COMPARATIVE EVALUATION OF SEALING ABILITY AND CYTOTOXICITY OF BONE CEMENT, MTA AND BIODENTINE AS RETRO FILLING MATERIAL -AN IN VITRO STUDY

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CONSERVATIVE DENTISTRY AND ENDODONTICS

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled

"COMPARATIVE EVALUATION OF SEALING ABILITY AND CYTOTOXICITY OF BONE CEMENT, MTA AND BIODENTINE AS RETRO FILLING MATERIAL -AN IN VITRO STUDY"

is a bonafide and genuine research work carried out by me under the guidance of **Dr.K.AMUDHALAKSHMI, Associate Professor,** Department Of Conservative Dentistry and Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai-600003.

DR. KRISHNA P. BISWAS

CERTIFICATE BY GUIDE

This is to certify that **Dr. KRISHNA P. BISWAS,** Post Graduate student (2014-2017) in the Department of Conservative Dentistry and Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai- 600003 has done this dissertation titled **"COMPARATIVE EVALUATION OF SEALING ABILITY AND CYTOTOXICITY OF BONE CEMENT, MTA AND BIODENTINE AS RETRO FILLING MATERIAL -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 Chennai -600032, for M.D.S., Conservative Dentistry and Endodontics (Branch IV) Degree Examination .

Dr.K.AMUDHALAKSHMI , M.D.S.
Associate Professor & Guide
Department of Conservative Dentistry and Endodontics.
Tamil Nadu Government Dental College and Hospital
Chennai- 600003

ENDORSEMENT BY HEAD OF THE DEPARTMENT / HEAD OF THE INSTITUTION

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Dr. M. KAVITHA, M.D.S.
Professor & HOD,
Dept. of Conservative Dentistry & Endodontics

Dr. B. SARAVANAN,M.D.S,PhD
Principal

Tamil Nadu Government Dental College and Hospital.
Chennai- 600003.

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NAME OF THE GUIDE	DR. K.AMUDHALAKSHMI
HEAD OF THE DEPARTMENT	DR. M. KAVITHA

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And

MR. DR. KRISHNA P. BISWAS aged 28 years currently studying as **Post Graduate student** in Department of Conservative Dentistry & Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai 3 (herein after referred to as the PG student and coinvestigator').

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1.

2

ABSTRACT

AIM:

The aim of this study was to comparatively evaluate the sealing ability and cytotoxicity of Bone cement, MTA and Biodentine as retro filling material.

MATERIALS AND METHODS:

SEALING ABILITY -

Fifty sound maxillary central incisors were chemico-mechanically prepared and obturated. Three millimeters of root end were resected and 3 mm retro cavity preparation was done using ultrasonic retro tips. The samples were divided into five groups of ten specimens each; Group A-Bone Cement, Group B-MTA and Group C-Biodentine, Group D- Positive Control, Group E- Negative Control. After retrofilling, the teeth were stored in humidifier and later coated with nail varnish except at apical 1 mm. After drying the specimens, they were immersed in 0.5% Rhodamine –B dye for 48 hours. The teeth were rinsed under water for 5 minutes and sectioned longitudinally. All the samples were evaluated under LSM 510 Meta confocal microscope for determining the dye penetration in micrometers.

CYTOTOXICITY-

Bone cement, MTA, Biodentine was evaluated for cytotoxicity by preparing their extracts and incubated at 37°C under control humidified atmosphere in an incubator for 24 hours till they set. The set materials were immersed in Dulbecco Modified Eagle culture medium for 24 hours. L929 mouse fibroblasts cultured in Dulbecco Modified Eagle medium were used as control group. The extracts of test materials were then separated and tested in culture wells in close proximity to growing cell culture and incubated for 24 hours. Cytotoxicity was estimated by MTT assay where the optical density was absorbed at 540 nm and evaluated under inverted phase contrast microscope. All statistical analysis was done using SPSS version 16 using Anova and Post-hoc test.

RESULTS- Evaluation of sealing ability revealed that the mean micro leakage was significantly higher in MTA followed by Bone cement and least with Biodentine.

Evaluation of cytotoxicity of three different cements revealed that the cell viability of Biodentine was greater than Bone cement followed by MTA.

CONCLUSION- Bone cement provided an excellent seal and biocompatibility and at the same time it provided comfortable handling properties, which could overcome potential disadvantages as faced with MTA. Thus promising it a good retrograde cement which can be used in future. However Biodentine remains to be the best in terms of both sealing ability and biocompatibility when compared with other two cements.

KEY WORDS: Bone cement, MTA, Biodentine, Sealing ability, Cytotoxicity, Confocal microscope.

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ABBREVIATIONS

MTA	MINERAL TRIOXIDE AGGREGATE
PMMA	POLY METHYL METHACRYLATE
GIC	GLASS IONOMER CEMENT
MTT	THIAZOLYL TETRAZOLIUM BROMIDE
NEC	NEW ENDODONTIC CEMENT
IRM	INTERMEDIATE RESTORATIVE MATERIAL
CLSM	CONFOCAL LASER SCANNING MICROSCOPY
SEM	SCANNING ELECTRON MICROSCOPE

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Introduction

INTRODUCTION

For successful endodontic therapy it is essential to have complete 3-Dimensional obturation of root canal system with fluid tight seal[54]. Several retrospective studies have evaluated the outcome of conventional non-surgical root canal therapy and success rates reported have ranged from 53% to 93%[47]. Also certain number of failure is evident due to persistence of bacteria and their by-products in root canal system[40]. In order to save the tooth sometimes it is necessary to intervene with surgical endodontics when orthograde treatment is not possible (eg. Non-negotiable ledges, instrument separation, calcific metamorphosis, failure of retreatment)[14,48]. Surgical endodontics includes exposure of root apex, root end resection, root end cavity preparation followed by filling the cavity by retrograde material[80]. The goal of endodontic therapy is successful regeneration of periodontal attachment which is functional and this will occur only when we select the retrograde material such that it not only seals the canal which will stop the egress of bacteria and their derivatives [65, 23, 78] but also forms healthy periodontium.

An ideal retrograde filling material should provide fluid tight seal in root canal system. It should also have properties like non- absorbable, radiopaque, biocompatibility, induce periapical healing, dimensionally stable, easy to use and it should not be affected by moisture[5] .Several materials have been used for this

purpose in endodontic surgery such as gold foil, Gutta percha, amalgam, cavit, glass ionomer cements, composite resins, carboxylate cements, intermediate restorative material (IRM), Super EBA, zinc phosphate cements, zinc oxide eugenol cements , Biodentine and Mineral trioxide aggregate (MTA)[48,65,23]. Due to certain disadvantages like corrosion, mercury toxicity, delayed expansion (zinc containing alloys), microleakage, usage of amalgam as retrograde filling is limited. In order to overcome this disadvantage various other materials were developed like super EBA and IRM[8]. Though MTA, Biodentine, Super EBA, Glass ionomer cement are used commonly but none of this material fulfils the requirement of ideal retrograde material[20].

