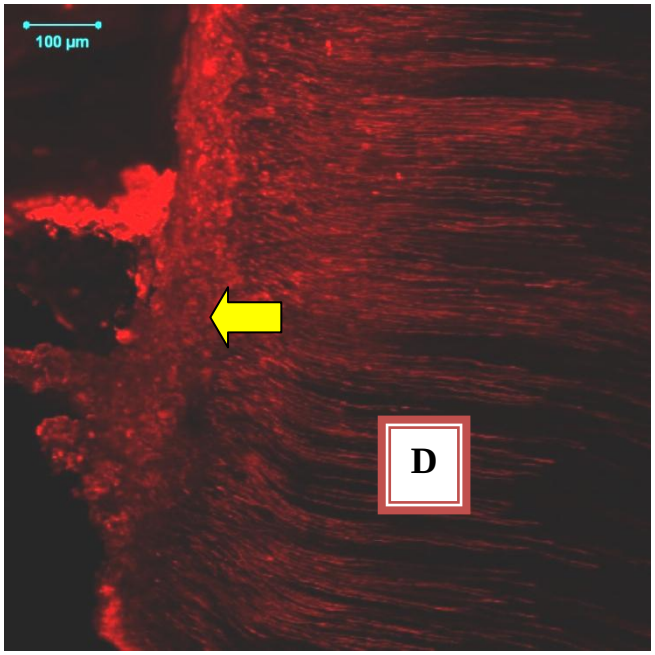
MINIMAL DYE LEAKAGE

GROUP V- GIC+ 30 wt% BIOACTIVE GLASS



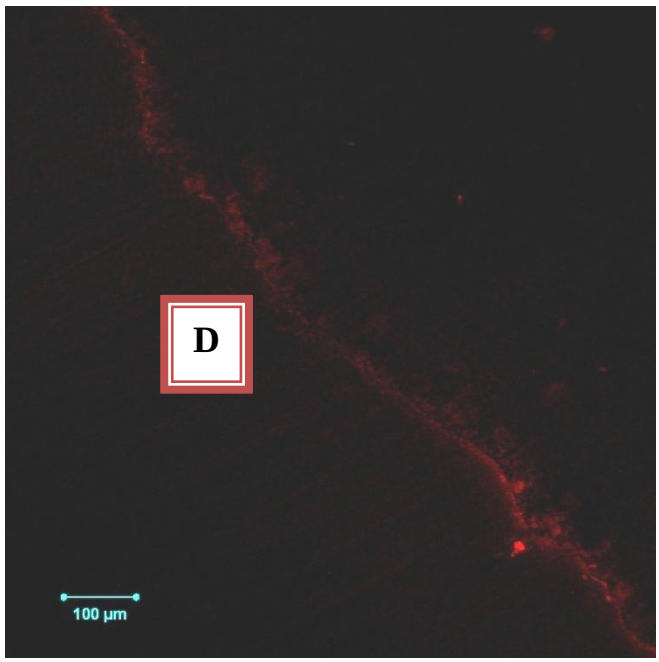
MINIMAL DYE LEAKAGE

GROUP VI –POSITIVE CONTROL

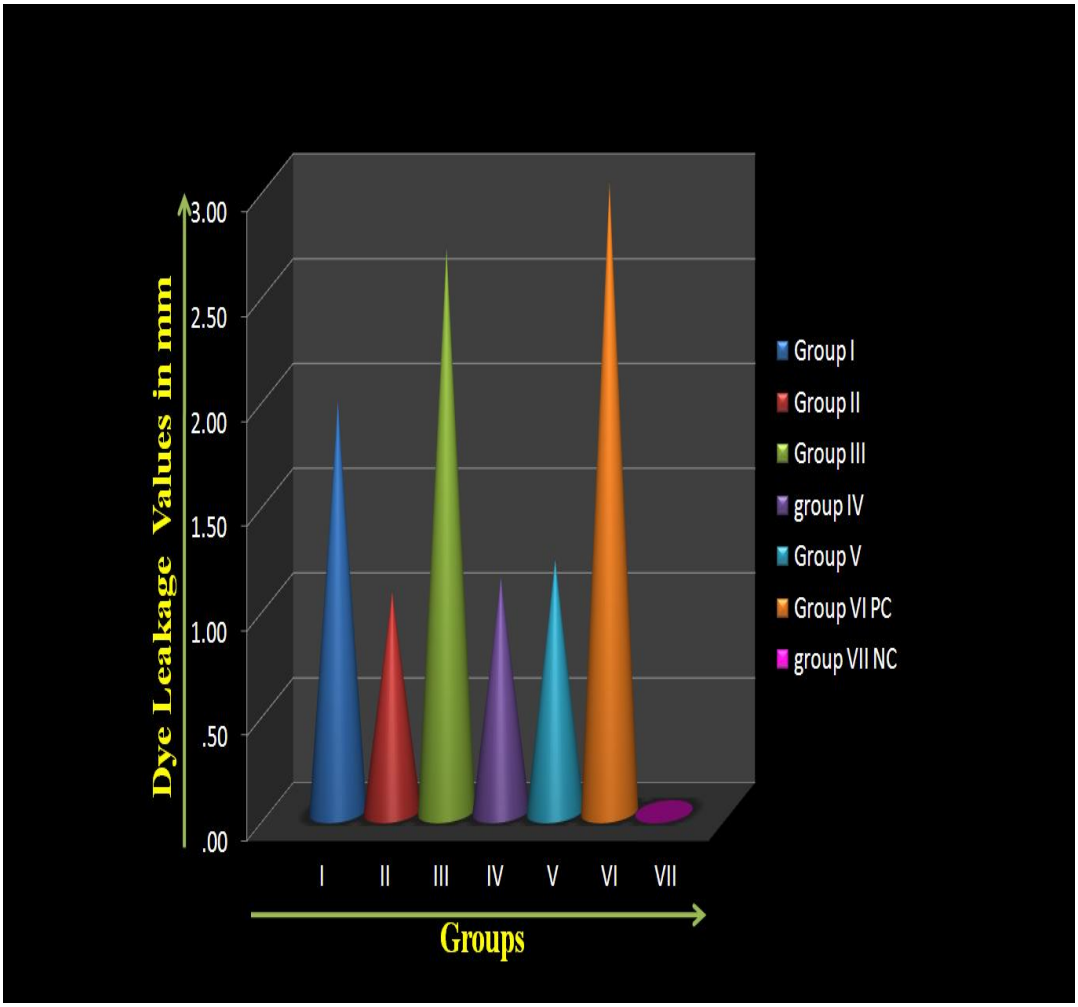


MASSIVE DYE LEAKAGE

GROUP VII-NEGATIVE CONTROL



NO DYE LEAKAGE



HISTOGRAM REPRESENTATION OF SEALING ABILITY (GRAPH 1)

(Group I-Conventional GIC, Group II –GIC + 10 v/v% Chitosan, Group III-GIC + 50 v/v % Chitosan, Group IV- GIC +10 wt % BAG, Group V- GIC + 30 wt % BAG, Group VI-Positve Control, Group VII- Negative control)

Analysis of mean values of the sealing ability at 0.05 level significance reveals

Positive control ≥ Group III > Group I > Group V ≥ Group IV ≥ Group II > negative control

OSTEOGENIC POTENTIAL

Table 4. Optical Density values for MTT assay

GROUPS	24 hrs	48 hrs	72hrs
I	0.853	0.821	0.751
	0.791	0.813	0.837
	0.837	0.856	0.973
II	2.510	2.791	3.134
	2.951	3.018	2.835
	2.862	2.681	2.721
III	2.816	3.015	3.018
	2.835	2.860	2.963
	2.770	2.781	2.799
IV	1.646	2.093	1.783
	1.687	1.759	2.333
	1.759	1.687	2.718
V	2.768	2.908	3.110
	2.843	2.704	2.800
	2.785	3.032	2.834
Control	1.582	0.878	0.947
	1.652	0.964	0.846
	1.497	0.854	0.828

The optical density values for MTT assay and Alkaline phosphatase activity expressed as μ moles of ρ-nitrophenol formed per min per microgram of protein were analysed using ONE WAY ANOVA and TUKEY HSD POST HOC multiple comparisons at 0.05 level significance.

Table 5. ONEWAY ANOVA FOR MTT ASSAY

Duration	Groups	Mean	Std. Deviation	F	Sig.
24 hrs	GROUP I	.82700	.032187	186.813	.000
	GROUP II	2.77433	.233204		
	GROUP III	2.80700	.033422		
	GROUP IV	1.69733	.057204		
	GROUP V	2.79867	.039323		
	CONTROL	1.57700	.077621		
	Total	2.08022	.790549		
48 hrs	GROUP I	.83000	.022869	143.197	.000
	GROUP II	2.83000	.171852		
	GROUP III	2.88533	.119039		
	GROUP IV	1.84633	.216632		
	GROUP V	2.88133	.165618		
	CONTROL	.89867	.057839		
	Total	2.02861	.932860		
72 hrs	GROUP I	.85367	.111935	56.156	.000
	GROUP II	2.89667	.213294		
	GROUP III	2.92667	.113931		
	GROUP IV	2.27800	.469920		
	GROUP V	2.91467	.170016		
	CONTROL	.87367	.064143		
	Total	2.12389	.965627		

Table 6. POST HOC TUKEY HSD TEST FOR MTT ASSAY

Multiple Comparisons for 24hrs

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Group I	.750000*	.086526	.000	.45937	1.04063
	Group II	-1.197333*	.086526	.000	-1.48797	-.90670
	Group III	-1.230000*	.086526	.000	-1.52063	-.93937
	Group IV	-.120333	.086526	.732	-.41097	.17030
	Group V	-1.221667*	.086526	.000	-1.51230	-.93103
Group I	Control	-.750000*	.086526	.000	-1.04063	-.45937
	Group II	-1.947333*	.086526	.000	-2.23797	-1.65670
	Group III	-1.980000*	.086526	.000	-2.27063	-1.68937

	Group IV	-.870333*	.086526	.000	-1.16097	-.57970
	Group V	-1.971667*	.086526	.000	-2.26230	-1.68103
Group II	Control	1.197333*	.086526	.000	.90670	1.48797
	Group I	1.947333*	.086526	.000	1.65670	2.23797
	Group III	-.032667	.086526	.999	-.32330	.25797
	Group IV	1.077000*	.086526	.000	.78637	1.36763
	Group V	-.024333	.086526	1.000	-.31497	.26630
Group III	Control	1.230000*	.086526	.000	.93937	1.52063
	Group I	1.980000*	.086526	.000	1.68937	2.27063
	Group II	.032667	.086526	.999	-.25797	.32330
	Group IV	1.109667*	.086526	.000	.81903	1.40030
	Group V	.008333	.086526	1.000	-.28230	.29897
Group IV	Control	.120333	.086526	.732	-.17030	.41097
	Group I	.870333*	.086526	.000	.57970	1.16097
	Group II	-1.077000*	.086526	.000	-1.36763	-.78637
	Group III	-1.109667*	.086526	.000	-1.40030	-.81903
	Group V	-1.101333*	.086526	.000	-1.39197	-.81070
Group V	Control	1.221667*	.086526	.000	.93103	1.51230
	Group I	1.971667*	.086526	.000	1.68103	2.26230
	Group II	.024333	.086526	1.000	-.26630	.31497
	Group III	-.008333	.086526	1.000	-.29897	.28230
	Group IV	1.101333*	.086526	.000	.81070	1.39197

* The mean difference is significant at the .05 level.

Table7. POST HOC TUKEY HSD TEST FOR MTT ASSAY

Multiple Comparisons for 48 hrs

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Group I	.068667	.116395	.990	-.32230	.45963
	Group II	-1.931333*	.116395	.000	-2.32230	-1.54037
	Group III	-1.986667*	.116395	.000	-2.37763	-1.59570
	Group IV	-.947667*	.116395	.000	-1.33863	-.55670
	Group V	-1.982667*	.116395	.000	-2.37363	-1.59170
Group I	Control	-.068667	.116395	.990	-.45963	.32230
	Group II	-2.000000*	.116395	.000	-2.39096	-1.60904
	Group III	-2.055333*	.116395	.000	-2.44630	-1.66437
	Group IV	-1.016333*	.116395	.000	-1.40730	-.62537
	Group V	-2.051333*	.116395	.000	-2.44230	-1.66037
Group II	Control	1.931333*	.116395	.000	1.54037	2.32230
	Group I	2.000000*	.116395	.000	1.60904	2.39096

	Group III	-.055333	.116395	.996	-.44630	.33563
	Group IV	.983667*	.116395	.000	.59270	1.37463
	Group V	-.051333	.116395	.997	-.44230	.33963
Group III	Control	1.986667*	.116395	.000	1.59570	2.37763
	Group I	2.055333*	.116395	.000	1.66437	2.44630
	Group II	.055333	.116395	.996	-.33563	.44630
	Group IV	1.039000*	.116395	.000	.64804	1.42996
	Group V	.004000	.116395	1.000	-.38696	.39496
Group IV	Control	.947667*	.116395	.000	.55670	1.33863
	Group I	1.016333*	.116395	.000	.62537	1.40730
	Group II	-.983667*	.116395	.000	-1.37463	-.59270
	Group III	-1.039000*	.116395	.000	-1.42996	-.64804
	Group V	-1.035000*	.116395	.000	-1.42596	-.64404
Group V	Control	1.982667*	.116395	.000	1.59170	2.37363
	Group I	2.051333*	.116395	.000	1.66037	2.44230
	Group II	.051333	.116395	.997	-.33963	.44230
	Group III	-.004000	.116395	1.000	-.39496	.38696
	Group IV	1.035000*	.116395	.000	.64404	1.42596

* The mean difference is significant at the .05 level.