It was in 1972 when a dentin substitute was introduced which was widely known as GIC for sealing of root end preparation because of its adhesive properties, biocompatibility and antibacterial action due to its component containing fluoride[35] .In 1993 mineral trioxide aggregate developed by Torabinejad, was introduced which overcome a number of disadvantages of other materials and made it a material of choice as it had good sealing ability and biocompatibility and also fulfilled most of the ideal requirements[74] .The main constituents are calcium and phosphorus which gives the factor of biocompatibility with the tissues and cells[82].However good, the material has its disadvantages include slow setting, initial powdery form and granular consistency and difficulty in handling[34,71].

As this mixture starts to desiccate, it loses its cohesiveness and crumbles[13]. Therefore it is advisable to keep moist cotton on setting MTA. Usually it takes 75 minutes to 4 hours before the dentist go to next step[87].

Due to certain drawbacks in MTA newer products like Biodentine and a newly launched product named as Bone cement has come up with promising results.

The newer material such as Biodentine is now used as retrograde filling material, having the same composition like MTA. Accelerators and softeners when added to powder of MTA can overcome certain disadvantages like poor handling characteristics and prolong setting time[5].

Bone cement also known as Poly methyl methacrylate (PMMA) .Bone cement is mainly used for filling bone defects, fixation of prosthesis, stabilizing of fractures in orthopaedics, filling of bur holes, vertebral reconstruction and in cases where bone grafts are necessary. This is because during polymerization, the cement increases to a maximum volume before shrinking slightly, although not to its initial volume and also the properties like unaffected by moist environment and blood contamination makes it favourable for use as a retrograde filling material[85]. Other properties of bone cement are good handling properties[76] and faster setting time, good load bearing capacity[48] and good marginal adaptation and thus can be used for filling in retrograde preparation.[15]

In the present study a comparative analysis of sealing ability and cytotoxicity of three different cements namely MTA, Biodentine, Bone Cement as retro filling materials have been assessed.

Aim and Objectives

AIM AND OBJECTIVES

AIM:

To compare the sealing ability and cytotoxicity of Bone cement, MTA and Biodentine as retro filling material.

OBJECTIVES:

1. To evaluate and compare the sealing ability of MTA, Biodentine, Bone cement as filling material in retrograde preparation in single rooted teeth.
2. To evaluate and compare the cytotoxicity of three different cements namely Bone cement, MTA, Biodentine.

Review of Literature

Torabinajed M, et al 1995[81] investigated the response of periradicular tissues of dogs to amalgam and MTA when it was placed as root end filling material by histologic evaluation, 2 to 5 and 10 to 18 weeks following periradicular surgery. They concluded that MTA showed less periradicular inflammation and more fibrous capsules adjacent to it when compared to amalgam. This may be due to its capability of inducing cementoblasts to produce cementum matrix and it does not prevent regeneration of dental and osseous tissues.

Aqrabawi in 2000[8] compared the apical seal of MTA with amalgam and Super EBA when used as a retrograde filling material using dye penetration method. The study concluded that MTA provides a better seal than amalgam and Super EBA when used as a retrograde filling material. The superior results produced by MTA are because of its property of producing hermetic seal.

Keiser Karl et al 2000[40] compared the cytotoxicity of MTA with Super EBA and amalgam using human PDL fibroblasts and MTT assay to check metabolic activity of cells after exposure to test materials both freshly mixed and after 24 hours. The MTT assay was selected as MTA being hydrophilic substance is likely to release ionic components which can interfere with intracellular enzyme activities. They concluded that MTA was less toxic than Super EBA and amalgam.

Zhu Qiang et al 2000[90] observed the adhesion of human osteoblasts on commonly used root end filling materials-IRM, MTA, Composites, amalgam with scanning electron microscopy. The result showed that osteoblasts attached and spread on MTA and composite by forming a monolayer. Osteoblasts are attached on amalgam but with few cells spreading and there was no attachment or spreading with IRM.

Lamb Edwin et al 2003[42] determined the minimum depth of MTA required to maintain an apical seal following root resection. MTA was obturated in the apical 6 mm of root canal and leakage was measured using the fluid filtration method before root resection and after 3,4,5,6 mm of apical resections. It was found that leakage increased after each resection without any statistical significance until 4 mm of apex was removed. Thus it was concluded that sealing ability of MTA was not significantly affected when at least 3 mm of MTA remained.

Ribeiro Daniel Araki et al 2005[62] evaluated the in vitro genotoxic effects of MTA and Portland cements in mouse lymphoma cells by single cell gel assay and cytotoxicity test was done using trypan blue inclusion test. The materials did not show any genotoxic effects on cells up to a concentration of 1000µg/ml and they did not show any cytotoxic effects. Both MTA and Portland cement contain the

same chemical elements and are manufactured from similar raw materials and this can be a reason that they both are not having genotoxic and cytotoxic effects.

Erkut Seluk et al 2005[23] compared the sealing ability of zinc phosphate cement, Gutta percha, amalgam, IRM and MTA using dye penetration test under stereomicroscope. The result showed least leakage in MTA when compared to IRM, amalgam and zinc phosphate cements though there was no significant difference between MTA and amalgam. The low leakage of MTA may be due to its superior marginal sealing ability.

Morais et al 2006[50] evaluated the biocompatibility of Portland cement mixed with iodoform compared to MTA. Biocompatibility was checked using polyethylene tubes filled with the material and subcutaneously implanted into Wistar albino rats and severity of inflammation was checked after 7,30 and 60 days. They concluded that there was no significant difference regarding inflammatory responses between MTA and Portland cement with iodoform after 7, 30 and 60 days. Iodoform was used as a radiopaque agent due to its availability and several studies have proved it to be harmless to pulp and periapical tissues.

Karimjee et al 2006[38] evaluated the cytotoxicity of white MTA mixed with KY jelly on human PDL fibroblasts and compared it with MTA mixed with water,

dental amalgam and resin modified GIC. KY jelly is a surgical lubricant which can accelerate the setting time of MTA. Cell viability was checked by measuring mitochondrial enzyme activity and cell lysis checked using lactate dehydrogenase assay. They concluded that MTA/KY jelly; MTA/water and amalgam showed similar biocompatibility.

Tina Ovir et al 2006[53] assessed the cell proliferation of immortalize murine cementoblasts and immortalized keratinocytes on grey MTA and white MTA with the DNA intercalating dye Hoechst 33342. The results showed that both types of cell grew significantly better on surface of WMTA compared to GMTA. The difference in proliferation rate between 2 cell types may be due to slight chemical difference in the surface chemical composition of WMTA and GMTA and also on the surface roughness and topography. They concluded that WMTA is more biocompatible than GMTA.