Table8. POST HOC TUKEY HSD TEST FOR MTT ASSAY

Multiple Comparisons for 72 hrs

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Group I	.020000	.189985	1.000	-.61814	.65814
	Group II	-2.023000*	.189985	.000	-2.66114	-1.38486
	Group III	-2.053000*	.189985	.000	-2.69114	-1.41486
	Group IV	-1.404333*	.189985	.000	-2.04248	-.76619
	Group V	-2.041000*	.189985	.000	-2.67914	-1.40286
Group I	Control	-.020000	.189985	1.000	-.65814	.61814
	Group II	-2.043000*	.189985	.000	-2.68114	-1.40486
	Group III	-2.073000*	.189985	.000	-2.71114	-1.43486
	Group IV	-1.424333*	.189985	.000	-2.06248	-.78619
	Group V	-2.061000*	.189985	.000	-2.69914	-1.42286
Group II	Control	2.023000*	.189985	.000	1.38486	2.66114
	Group I	2.043000*	.189985	.000	1.40486	2.68114
	Group III	-.030000	.189985	1.000	-.66814	.60814
	Group IV	.618667	.189985	.059	-.01948	1.25681
	Group V	-.018000	.189985	1.000	-.65614	.62014
Group III	Control	2.053000*	.189985	.000	1.41486	2.69114
	Group I	2.073000*	.189985	.000	1.43486	2.71114

	Group II	.030000	.189985	1.000	-.60814	.66814
	Group IV	.648667*	.189985	.046	.01052	1.28681
	Group V	.012000	.189985	1.000	-.62614	.65014
Group IV	Control	1.404333*	.189985	.000	.76619	2.04248
	Group I	1.424333*	.189985	.000	.78619	2.06248
	Group II	-.618667	.189985	.059	-1.25681	.01948
	Group III	-.648667*	.189985	.046	-1.28681	-.01052
	Group V	-.636667	.189985	.051	-1.27481	.00148
Group V	Control	2.041000*	.189985	.000	1.40286	2.67914
	Group I	2.061000*	.189985	.000	1.42286	2.69914
	Group II	.018000	.189985	1.000	-.62014	.65614
	Group III	-.012000	.189985	1.000	-.65014	.62614
	Group IV	.636667	.189985	.051	-.00148	1.27481

* The mean difference is significant at the .05 level.

INTERPRETATION OF RESULTS FOR MTT ASSAY

Analysis of mean values at 0.05 level significance reveals

At 24hrs, 48 hrs and 72 hrs

- Groups II, III, IV,V were significantly greater than Group I ($p<0.05$).
- There was no significant difference between Group II, Group III and Group V.
- At 24 & 48 hrs, Group IV was significantly less than Group II, III and V ($p<0.05$)

At 24 hrs

- Group I was significantly less than control ($p<0.05$).
- There was no significant difference between Group IV and control

Group III ≥ Group V ≥ Group II > Group IV ≥ Control >Group I.

At 48 hrs

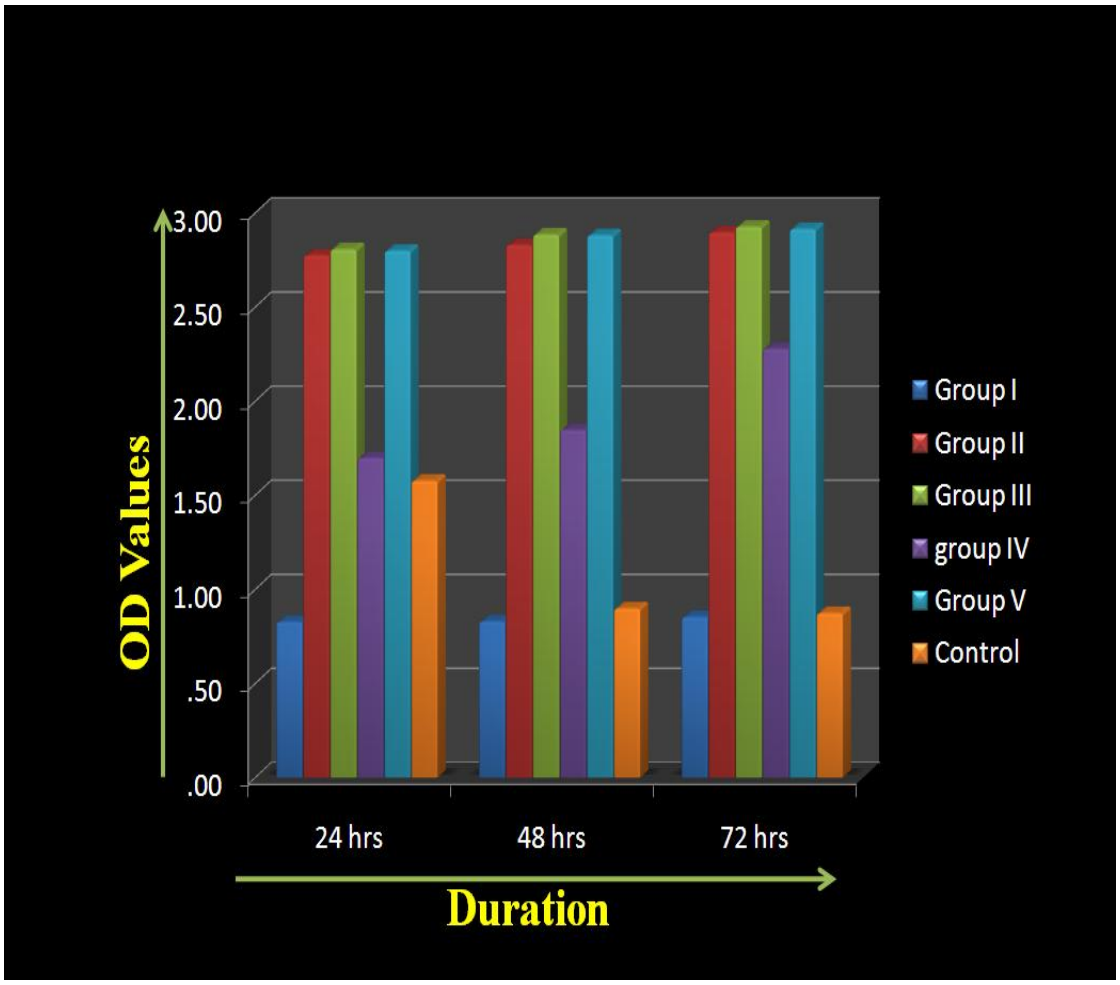
- There was no significant difference between Group I and control
- Group IV was significantly greater than control ($p<0.05$).

Group III ≥ Group V ≥ Group II > Group IV > Control ≥ Group I.

At 72 hrs

- There was no significant difference between Group IV,II & V.

Group III ≥ Group V ≥ Group II ≥ Group IV > Control ≥ Group I.



HISTOGRAM REPRESENTATION OF MTT ASSAY (GRAPH 2)

(Group I-Conventional GIC, Group II –GIC + 10 v/v% Chitosan, Group III-GIC + 50 v/v % Chitosan, Group IV- GIC +10 wt % BAG, Group V- GIC + 30 wt % BAG)

Analysis of mean values at 0.05 level significance reveals

<u>At 24 hrs</u>

Group III ≥ Group V ≥ Group II > Group IV ≥ Control >Group I.

<u>At 48 hrs</u>

Group III ≥ Group V ≥ Group II > Group IV > Control ≥ Group I.

<u>At 72 hrs</u>

Group III ≥ Group V ≥ Group II ≥ Group IV > Control ≥ Group I.

Table 9. Alkaline phosphatase values expressed as μ moles of ρ-nitrophenol formed per min per microgram of protein

Groups	7 days	14 days	21days
I	254.4	315.2	394.7
	241.4	345.3	383.0
	310.3	295.8	431.1
II	448.3	534.1	594.1
	388.6	594.8	580.3
	493.9	498.5	548.9
III	449.8	489.2	568.2
	397.6	544.6	582.0
	498.4	596.8	598.5
IV	385.6	556.6	566.2
	436.8	528.5	589.5
	445.2	496.1	537.4
V	431.5	536.5	584.6
	420.4	533.1	581.2
	483.1	560.8	564.7
Control	394.2	493.1	522.3
	386.5	399.8	451.9
	398.0	473.2	458.5

Table 10. ONEWAY ANOVA FOR ALKALINE PHOSPHATASE ASSAY

Duration	Groups	Mean	Std. Deviation	F	Sig.
7 DAYS	GROUP I	268.700	36.6083	9.751	.001
	GROUP II	443.600	52.8071		
	GROUP III	448.600	50.4107		
	GROUP IV	422.533	32.2598		

	GROUP V	445.000	33.4591		
	CONTROL	392.900	5.8592		
	Total	403.556	72.6631		
14 DAYS	GROUP I	455.37	49.14	15.378	.000
	GROUP II	318.77	24.94		
	GROUP III	542.47	48.69		
	GROUP IV	543.53	53.81		
	GROUP V	527.07	30.28		
	CONTROL	543.47	15.11		
	Total	488.44	90.73		
21 DAYS	GROUP I	402.933	25.0847	26.297	.000
	GROUP II	574.433	23.1641		
	GROUP III	582.900	15.1700		
	GROUP IV	564.367	26.0983		
	GROUP V	576.833	10.6444		
	CONTROL	477.567	38.8805		
	Total	529.839	72.1643		

Table11. POST HOC TUKEY HSD TEST FOR ALP
Multiple Comparisons -7 DAYS

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Group I	124.2000*	31.38388	.018	18.7840	229.6160
	Group II	-50.7000	31.38388	.605	-156.1160	54.7160
	Group III	-55.7000	31.38388	.514	-161.1160	49.7160
	Group IV	-29.6333	31.38388	.927	-135.0493	75.7826
	Group V	-52.1000	31.38388	.579	-157.5160	53.3160
Group I	Control	-124.2000*	31.38388	.018	-229.6160	-18.7840
	Group II	-174.9000*	31.38388	.001	-280.3160	-69.4840
	Group III	-179.9000*	31.38388	.001	-285.3160	-74.4840
	Group IV	-153.8333*	31.38388	.004	-259.2493	-48.4174
	Group V	-176.3000*	31.38388	.001	-281.7160	-70.8840
Group II	Control	50.7000	31.38388	.605	-54.7160	156.1160
	Group I	174.9000*	31.38388	.001	69.4840	280.3160
	Group III	-5.0000	31.38388	1.000	-110.4160	100.4160
	Group IV	21.0667	31.38388	.982	-84.3493	126.4826
	Group V	-1.4000	31.38388	1.000	-106.8160	104.0160
Group III	Control	55.7000	31.38388	.514	-49.7160	161.1160
	Group I	179.9000*	31.38388	.001	74.4840	285.3160

	Group II	5.0000	31.38388	1.000	-100.4160	110.4160
	Group IV	26.0667	31.38388	.956	-79.3493	131.4826
	Group V	3.6000	31.38388	1.000	-101.8160	109.0160
Group IV	Control	29.6333	31.38388	.927	-75.7826	135.0493
	Group I	153.8333*	31.38388	.004	48.4174	259.2493
	Group II	-21.0667	31.38388	.982	-126.4826	84.3493
	Group III	-26.0667	31.38388	.956	-131.4826	79.3493
	Group V	-22.4667	31.38388	.976	-127.8826	82.9493
Group V	Control	52.1000	31.38388	.579	-53.3160	157.5160
	Group I	176.3000*	31.38388	.001	70.8840	281.7160
	Group II	1.4000	31.38388	1.000	-104.0160	106.8160
	Group III	-3.6000	31.38388	1.000	-109.0160	101.8160
	Group IV	22.4667	31.38388	.976	-82.9493	127.8826

* The mean difference is significant at the .05 level.

Table 12. POST HOC TUKEY HSD TEST FOR ALP
Multiple Comparisons 14 DAYS

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Group I	136.6000*	32.40009	.012	27.7707	245.4293
	Group II	-87.1000	32.40009	.149	-195.9293	21.7293
	Group III	-88.1667	32.40009	.141	-196.9960	20.6627
	Group IV	-71.7000	32.40009	.299	-180.5293	37.1293
	Group V	-88.1000	32.40009	.142	-196.9293	20.7293
Group I	Control	-136.6000*	32.40009	.012	-245.4293	-27.7707
	Group II	-223.7000*	32.40009	.000	-332.5293	-114.8707
	Group III	-224.7667*	32.40009	.000	-333.5960	-115.9373
	Group IV	-208.3000*	32.40009	.000	-317.1293	-99.4707
	Group V	-224.7000*	32.40009	.000	-333.5293	-115.8707
Group II	Control	87.1000	32.40009	.149	-21.7293	195.9293
	Group I	223.7000*	32.40009	.000	114.8707	332.5293
	Group III	-1.0667	32.40009	1.000	-109.8960	107.7627
	Group IV	15.4000	32.40009	.996	-93.4293	124.2293
	Group V	-1.0000	32.40009	1.000	-109.8293	107.8293
Group III	Control	88.1667	32.40009	.141	-20.6627	196.9960
	Group I	224.7667*	32.40009	.000	115.9373	333.5960
	Group II	1.0667	32.40009	1.000	-107.7627	109.8960
	Group IV	16.4667	32.40009	.995	-92.3627	125.2960
	Group V	.0667	32.40009	1.000	-108.7627	108.8960
Group IV	Control	71.7000	32.40009	.299	-37.1293	180.5293

	Group I	208.3000*	32.40009	.000	99.4707	317.1293
	Group II	-15.4000	32.40009	.996	-124.2293	93.4293
	Group III	-16.4667	32.40009	.995	-125.2960	92.3627
	Group V	-16.4000	32.40009	.995	-125.2293	92.4293
Group V	Control	88.1000	32.40009	.142	-20.7293	196.9293
	Group I	224.7000*	32.40009	.000	115.8707	333.5293
	Group II	1.0000	32.40009	1.000	-107.8293	109.8293
	Group III	-.0667	32.40009	1.000	-108.8960	108.7627
	Group IV	16.4000	32.40009	.995	-92.4293	125.2293

* The mean difference is significant at the .05 level.