Hashem RA et al 2008[31] evaluated the sealing ability of Grey Pro Root MTA, Grey MTA Angelus and IRM to repair large furcation perforations, with or without internal matrix. They used dye leakage test and dye absorbance was measured using spectrophotometer. They concluded that Pro Root MTA with and without internal matrix and MTA Angelus with internal matrix showed least dye absorbance. MTA Angelus showed higher dye absorption as it lacks calcium

sulphate and has lower percentage of bismuth oxide, which leads to decreased setting time but may have prevented better wetting and adaptation to cavity walls.

Saini D et al 2008[65] compared the micro leakage of three root end filling materials –MTA, GIC, and Miracle Mix using dye penetration technique under stereomicroscope. They concluded that micro leakage was found to be significantly less in MTA when compared to GIC and Miracle Mix. This may be due to hard tissue barrier formation induced by MTA which is due to presence of calcium and phosphorus ions in the material which is similar to principal ions present in dental hard tissues.

Zou L et al 2008[91] evaluated the effect of matrix on MTA when used to repair furcal perforations. The micro leakage was detected using glucose penetration test in samples with and without collaplug or calcium sulphate as internal matrix. They concluded that there was no significant difference in leakage or overfilling. Calcium sulphate showed no extrusion of MTA. This might be due to high compressive strength of calcium sulphate barriers. Collaplug did not affect the sealing ability of MTA due to its physical and chemical characteristics, but may not serve as a good barrier against overextension as it is not pressure resistant.

Ghoddusi Jamileh et al 2008[29] compared the cytotoxicity of MTA and New Endodontic Cement (NEC) on L929 mouse fibroblast. Cell viability was assessed using MTT assay at 3 time intervals 24, 48, 72 hrs. The result showed that there was no significant difference in cytotoxicity among the materials, however there was a significant difference between different time materials within each group. This may be because materials when freshly mixed release substances during chemical setting which can cause cytotoxic effects. However, when the setting reaction is complete, materials whole structure becomes chemically fixed and may have less cytotoxicity. They concluded that NEC and MTA have similar cytotoxicity on L929 cell culture.

P. Ghaziani 2008[28] compared the sealing ability of Biocalex with White MTA, Grey MTA and amalgam as root end fillings using dye penetration technique under stereomicroscope. The result stated that Biocalex showed less leakage compared to other materials and white MTA showed less leakage than grey MTA and amalgam. This is because calcium oxide in Biocalex penetrates dentinal tubules and reduces the dentin material interface to a minimum allowing stable micromechanical intratubular attachment. Another property enhancing sealing ability of Biocalex is due to its setting expansion. They concluded that Biocalex can be used as an alternative to MTA and amalgam as a retrograde filling material.

Tae Hong –Seong et al 2008[33] evaluated the micro leakage of accelerated MTA and Portland cement using an in vitro apexification model by flow photometry analysis. 10% calcium chloride was used as an accelerator. They concluded that after 48 hrs. of obturation , the maximum and mean flow pore diameters of accelerated samples were significantly reduced compared with normal samples. This is because of addition of accelerator like $CaCl_2$ can reduce setting time and hence would minimize initial micro leakage in an in vitro apexification model. Hence the results imply that an accelerated MTA can be useful in single visit apexification procedure.

Ding Jyn Shinn et al 2008[71] evaluated the physicochemical and cytological properties of white MTA mixed with distilled water and disodium hydrogen orthophosphate (Na_2HPO_4) buffer solution. They evaluated cytological properties tested using mitochondrial tetrazolium bromide colorimetric assay on mouse cell fibroblast lines. They concluded that there was no statistical significant difference between the groups in cell survival rate.

Ghoddusi Jamileh et al 2008[29] compared the cytotoxicity of MTA and a New Endodontic Cement on L929 mouse fibroblast cells. The results showed that there was no significant difference in cytotoxicity among the materials of test and

between them and the control group. It was concluded that MTA and New Endodontic Cement have similar cytotoxic effects on L929 cell culture.

Jafarnia Behnam et al 2009[36] evaluated the cytotoxicity of grey and white Pro Root MTA in freshly mixed and set forms when mixed with additives like sterile water, saline, 2% lidocaine, 5% calcium chloride, KY liquid and 3% sodium hypochlorite gel. Cell viability was evaluated by MTT assay. The results showed that there was no significant difference in cell viability for set forms of MTA and for freshly mixed MTA. Initially 3% NaOCl gel showed lower cell viability which may be due to free chlorine ion leaching out which can pose a toxic effect, which no longer release when MTA is set as it is taken in MTA setting reaction . They concluded that various additives added to MTA have no cytotoxic effect when set.

Chong BS, et al 2009[17] assessed the success rate of root end filling materials, MTA and IRM. Referred adult patients were recruited using strict entry criteria and randomly allocated to receive MTA or IRM. A standardized surgical technique was employed, the root end was resected perpendicularly and a root end cavity was prepared in ultrasonic and filled. Thus it was concluded that that use of MTA as a root end filling material resulted in high success rate that was not significantly better than obtained using IRM.

Sepet Elif et al 2009[70] examined the cytotoxicity of MTA and calcium hydroxide in 3T3Fibroblastic cell line at different time intervals. The results showed that there was no difference in morphology of cells of either of test materials. A statistically significant difference was seen in number of viable cells between test groups at 48 hrs This may be due to high pH values of test materials and the elution components during their fresh and setting states which would seem to cause a delay in the “s” phase of cell cycle. They concluded that MTA and calcium hydroxide were cytostatic for 24 hrs and 48 hrs which were reversible as the incubated cells showed normal cell proliferation at 48 hrs and 7 days . MTA showed a shorter cytotoxic effect on cells.

Belchior Rosana Miranda et al 2009[49] evaluated the cytotoxicity of Grey MTA, White MTA and experimental epoxy resin and calcium hydroxide based cement (MBPc) by comparing their effect on L929 cells, using the agar overlay method with neutral red dye. The results showed grade 1 (slight cytotoxicity) for both types of MTA and grade 2 (mild cytotoxicity) for MBPc. This is probably because the MBPcs used reduced its toxicity in a considerable way. They concluded that MBPc can be used as an alternative material to repair root perforations.

Costa AT et al 2009[19] evaluated marginal adaptation of silver amalgam without zinc, white MTA Angelus, white Portland cement (PC), Vitremer, and GC Fuji Ortho LC. Scanning electron microscopy (SEM) was used to determine gaps in the adaptation of the root end filling materials at the interface between them and the dentin. The results showed materials containing calcium oxide (MTA and PC) showed similar results. Resin modified glass ionomer cements (GICs) presented similar variations in marginal adaptation, but Vitremer showed significantly greater marginal adaptation when compared to GC Fuji Ortho LC.

Amer Z. et al 2010[2] evaluated the cytotoxicity of Endosequence root repair material (ERRM) and compared it with grey MTA, white MTA, and AH26 using L929 mouse fibroblasts using MTT assay. They concluded that there was no significant difference in cell viability among GMTA, WMTA, ERRM, whereas AH26 showed decreased cell viability. MTT assay is a standard assay to evaluate the cytotoxicity of endodontic materials with advantages of being quantitative, reproducible and its ability to test fresh and set materials at various stages.

Kazem Mazid et al 2010[39] investigated the bacterial and dye microleakage of for different root end filling materials (Root MTA, White MTA, Calcium enriched Mixture and amalgam) and compared the efficacy of these two methods.

Bacterial leakage was investigated using Trypticase Soy Broth containing E. faecalis and dye penetration test was done using 1% methylene blue. They concluded that there was no significant difference in the leakage between the materials and between the various methods.

Rashmi Chordiya et al 2010[58] evaluated the sealing ability of bone cement as furcation perforation material and compared it with MTA and calcium Phosphate cement. They concluded that furcation perforation repaired with MTA showed minimum microleakage (mean 54.5%), calcium phosphate cement showed maximum microleakage (100%), and bone cement showed moderate dye leakage (87.8%).

Amany E. Badr et al 2010[5] evaluated the marginal adaptation and cytotoxic effect of polymethylmethacrylate (PMMA) bone cement, mineral trioxide aggregate (MTA), and amalgam as root end filling materials. The results suggested that both bone cement and MTA exhibited a better adaptation to the dentinal walls than that of amalgam. Also, the cytotoxicity testing showed that bone cement had a comparable cytotoxic effect on fibroblast cells with MTA; both root end filling materials showed less cytotoxicity than that of amalgam.

Ma J et al 2011[45] evaluated the biocompatibility of 2 root end filling materials, Endosequence Root Repair Material Putty (ERRM Putty) and Paste (ERRM Paste) and compare them with grey mineral trioxide aggregate (MTA). For cytotoxicity assay, human gingival fibroblasts were incubated for 1, 3, and 7 days with extracts of varying concentrations from materials set for 2 days or 7 days. Cell viability was evaluated by methylthiazoltetrazolium (MTT) assay. They concluded that ERRM Putty and ERRM Paste displayed similar cell viabilities to MTA at all experimental conditions, except that fresh samples of ERRM Paste showed slightly lower cell viabilities than MTA.

El Syed Ma et al 2012[22] compared sealing ability of Diadent bioaggregate (DBA) versus amalgam, intermediate restorative material and white MTA, using methylene blue dye penetration technique. Results revealed significant difference in sealing ability among four tested materials. Microleakage was seen through all materials, but significantly less in DBA when compared to white MTA, IRM and amalgam

Sabari et al 2013[64] compared the sealing ability of MTA, polymethylmethacrylate (PMMA) bone cement and CHITRA Calcium phosphate cement (CPC) when used as root end filling material using Rhodamine B dye evaluated under a confocal laser scanning microscope and to compare the seal of

root ends prepared using an ultrasonic retroprep tip and an Er: YAG laser using three different root end filling materials. They concluded that all the three materials, namely MTA, PMMA BONE CEMENT and CHITRA CPC, showed micro leakage. The amount of dye penetration was found to be lesser in root ends prepared using Er: YAG laser when compared with ultrasonic, but the difference was found to be not statistically significant.

Prabath Singh et al 2013[56] evaluated the sealing ability of MTA, Calcium phosphate cement, GIC in the repair of furcation perforations. They concluded that GIC had the greatest dye penetration followed by CPC and MTA. Mineral trioxide aggregate and calcium phosphate cement had comparatively better sealing ability than glass ionomer cement.

Janani Balachandran et al 2013[37] compared the sealing ability of Bioactive Bone cement, Mineral trioxide aggregate and Super EBA as furcation repair materials in mandibular molars using a dye extraction leakage model. MTA and bioactive bone cement showed almost similar and lower absorbance values in comparison to Super EBA. They concluded Bioactive bone cement provided an excellent seal for furcal perforation repair and at the same time it provided comfortable handling properties, which could overcome the potential disadvantages as faced with MTA.

Sirisha Gundam et al 2014[75] compared the marginal adaption of Mineral Trioxide Aggregate (MTA), Glass Ionomer Cement (GIC) and Intermediate Restorative Material (IRM) as root end filling materials in extracted human teeth using Scanning Electron Microscope (SEM). They concluded that MTA showed least gap size when compared to IRM and GIC suggesting a better marginal adaptation.

Saravanapriyan Soundappan et al 2014[67] evaluated the marginal adaptation of Biodentine in comparison with Mineral Trioxide Aggregate (MTA) and Intermediate Restorative Material (IRM), as a root end filling material, using Scanning Electron Microscopy (SEM). They concluded that MTA and IRM were significantly superior when compared to Biodentine in terms of marginal adaptation, when used as retrograde filling material.

Ravichandra P.V. et al 2014[60] evaluated the marginal adaptation of three root-end filling materials Glass ionomer cement, Mineral trioxide aggregate and Biodentine. The CLSM examination of the transverse sections of the root end-filled teeth showed marginal gaps at the dentin-filling interface. They concluded that lowest mean gap area of 11143.42±967.75μm^2 was found in Biodentine followed by MTA and GIC which had the largest mean gap area of 33388.17±12155.90μm^2 and poorest adaptation among the three materials.

Noushin Shokouhinejad et al 2014[52] compared the marginal adaptation of new bioceramic materials, EndoSequence root repair material (ERRM putty and ERRM paste) , Mineral Trioxide Aggregate as root end filling materials. The gaps at the material/dentin interface was measured using scanning electron microscope (SEM). Transverse , longitudnal, overall gap sizes were measured for each specimen. In transversal sections, no significant difference was found between MTA, ERRM putty, ERRM paste. However in longitudnal section , larger gaps were evident between ERRM paste and dentinal walls compared to MTA and ERRM putty. Thus it was concluded marginal adaptation of ERRM paste and putty was comparable to that of MTA.

Abdollah Ghorbanzadeh et al 2014[1] compared the marginal adaptation of mineral trioxide aggregate (MTA) and MTA-like materials as root-end fillings after incubation in phosphate buffer saline (PBS), a synthetic tissue fluid, for either 1 week or 2 months. They concluded that there were no significant differences between the marginal adaptation of Pro-Root MTA, Retro MTA, and Ortho MTA in both transverse and longitudinal sections after incubation for either 1 week or 2 months ($p > 0.05$).