Table 13. POST HOC TUKEY HSD TEST FOR ALP
Multiple Comparisons 21 DAYS

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Group I	74.6333*	20.28154	.029	6.5093	142.7574
	Group II	-96.8667*	20.28154	.005	-164.9907	-28.7426
	Group III	-105.3333*	20.28154	.002	-173.4574	-37.2093
	Group IV	-86.8000*	20.28154	.011	-154.9241	-18.6759
	Group V	-99.2667*	20.28154	.004	-167.3907	-31.1426
Group I	Control	-74.6333*	20.28154	.029	-142.7574	-6.5093
	Group II	-171.5000*	20.28154	.000	-239.6241	-103.3759
	Group III	-179.9667*	20.28154	.000	-248.0907	-111.8426
	Group IV	-161.4333*	20.28154	.000	-229.5574	-93.3093
	Group V	-173.9000*	20.28154	.000	-242.0241	-105.7759
Group II	Control	96.8667*	20.28154	.005	28.7426	164.9907
	Group I	171.5000*	20.28154	.000	103.3759	239.6241
	Group III	-8.4667	20.28154	.998	-76.5907	59.6574
	Group IV	10.0667	20.28154	.995	-58.0574	78.1907
	Group V	-2.4000	20.28154	1.000	-70.5241	65.7241
Group III	Control	105.3333*	20.28154	.002	37.2093	173.4574
	Group I	179.9667*	20.28154	.000	111.8426	248.0907
	Group II	8.4667	20.28154	.998	-59.6574	76.5907
	Group IV	18.5333	20.28154	.936	-49.5907	86.6574
	Group V	6.0667	20.28154	1.000	-62.0574	74.1907

Group IV	Control	86.8000*	20.28154	.011	18.6759	154.9241
	Group I	161.4333*	20.28154	.000	93.3093	229.5574
	Group II	-10.0667	20.28154	.995	-78.1907	58.0574
	Group III	-18.5333	20.28154	.936	-86.6574	49.5907
	Group V	-12.4667	20.28154	.988	-80.5907	55.6574
Group V	Control	99.2667*	20.28154	.004	31.1426	167.3907
	Group I	173.9000*	20.28154	.000	105.7759	242.0241
	Group II	2.4000	20.28154	1.000	-65.7241	70.5241
	Group III	-6.0667	20.28154	1.000	-74.1907	62.0574
	Group IV	12.4667	20.28154	.988	-55.6574	80.5907

* The mean difference is significant at the .05 level.

INTERPRETATION OF RESULTS FOR ALP ASSAY

At 7days, 14 days and 21 days

- Group I was significantly less than control ($p<0.05$).
- Groups II,III,IV,V were significantly greater than Group I ($p<0.05$).

At 7 days and 14 days

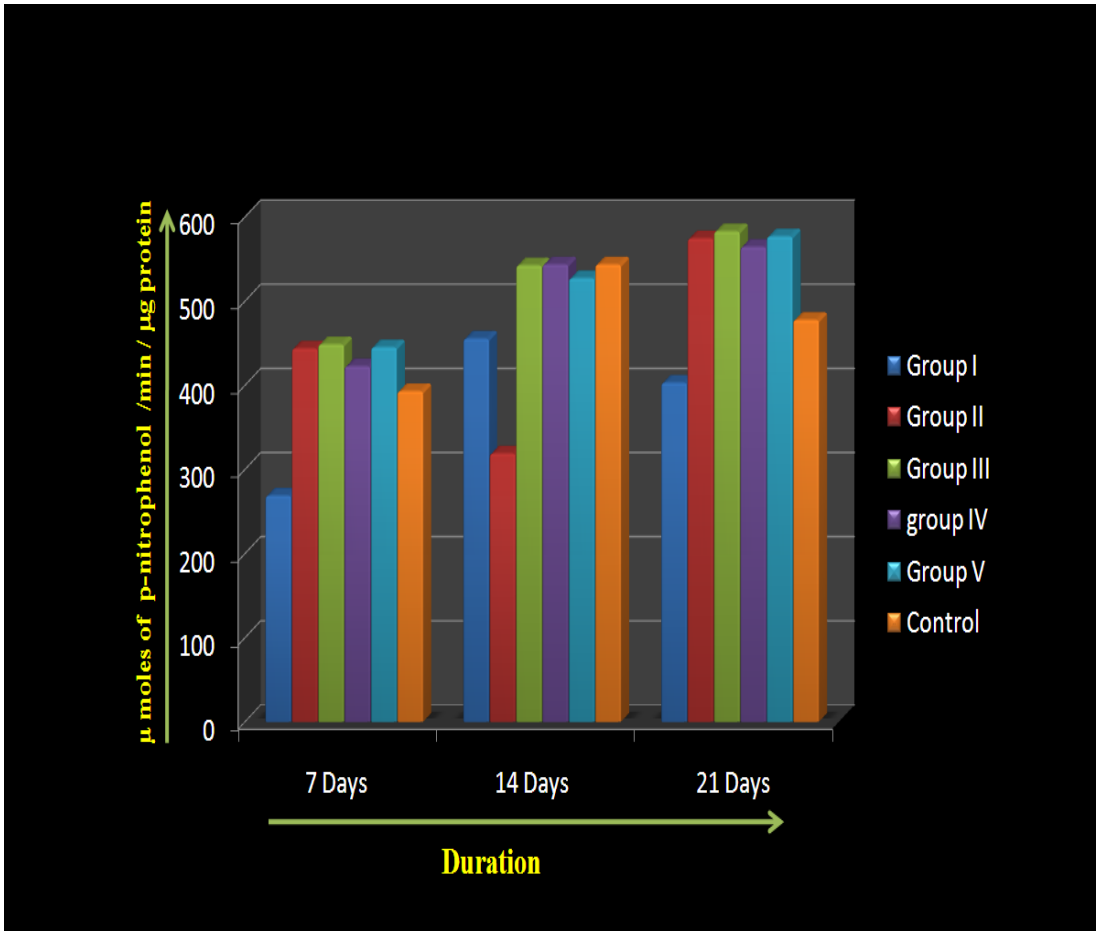
- There was no significant difference between Control, Group II, Group III, Group IV and Group V.

Group III ≥ Group V ≥ Group II ≥ Group IV ≥ Control >Group I.

At 21 days

- There was no significant difference between Group II, Group III, Group IV and Group V.
- Group II, Group III, Group IV and Group V significantly greater than control ($p<0.05$).

Group III ≥ Group V ≥ Group II ≥ Group IV > Control > Group I



HISTOGRAM REPRESENTATION OF ALP ASSAY (GRAPH 3)

(Group I-Conventional GIC, Group II –GIC + 10 v/v% Chitosan, Group III-GIC + 50 v/v % Chitosan, Group IV- GIC +10 wt % BAG, Group V- GIC + 30 wt % BAG)

Analysis of mean values at 0.05 level significance reveals

<u>At 7 days and 14 days</u>

Group III ≥ Group V ≥ Group II ≥ Group IV ≥ Control >Group I.