Susanne Jung et al 2014[79] compared the biological interaction of human osteoblasts and cells of the human periodontal ligament (PDL) with different

endodontic restorative material as Mineral Trioxide Aggregate (MTA), Biodentine, amalgam and composite over a time period of 20 days. They concluded that MTA and Biodentine showed a good biocompatibility in contact with the human osteoblasts and cells of the periodontal ligament. Regarding cell survival and proliferation particularly of PDL cells, Biodentine showed good results and can be considered as a well-tolerated bioactive endodontic material.

Young-Eun Jang et al 2014[88] evaluated the cytotoxicity, setting time and compressive strength of MTA and two novel tricalcium silicate-based endodontic materials, Bioaggregate (BA) and Biodentine (BD). BA and BD were biocompatible, and they did not show any cytotoxic effects on human periodontal ligament fibroblasts. BA showed comparable cytotoxicity to MTA but inferior physical properties. BD had somewhat higher cytotoxicity but superior physical properties than MTA.

Claudio Poggio et al 2014 [18] evaluated the biocompatibility of a new pulp capping material (Biodentine, Septodont) compared with reference pulp capping materials: Dycal (Dentsply), ProRoot MTA (Dentsply) and MTA Angelus Angelus) by using murine odontoblast cell line and Alamar blue and MTT cytotoxicity tests. Thus Biodentine and MTA based products show lower

cytotoxicity varying from calcium hydroxide based material which presents higher cytotoxicity.

Eppala Jeevani et al 2014[21] evaluated the sealing ability of MICRO-MEGA Mineral Trioxide Aggregate, Endosequence, Biodentine as furcation repair materials using a dye extraction leakage method. They concluded that Biodentine showed highest dye absorbance, whereas Endosequence showed lowest dye absorbance when compared with other repair materials. Endosequence showed better sealing ability when compared with other root repair materials.

Nevil Mathews et al 2015[51] compared the cytotoxicity of potential retrograde filling materials against on Human Gingival Fibroblasts cell line by means of the Sulforhodamine B assay. Cells exposed to extracts from MTA Angelus and Biodentine showed the highest survival fraction percentage after 24 hrs & 48 hrs at all elute concentrations, whereas cells exposed to Glass-ionomer cement type IX extracts displayed the lowest survival fraction percentage. It was concluded that on serial dilution of cement extracts, Survival fraction percentage continued to increase on further dilution and showed better results for cell viability and compatibility of the entire root filling materials tested. The degree of cytotoxicity in descending order was Glass-ionomer cement type IX, MTA

Angelus & Biodentine in the cell line tested for both 24hr and 48 hrs exposure period of the study.

Ankita Khandelwall et al 2015[6] compared sealing ability of mineral trioxide aggregate (MTA) and Biodentine as root end filling material, and also to compare the effect of different retro preparation techniques i.e. conventional bur v/s ultrasonic tips on sealing ability of both the root end filling materials. It was concluded that Biodentine and ultrasonic preparations showed significantly less microleakage than MTA and bur preparations. Therefore Biodentine can be used as a replacement for MTA, as a root end filling material.

Behman Bolhari et al 2015[12] evaluated the marginal adaptation of mineral tri-oxide aggregate (MTA), calcium enriched mixture (CEM) cement, Biodentine and BioAggregate in presence of normal saline and human blood. There were no significant differences in marginal adaptation of the eight tested groups ($P>0.05$). Based on the results, blood contamination does not affect the marginal adaptation of MTA, CEM cement, Biodentine or Bio Aggregate.

Sakshi Malhotra et al 2015[66] evaluated the marginal seal of the following materials when used as root-end filling materials, MTA Angelus, White ProRoot MTA, Biodentine and Glass ionomer cement (GIC). They concluded that micro

leakage was present in all the samples. Least amount of apical dye micro leakage was seen in Biodentine with mean value of 0.16 mm followed by ProRoot MTA 0.68 mm, MTA Angelus 0.74 mm, and GIC 1.53 mm. The best sealing ability was seen in Biodentine, and this difference was statistically significant.

Varol Basak et al 2016[83] investigated the effects of calcium silicate-based products on cytotoxicity in the 3T3 fibroblast and gelatinolytic activity of matrix metalloproteinases (MMPs). It was concluded that Ortho MTA and Biodentine had mild cytotoxicity. No cytotoxicity was observed with Retro MTA, BioAggregate, MTA Angelus, MTA Plus and MTA Cerkamed. However, BioAggregate showed better cell viability compared with MTA-derived materials. Thus, BioAggregate appears to be a possible alternative to MTA for root repair treatment. There was no effect on the MMP-9 in 3T3 fibroblasts.

Eshagh A. Saberi et al 2016[24] compared the effects of mineral trioxide aggregate (MTA), calcium enriched mixture (CEM) cement, Biodentine (BD) and octacalcium phosphate (OCP) on the viability of human gingival fibroblasts (HGFs). It was concluded that cytotoxicity of MTA, CEM, Biodentine and OCP against HGFs was similar to that of the control group at 24 and 48 hours. Over time, MTA and Biodentine exhibited less cytotoxicity than other materials.

Silva Ej et al 2016[73] evaluated the cytotoxic effects of Biodentine, using a three dimensional (3D) cell culture associated with an in situ root end filling experimental model. White mineral trioxide aggregate (MTA) and zinc oxide cement were used as reference for comparison. IL1α and TNFα cytokine production were also evaluated. They concluded that Biodentine and MTA had similar cytocompatibility in a 3D cell culture model associated with an in situ root end filling model. The methodology could be used as an alternative to assess the cytocompatibility of endodontic cements because it is more closely related to the in vivo situation.

Anurag Jain et al 2016[7] compared the sealing ability of four root-end filling materials MTA, Portland cement, IRM, RMGIC in teeth with root apices resected at 0 and 45 angle using dye penetration method under fluorescent microscope. The root apex sealing ability of Mineral Trioxide Aggregate (MTA) was superior to Portland cement, Intermediate Restorative Material (IRM) and LC GIC. IRM demonstrated the maximum apical leakage value among all the materials. Portland cement and LC GIC showed comparable sealing ability. They concluded that the angulation whether 0° or 45° angle did not affect the sealing ability of all the four materials used, MTA proved to be one of the superior materials for root-end filling.

Materials and Methods

ARMAMENTARIUM

PREPARATION OF SPECIMEN

50 Maxillary Incisors

Distilled Water

Ultrasonic Scaler (Wood Pecker-UDS P Scaler)

Glass Beaker-100ml

0.5% Chloramine T (Explicit Chemicals,Pune,India)

FOR ROOT CANAL PREPARATION:

Airoter Hand Piece (NSK)

Long Straight Fissure Diamond Bur (SF 12C, MANI Corporation, Japan)

Round Bur (BR-31, MANI Corporation, Japan)

Finishing Diamond Burs (FO-42EF, MANI, Nakakusu, Japan)

K Files 15-40 sizes (Mani, Inc., Tochigi, Japan)

Protaper Universal rotary system (SX, S1, S2, F1, F2, F3, F4, F5)

Canal Irrigants – 2.5% sodium hypochlorite (Chenchems Pvt Ltd, Chennai)

Normal Saline (Fresinius Kabi India Pvt Ltd)

Gutta Percha - Protaper F5 (Denstply Maillefer, Switzerland: Batch no. 061209)

AH plus sealer (Root canal sealing material (Dentsply, India; lot no:1602000162)

FOR RETROGRADE PREPARATION:

Diamond Disc (Indogem pvt ltd. Mumbai, India)

Diamond Bur –Round (BR-46, Mani, Inc., Tochigi, Japan)

Ultrasonic tip (Satelec P14D, France: Batch no. 601439)

FOR DYE PENETRATION:

Nail Varnish (MAC)

Rhodamine B Dye (Reachem Laboratory Chemicals Pvt Ltd, Chennai, India)

FOR MOUNTING SPECIMENS:

PVC Tubes (18mm X 30mm)

Addition Silicone Rubber Base Impression Material (Dentsply, India)

FOR TESTING MICROLEAKAGE

Hard Tissue Microtome (Leica SP 1600 Leica Biosystems, Melbourne, Australia)

Jig

LSM 510 Meta Confocal Laser Scanning Microscope (Carl Zeiss)

EXPERIMENTAL MATERIALS :(Table 1) (Fig 1)

Material	Manufacturer	Composition
Bone Cement	SURGIWEAR	GBCEM1 Powder- calcium phosphate powder Tri calcium phosphate Tetra calcium phosphate Liquid- Sodium and calcium Salts
MTA	ANGELUS	Powder -Dicalcium silicate, Tricalcium silicate, Tricalcium aluminate, Tetracalcium aluminate. Trace amounts of silicon dioxide, calcium oxide,

		magnesium oxide, potassium sulphate and sodium sulphate. Liquid- Distilled water
Biodentine	SEPTODONT	Powder-Tricalcium silicate, Dicalcium silicate, Calcium carbonate, Iron oxide, Zirconium dioxide. Liquid- Calcium chloride , Hydrosoluble polymer
Abbreviations: MTA- Mineral trioxide Aggregate		

METHODOLOGY:

SPECIMEN SELECTION

Fifty sound human maxillary central incisors were extracted for periodontal reasons (Fig 2), cleaned by ultrasonic scaler and disinfected in 0.5% chloramine solution for 2 weeks. The teeth were then stored in distilled water until use.

Single rooted maxillary central incisor with single root canal and apical foramen were selected and IOPA (Intra oral periapical) radiograph was taken to confirm the root canal morphology. Teeth with any sort of defects like internal and external resorptions, root caries, dilacerated roots, open apices and previous endodontic therapy were excluded from this study.

GROUPS:

The teeth were randomly divided into five groups of 10 teeth each.

GROUP A: BONE CEMENT

GROUP B: MTA

GROUP C: BIODENTINE

GROUP D: POSITIVE CONTROL- No cements placed as retrograde filling material

GROUP E: NEGATIVE CONTROL- The teeth were coated with nail varnish at the root apex.

The above mentioned groups were evaluated for the following study parameter.

Study parameter 1- Sealing ability

The teeth were decoronated at the cementoenamel junction using a diamond disc to obtain a standard root length of 14 mm (Fig 3). A round bur (BR-31, MANI Corporation, Japan) was used to gain access and straight line entry to the root canal was obtained. A size 15 K file was introduced into root canal until the tip was visible at major apical foramen and the working length was measured by Ingle's method. Cleaning and shaping was done using nickel titanium rotary system (Protaper rotary system Dentsply / Maillefer, Tulsa, OK, USA) upto F5.After each instrumentation the canal was irrigated with 2 ml of 2.5% sodium hypochlorite solution and 2 ml of normal saline with a 27 – gauge needle. Then

the canals were obturated with Gutta- percha Protaper F5 and AH plus sealer. After 24 hrs the apical third of each root was resected at 3 mm level perpendicular to the long axis of the root using a high speed hand piece with a diamond disk (Indogem pvt ltd. Mumbai, India) and copious water supply (Fig 4).

Retrograde preparation of 1.5 mm diameter and 3 mm depth was prepared using an ultrasonic tip (Satelec P14D) (Fig 5, 6) and the cavity was filled with test materials as per manufacturer's instruction. The teeth were divided into 5 groups of 10 teeth each; Groups A, B, C were the experimental groups.

Group D was positive control (teeth without retrograde filling material) and Group E was negative control (teeth without retrograde filling material whose root surface was coated entirely with nail varnish). The external surface of the experimental group specimens were covered with two coats of nail varnish, except the root end filling to prevent penetration of the dye through the dentinal tubules and the accessory canals (Fig 7,8) .

DYE PENETRATION:

The specimens with the respective groups having different cements as retrograde filling material and the controls were immersed in the 0.5% aqueous rhodamine dye (5mg of Rhodamine B powder is mixed in 100ml of distilled water) for 48 hrs (Fig 9) . After 48 hrs the specimens were rinsed under running water for 5 mins and allowed to dry.

TOOTH SECTIONING:

The teeth were then mounted in acrylic block of 18mm X 30mm dimensions (Fig 10). The block was then placed in the jig (Fig 11) and then sectioned along the long axis of tooth (Fig 12) to get section of 1mm thickness using a hard tissue microtome (Leica SP 1600 Leica Biosystems, Melbourne, Australia) (Fig 14) till the centre of root canal with obturating material and retrograde material is obtained (Fig 13), so that the particular section what we are selecting should have the interface of obturating material , retrograde material and dentin interface.

MICROLEAKAGE TESTING:

The extent of dye penetration was measured using LSM 510 Meta confocal microscopy (Carl Zeiss) (Fig 15) at 10× magnification in fluorescent mode. The amount of dye penetration was measured in μm using the ZEISS LSM IMAGE BROWSER SOFTWARE (Version 4.2.0.121). Statistical analysis was done using one way ANOVA, intergroup comparison using Tukey's post hoc test with SPSS Version 16 for Windows.

STUDY PARAMETER 2- CYTOTOXICITY

Mouse subcutaneous connective tissue fibroblast (L929) cell line was obtained from National Centre for Cell Science (NCCS), Pune. Dulbecco's modified Eagle's medium (DMEM) (Fig 18) was obtained from Lonza, Switzerland. Thiazolyl tetrazolium bromide (MTT) was purchased from Sigma Aldrich. Fetal bovine serum (FBS),

antibiotic- antimycotic cocktail solution (100X) and phosphate buffered saline (10X) were purchased from Invitrogen Life Technologies, USA. Trypsin-EDTA solution (0.05%) and dimethyl sulfoxide (DMSO) were purchased from Hi-Media, India. All other reagents used were of analytical grade and were purchased from Fischer Scientifics, India.