<u>At 21 days</u>

Group III ≥ Group V ≥ Group II ≥ Group IV > Control > Group I

DISCUSSION

Periradicular surgery involves surgical debridement of pathological periradicular tissue, apical root-end resection, root-end cavity preparation and the placement of a root-end filling in an attempt to seal the root canal. The ideal healing response after periradicular surgery is the re-establishment of an apical attachment apparatus and osseous repair.[19]

The basic properties of GICs, such as adhesion, antimicrobial effects, ease of application and biocompatibility make them suitable for use during endodontic surgery. A number of in vitro and in vivo studies have compared GIC with Amalgam, EBA, MTA to be used as root end filling material[18,39,72,76]. It would definitely be an advantage if glass ionomer cement could possess bioactivity because currently, there is a trend for the development of biomaterials that have therapeutic or biologic functions in addition to their inherent properties.

Chitosan(CH) is a biocompatible, natural biopolymer that is a copolymer of glucosamine and N-acetylglucosamine derived from chitin. Chitosan nanoparticle has antibacterial property, which has been suggested for root canal disinfection [44]. Studies are being carried out considering the importance of the biomaterials containing either CH or CH derivatives for the regeneration of damaged bone, cartilage, and nerve tissue[30].

Denise F.S.Petri et al.[24] (2007) found that CH chains carry many hydroxyl groups and acetamide groups, which are able to bind to the hydroxyl groups and to Polyacrylic acid carboxylic groups of GIC by hydrogen bonding. The addition of 10 v/v% CH led to a significant increase in the flexural resistance and contents higher

than 25v/v% (50v/v% & 100v/v%) led to a poor performance and in the presence of chitosan, the release of fluoride ions from glass ionomer restoratives was catalyzed.

Bioactive glasses (BAG) are surface-active glasses with which bone minerals are able to bond chemically. The bioactive nature of BAG and glass-ceramics is related to their ability to form a bone-like apatite layer on their surfaces in the body environment[37].**Waltimo et al.** [89] showed that BAG nanoparticulate 45S5 was able to kill 99% of *E.faecalis* when incubated in simulated body fluid for 100 minutes.

Matsuya S et al. [51](1999) prepared a new glass ionomer cement using bioactive glass and investigated its setting process using Fourier Transform Infra Red Spectroscopy (FT-IR) and Mass Spectrometry Nuclear Magnetic Resonance(MAS NMR) and found that the setting mechanism of the cement was essentially the same as in conventional glass ionomer cement. **Helena Yli-Urpo et al.[36] (2005)** found that addition of BAG to GIC compromises the mechanical properties of the materials to some extent when added in proportions of 10-30 wt %.

Hench et al.[37](1972) defined bioactive material as "one that elicits a specific biological response at the interface of the material that results in the formation of a bond between the tissues and the material".

This study attempted to enrich GIC with bioactivity by addition of CH and BAG. Earlier studies analysed the mechanical properties on modifying GIC with Chitosan and Bioactive glass. This study is the first attempt to analyse the **sealing ability and osteogenic potential** to be used as a root end filling material. A comparative analysis of

the above mentioned properties by addition of 10v/v% and 50v/v% of chitosan and 10wt% and 30 wt% bioactive glass (concentrations as suggested by earlier studies) to conventional Glass ionomer cement was made.

SEALING ABILITY

Fluorescent dye is one of the dyes used for microleakage test. **D'Alpino et al.[21](2006)** stated that fluorescent dyes are useful as tracers because they are detectable in dilute concentrations, inexpensive, and non-toxic, allowing use in clinical as well as laboratory investigations . Rhodamine B fluoresces when excited by red light of a particular wavelength (546 nm).[41]

Watson (1997) [94] stated that that microleakage studies performed with fluorescent dyes and examined using confocal microscopy may provide a more accurate description of restorative failure. Earlier studies that used fluorescing agents did not use confocal microscopy to test for microleakage. Instead, normal, low-resolution optical microscopy was used.

As shown by previous studies, the use of confocal laser scanning microscope (CLSM) has shown to be a simple method to test the adaptation of dental materials to the dentin [62,65,92,93,94]. The use of non-decalcified or hard tissue samples that does not require a specific section technique is a clear advantage of confocal analysis [92]. In spite SEM being used extensively for analysis of dental material interfaces, the preparation of the specimens, as the sputter-coating procedure, may increase the possibility of artifacts. CLSM has added advantage of the ability to control depth of field, elimination or reduction of background information away from the focal plane, capability to collect

serial optical sections from thick specimens with significant improvement in resolution and the microleakage testing rely on the fluorescence criteria of the dye rather than the color.

For all these reasons a dye leakage study using 0.5% Rhodamine B dye using Confocal Laser Scanning microscopy was utilized to detect microleakage in this study.

A controversial topic in the literature is the moment at which the specimens should be immersed in the dye solution[66]. In the present study immediate immersion in dye by passive dye penetration technique at 37 ^{0} C and 100% humidity for 24 hrs was selected based on the fact that in clinical situation the root end filling material will be in contact with secretions like blood, soon after the insertion in the cavities.

Apical ramifications and lateral canals are very common near the root tip and the preferred depth of root resection is 3mm[74]. Root resection at the depth of 3mm reduces apical ramifications by 98% and lateral canals by 93%.

Apical root resections at 90^{o} to the long axis of the tooth has been approved to be most acceptable by earlier studies. The inclined plane sectioning at 30^{o} or 40^{o} could have disadvantages like open dentinal tubules, errors in post operative radiographs, more mechanical stress and loss of dentin, cementum and bone could result in compromised healing[74]. The plane of sectioning also affects the degree of microleakage, so a root resection angle of 90^{0} was selected for this study.

Plain fissure burs, both high- and low speed, produced the smoothest resected root surface, with, plain fissure burs and a low-speed handpiece resulting in the least gutta-

percha distortion[58]. In this study root end resection was done with #701 fissure bur in a high-speed hand piece and water coolant.

Ultrasonic root-end cavity instrumentation produces conservative, smooth, nearly parallel walled preparations that followed the direction of root canal more closely and have been reported to be contaminated with less debris and smear layer than those prepared using a bur [32,95,45,28]. A success rate of 92.4% was reported in a study that evaluated the success/ failure rate of periradicular surgeries performed on 157 teeth involving root-end cavity preparations using ultrasonic instrumentation and was concluded that ultrasonic root-end preparation provides excellent clinical results[79]. In this study, root end cavity of 3mm depth was prepared with S12-90ND ultrasonic tip under medium power settings as recommended by the manufacturer.

The results showed that the positive control samples showed dye leakage throughout the length of the canals, while the negative control samples had no dye penetration.

There was significant leakage in all groups when compared with Group VII (negative control) ($p<0.05$). Group II (GIC +10 v/v% CS), Group IV (GIC +10 wt% BAG), GroupV (GIC+30 wt% BAG) showed less linear dye penetration when compared with Group I (Conventional GIC) which was statistically significant ($p<0.05$). But there was no significant difference in leakage among Group II, Group IV and Group V.

Group III (GIC +50 v/v% CH) significantly showed more leakage than Group I (Conventional GIC).This was in accordance with the study done by

Denise F.S.Petri et al.[24] (2007) who found that the addition of 10 v/v% of CH led to a significant increase in the flexural resistance and CH contents higher than 25v/v% (50 v/v% & 100 v/v%) led to poor performance. This effect can be explained considering that some CH chains segregate, interacting with each other, and no longer with Polyacrylic acid (PAA) or the particle surface. So a 50 v/v % CH addition to GIC could have considerably affected the original sealing ability of GIC resulting in more leakage.

Group II (GIC +10 v/v% CH) showed less leakage than Group I (Conventional GIC).The probable reason could be increased crosslinking network formed by CH and Polyacrylic acid around the inorganic particles that might reduce the interfacial tension among the GIC components, improving mechanical performance[24].

Also this study showed decreased linear dye penetration in Group IV (GIC+10wt% BAG) and GroupV (GIC+30wt% BAG) than Group I (Conventional GIC). This was not in accordance with earlier studies[36,67] which stated that addition of BAG to GIC decreases the compressive strength (CS), modulus of elasticity and surface microhardness because the BAG particles might be only loosely attached to the GIC matrix and BAG particles probably acted as fillers that had not been adhered into the matrix of GIC leading to decreased CS and modulus of elasticity.

Matsuya et al.[51] (1999) stated that calcium was released from the bioactive glass to form carboxylate salt and the degree of polymerization in the silicate network increased. This could be the reason for improved sealing ability with BAG (10 wt % and 30 wt %) modified GIC obtained in our study.

OSTEOGENIC POTENTIAL

Osteogenic potential can be evaluated by invivo or invitro tests. An in vitro comparison using human osteoblasts could elicit more consistent, clear results and allow more detailed information than in vivo experiments[98]. In this study, therefore, we evaluated the cell proliferation and differentiation using human osteoblasts on the surface of set GIC samples.

SaOS-2 cell line was selected as they are characterized by their homogeneity, unlimited number of cells, cytokine and growth factor expression profile similar to human osteoblast cells, sensitivity to hormonal administration, matrix mineralization, no interspecies difference[20].

MTT ASSAY

Cell proliferation was assessed using **MTT assay**. The MTT assay is dependent on the intact activity of the mitochondrial enzyme, succinate dehydrogenase, which is impaired after exposure of cells to toxic surroundings. The test involves the conversion of a tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to an insoluble formazan product, which can be quantified by UV spectrophotometry[56].To assess immediate and late toxic effects of the test materials on cell viability the MTT assay was carried out at 24hrs, 48hrs and 72hrs.

The results showed increased proliferative activity than control in all groups except Group I (Conventional GIC) at the end of 24 hrs. After 48 hrs & 72 hrs, Group I was not significantly different from control. This was in accordance with many previous

in vitro evaluations of GIC biocompatibility.[9,25,73] . This could be explained by the fact that the in vitro toxicity of GICs was due to a complex mechanism based on both ion release in particular, Aluminium and Fluoride ions and pH effects. **Devlin et al. [25](1998)** stated that aluminum ions contributed to the in vitro toxicity of Ionomeric cements. **Oliva et al.[61] (1996)** stated the polyacid component (e-g. acrylic, maleic, tartaric and benzoic acids) can also create a decreased pH in the immediate environment, further contributing to a cytotoxic response. There was statistically significant increase in proliferative activity in Group II, III, IV and V than Group I (Conventional GIC) at 24, 48 & 72 hrs ($p<0.05$). GROUP II (GIC + 10 v/v% CH), Group III (GIC +50v/v% CH) and Group V (GIC + 30wt % BAG) showed higher proliferative activity than all groups with no significant difference between them.

The increased cell proliferative activity in GROUP II (GIC + 10 v/v% CH), Group III (GIC +50v/v% CH) was in accordance with the following studies. **Shi SF et al.[77] (2012)** showed Chitosan-coated iron oxide nanoparticles enhanced osteoblast proliferation, decreased cell membrane damage, and promoted cell differentiation, as indicated by an increase in alkaline phosphatase and extracellular calcium deposition. **Pawlowska E et al.[63] (2010)** showed that chitosan can reduce the percentages of DNA damage caused by 2-hydroxyethyl methacrylate. **Limapornvanich A et al.[47] (2009)** discovered a novel chitosan - fluoroaluminosilicate GIC that can prolong the release of Bovine serum Albumin (BSA) without alteration of its molecular weight, and this cement did not increase toxicity to pulp cells.

The higher proliferative activity of Group IV (GIC +10wt% BAG) and GroupV (GIC +30wt % BAG) than Group I (Conventional GIC) could be explained by the fact that Bioglass has the ability to stimulate cell cycling and subsequently enhance osteoblastic turnover of human primary osteoblasts in vitro as reported by **Xynos et al.[97] (2000)**. Also this was in accordance with the studies made by **Bielby et al. [5](2004)** and **Foppiano et al. [29] (2004)** who stated that bioactive glass extracts in cell culture medium have been shown to increase osteoblast cell proliferation. At the end of 24hrs, the proliferative activity of Group IV was not significantly different from control. But at 48 hrs and 72 hrs Group IV showed higher proliferative activity than control. The probable reason could be less amount of BAG (10 wt %) added to GIC. With time, the reactivity of BAG increased resulting in release of Calcium and Phosphate ions on the surface that lead to increased osteoblastic proliferation.

ALKALINE PHOSPHATASE ASSAY

Alkaline phosphatase is an enzyme participating in bone tissue mineralization and an important marker of osteogenic cell differentiation[70]. ALP activity was tested at 7,14,21 days to assess long term bioactivity of the test materials.

The results showed increased ALP activity in all groups over a period of 7, 14 and 21 days. Throughout the observation period GroupII , Group III , Group IV, Group V showed higher ALP activity than Group I (Conventional GIC) and it was statistically significant ($p<0.05$). At the end of 21days, Group II, Group III, Group IV and Group V showed significantly greater ALP activity than control ($p<0.05$).

The increased ALP activity as observed in GROUP II (GIC + 10 v/v% CH), Group III (GIC +50v/v% CH) was in accordance with the following studies. Chitosan (CH) and hydroxyapatite are among the best bioactive biomaterials in bone tissue engineering and renowned for their excellent biocompatibility with the human body environment[90]. **Mathews S et al. [50](2011)** stated that Chitosan upregulated genes associated with calcium binding and mineralization such as, collagen type 1 alpha 1, integrin-binding sialoprotein, osteopontin, osteonectin and osteocalcin, significantly. **Lee SK et al. [46](2010)** found that the addition of chitosan to calcium phosphate cement can increase compressive strength of the cement as well as promote odontoblastic differentiation in human dental pulp cells. **Nitra Rakkiettiwong et al.[60] (2011)** devised a BIO-GIC(chitosan-fluoroaluminosilicate GIC with Albumin) with potential for the retained effect of added TGF-beta1, which was longer than the conventional GIC for its applications in regenerative endodontics or for use as vital pulp therapy material, which could promote repair of the dentin pulp complex. All these studies explains the increased cell differentiation potential of chitosan.