Reagents

Dulbecco's Modified Eagle's Medium (DMEM) with 10% FBS

Dulbecco's modified Eagle's medium (DMEM) with 10% FBS was prepared by mixing 100 ml of heat-inactivated sterile-filtered (0.22 µm) FBS with 890 ml of ready-to-use commercially available DMEM (Lonza, Switzerland). To this, 10 ml of antibiotic antimycotic (penicillin – 100 U/ml; streptomycin – 100 µg /ml; amphotericin B – 250 µg/ml) cocktail solution (100X) (Invitrogen, USA) was added, mixed well and stored at 2 – 8°C for use.

Phosphate-buffered Saline (PBS) [1X; pH 7.4]

Phosphate-buffered saline (1X) was prepared by mixing 1 volume of 10X PBS (pH 7.4) with 9 volumes autoclaved Milli Q water.

Trypsin– EDTA Solutions (1X)

Ready-to-use commercially available trypsin-EDTA solution (0.05%) from Hi-Media, India was used for trypsinization of the cells during subculturing processes.

Thiazolyl Tetrazolium Bromide (MTT) (5 mg/ml Stock)

One hundred milligrams (100 mg) of MTT was dissolved in 20 ml of 1X PBS (pH 7.4) thoroughly, filtered through 0.22 μ syringe filter and stored at 2 – 8°C for use.

Preparation of Extracts of Dental Cements

The extracts of dental cements were prepared as follows: The three dental cements to be tested for cytotoxicity were manipulated as per the manufacturer's instruction by mixing the cement powder with diluents provided by the manufacturer, mixed thoroughly (Fig 16) , were placed in individual wells in a 6-well culture plate and incubated at 37°C under control humidified atmosphere in an incubator for 24 h to allow them to set. At the end of 24 h incubation period, 1 ml of DMEM was added to the set dental cements and again incubated for 24 h at 37°C under control humidified atmosphere in an incubator in order to prepare the extracts of dental cements. At the end of 48 h, media from each well containing three different dental cements was collected, sterile filtered through 0.22 μm syringe filter (Fig 17) into appropriately labeled 1.5 ml micro centrifuge tubes

.

Dilution of Dental Cement Extracts

The different dilutions (1:1, 1:2, 1:4, 1:8, 1:16 & 1:32) of dental cement extracts were then prepared in 96-well culture plate by serial dilution using DMEM from the undiluted concentrated extract . Serial dilution was performed by transferring one volume of the concentrated extract to the well containing an equal volume of media to prepare 1:1 dilution of the dental cement extract and subsequent dilutions were prepared by the transfer and mixing of an equal volume of the previous dilution with an equal volume of culture media (Fig 24).

EXPERIMENTAL PROCEDURE

Culture of Mouse Subcutaneous Connective Tissue Fibroblast (L929) Cells

Mouse subcutaneous connective tissue fibroblast (L929) cells were grown in DMEM containing 10% FBS, 100 IU/ml penicillin, 100 mg/ml streptomycin and 250 µg/ml amphotericin B in a 25cm^2-culture flask (Fig 19) in a CO_2 incubator (Fig 20) at 37°C and 5% CO_2 under controlled humidified atmosphere. Once the cells reached ~90% confluency, they were trypsinized using trypsin (0.05%) – EDTA (0.54 mM) solution, washed thoroughly with media and subcultured into a 75-cm^2 culture flask for expansion. This process was repeated twice till the cells attained a consistent growth phase. Once after the cells attained consistent growth phase, they were trypsinized at 80% confluency and then utilized for the assay (Fig 21).