The greater alkaline phosphatase activity of Group IV (GIC +10 wt% BAG) and GroupV (GIC +30wt % BAG) was in accordance with the following studies. **Bosetti and Cannas[6] (2005)** and **Lossdorfer et al. [48](2004)** have showed that bioactive glass extracts in cell culture medium induce osteogenic differentiation and mineralization. Bioactive glass surface reaction is initiated after contact with body fluids and subsequently, rapid ion exchange of Na^+ and K^+ from the bioactive glass with H^+ and H_3O^+ from the extracellular fluids and the network structure dissolutes, Na^+, Ca^{2+}, Mg^{2+}, P^{5+} and Si^{4+}

leach, and Ca^{2+} and PO_4^{3+} precipitate from the extracellular fluids onto the Si-rich layer[85]. Thus Bioactive glasses act as a template for osteoblast differentiation of bone marrow stromal cells that could be encouraged by ion exchange at the surface of the bioactive glass. This explains increased osteogenic potential with addition of BAG (10wt % and 30 wt %) to GIC as found in our study.

Considering the mechanical properties on modifying GIC with Chitosan, a study was conducted in our department which revealed increased mean flexural strength, microshear bond strength for 10 v/v% Chitosan modified GIC than conventional GIC. While 50 v/v % Chitosan modified GIC showed increased fluoride release but poor mechanical properties.

Correlating the results obtained on testing mechanical properties, sealing ability and bioactivity,10 v/v % Chitosan modified GIC exhibited improved mean flexural strength, microshear bond strength, sealing ability and bioactivity so that it could be suggested for use as a restorative material and also as a root end filling material.

5 0 v/v % Chitosan modified GIC had compromised mechanical properties and sealing ability but excellent osteogenic potential. So further studies need to be conducted to utilize its bioactivity.

As reported by previous studies[36,67], addition of Bioactive Glass compromised the mechanical properties of GIC to some extent. In our study, improved sealing ability and osteogenic potential was obtained on modifying GIC with 10wt % and 30 wt% Bioactive Glass. This suggests the usage of GIC modified with 10 wt % or 30 wt %

Bioactive Glass as a root end filling where increased mechanical strength is not among the ideal requisite.

Within the limitations of this study it can be concluded that GIC modified with 10 v/v % of Chitosan and GIC with 10 wt % or 30 wt % Bioactive Glass has improved sealing ability as well as osteogenic potential when compared with conventional GIC to be used as a root end filling material. Further long term and in vivo studies and studies regarding regenerative potential could be conducted to confirm the beneficial effect of adding these bioactive materials.

SUMMARY

The aim of this study was to evaluate the sealing ability and osteogenic potential of Glass ionomer cement modified with Chitosan and Bioactive glass and to compare it with conventional Glass ionomer cement.

Chitosan solution was prepared and mixed with Glass ionomer liquid in concentration of 10 v/v% and 50 v/v % to produce Chitosan modified GIC. Similarly Bioactive glass was added to Glass ionomer powder in concentration of 10 wt % and 30 wt % to produce Bioactive Glass modified GIC.

The experimental groups considered were

Group I - Conventional Glass ionomer cement

Group II - Glass ionomer cement containing 10 v/v% Chitosan

Group III - Glass ionomer cement containing 50 v/v% Chitosan

Group IV - Glass ionomer cement containing 10 wt% Bioactive Glass

Group V - Glass ionomer cement containing 30 wt% Bioactive Glass

SEALING ABILITY

Dye leakage using 0.5% Rhodamine B dye and Confocal Laser Scanning microscopy was utilized to assess the sealing ability. Sixty freshly extracted human maxillary central incisors were instrumented and obturated with gutta-percha using lateral condensation technique & access cavities were sealed with composite. For 55 teeth samples, 3 mm apical root resections at 90 degrees to the long axis of the tooth was done and a 3 mm deep retrograde cavity was prepared with an ultrasonic tip, irrigated & dried. Five instrumented roots with retro-preparations received no retrograde filling, and these

were used as positive controls. Another five roots were instrumented and obturated with gutta-percha and sealer without retro preparation and their entire root surfaces were covered with two coats of nail polish and were used as negative controls. The teeth were divided into following groups:

Group I - Conventional GIC (n=10)

Group II - GIC + 10 v/v% Chitosan (n=10)

Group III - GIC + 50 v/v% Chitosan (n=10)

Group IV - GIC + 10 wt% Bioactive Glass (n=10)

Group V - GIC + 30 wt% Bioactive Glass (n=10)

Group VI - Positive control (n=5)

Group VII - Negative control (n=5)

The cavities were filled and specimens were stored in moist cotton at room temperature. Samples were coated with three coats of nail varnish except at the apical 1 mm of the resected root, and then were allowed to dry. All the specimens were suspended in 0.5% Rhodamine B dye for 24 hours and rinsed for 1 hr under tap water. The teeth were mounted in acrylic blocks and split longitudinally with a hard tissue microtome using a water coolant and the specimens were examined under confocal laser scanning microscope at 10X magnification and microleakage was evaluated in millimeters.

OSTEOGENIC POTENTIAL

MTT ASSAY

The SaOS-2 cells were plated in 24 well plates at a concentration of 3 x 10^4 cells/well. Round-shaped samples measuring 2mm thick and 5mm in diameter (9 for every experimental material) were prepared and rinsed three times with (PBS) and α-MEM medium & placed into 24-well plates for 24, 48 and 72 hrs and incubated at 37º C. The medium was discarded and 100 µl of MTT containing DMEM medium was added to each well and incubated for 3 hours. The MTT containing medium was then discarded, washed with PBS (200 µl) and the crystals were then dissolved by adding 1 ml of DMSO. The optical density values were measured in an ELISA reader at 545 nm.

ALKALINE PHOSPHATASE ASSAY

For Alkaline phosphatase assay, the test material discs were prepared & divided into 5 groups of 9 wells each as mentioned for MTT assay. 2×10^4 SaOS-2 cells were seeded on test material discs in osteogenic medium. Cell lysate was obtained & ALP activity was determined by using p-nitrophenyl phosphate as the substrate. The reaction was stopped by the addition of 1 N NaOH to reaction mixture and the absorbance at 405 nm (OD value) was measured using auto analyser and alkaline phosphatase activity was expressed as µ moles of ρ-nitrophenol / min / µg protein.

Data were recorded and statistically analyzed using ONE WAY ANOVA and TUKEY HSD POST HOC multiple comparisons.

Considering sealing ability, Group II (GIC +10 v/v% CH), Group IV (GIC +10 wt% BAG), GroupV (GIC +30wt % BAG) showed less linear dye penetration when compared with Group I (Conventional GIC). Group III (GIC +50 v/v% CH) showed more leakage than Group I.

Cell culture studies showed significant increase in proliferative activity in Group II, III, IV and V than Group I (Conventional GIC) at 24, 48 & 72 hrs. Also, there was increased ALP activity in Group II, III, IV and V than Group I (Conventional GIC) at 7, 14 and 21 days of evaluation.

CONCLUSION

From the results of this study, the following conclusions can be arrived-

- ✓ GIC modified with 10 v/v % of Chitosan and GIC with 10 wt % or 30 wt % Bioactive Glass has improved sealing ability as well as osteogenic potential when compared with conventional GIC to be used as a root end filling material.
- ✓ 50 v/v % Chitosan modified GIC had compromised sealing ability but excellent osteogenic potential.
- ✓ Further long term and in vivo studies and studies regarding regenerative potential could be conducted to confirm the beneficial effect of adding these bioactive materials in GIC.

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