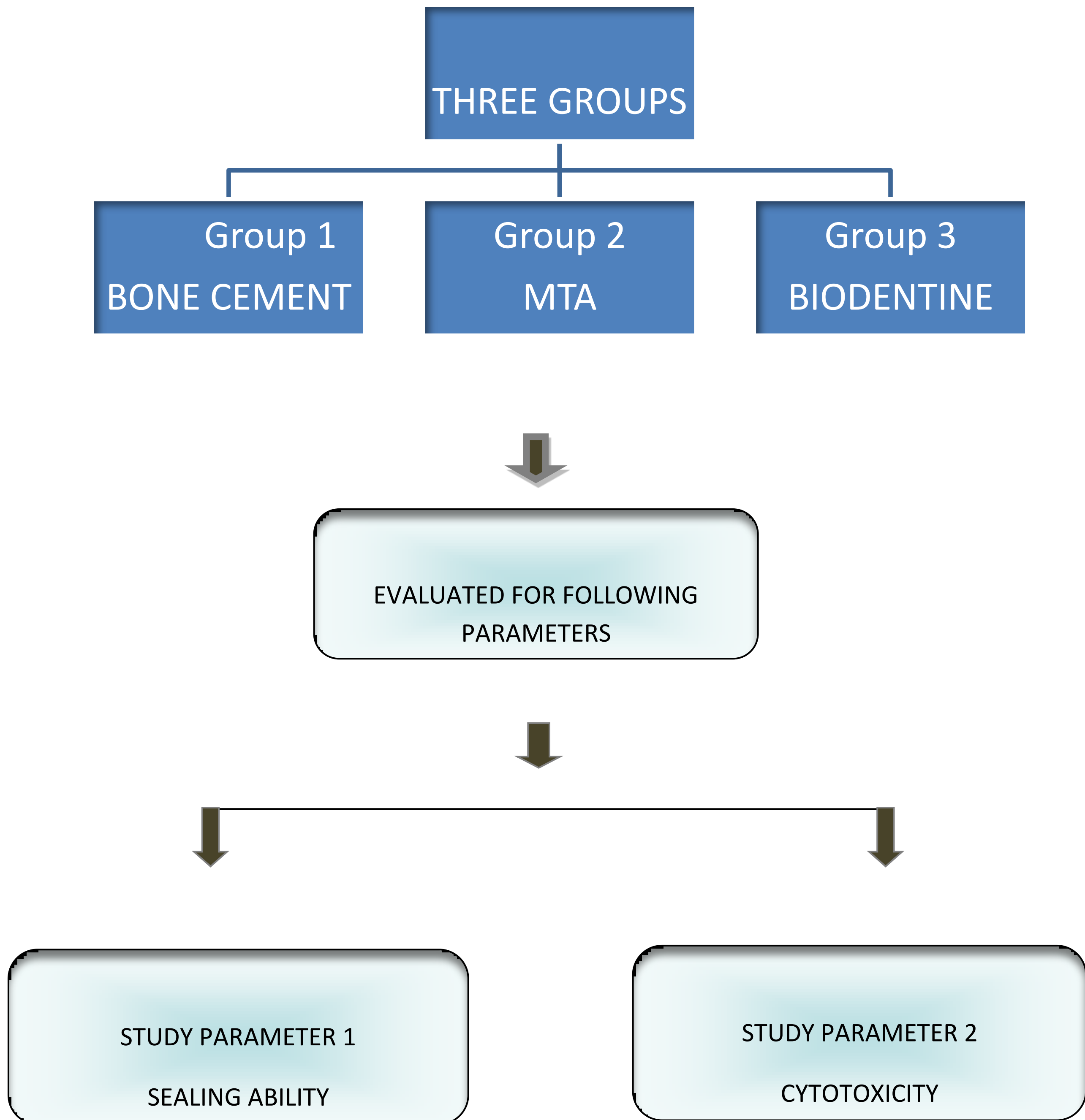
Assessment of Cytotoxicity by MTT Assay

The cells were trypsinized when they were at 80% confluence and seeded in a 96-well plate at the density of 7 x 10^3 cells / well (Fig 22, 23) . The cells were incubated in a CO_2 incubator (Fig 19) at 37°C and 5% CO_2 under controlled humidified atmosphere overnight to allow them to attach to the plate (Fig 27,28). After overnight incubation, the cells were exposed to different dilutions (0, 1, 1:1, 1:2, 1:4, 1:8, 1:16 & 1:32) of the test items A,B,C for 48 h (Fig 24, 25, 26). At the end of 48 h, 50 µl MTT (5 mg/ml stock) was added to the cells and further incubated for 3 h at 37°C (Fig 20).

At the end of incubation period, the contents of the plates were discarded by simple decantation and the plates were dried overnight at room temperature. The purple colored formazon crystals formed were dissolved in 100 µl of dimethyl sulfoxide by shaking at 400 rpm for 15 min at room temperature in a thermo shaker. The intensity of the colour i.e. OD (optical density) developed was absorbed at 540 nm in a multimode microplate reader and checked under inverted phase contrast microscope (Fig 29). The percentage growth / viability of cells were calculated using the following formula:

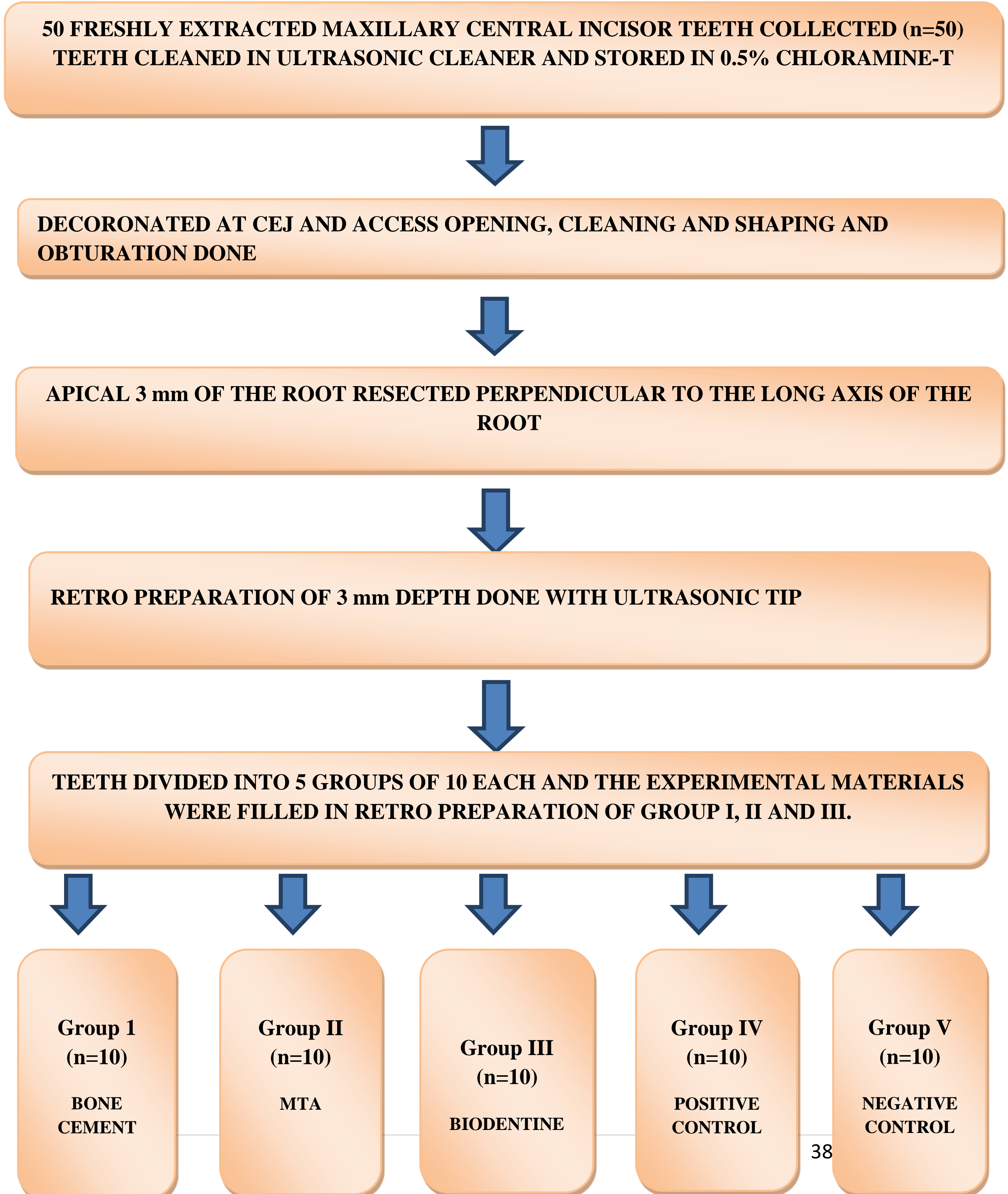
$$\text{Percentage (\%) Growth/ Viability} = \frac{\text{OD OF TEST}}{\text{OD of the Control}} \times 100$$

METHODOLOGY FLOW CHART



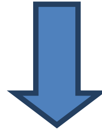
PROCEDURAL FLOWCHART:

STUDY PARAMETER 1- SEALING ABILITY



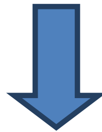
MICROLEAKAGE TESTING:

AFTER APPLICATION OF NAIL VARNISH, RESTORED TOOTH SAMPLES WERE IMMERSED IN 0.6% RHODAMINE DYE FOR 48 HOURS.



TEETH WERE MOUNTED IN STANDARDIZED SELFCURED ACRYLIC BLOCKS

TEETH WERE SECTIONED LONGITUDINALLY AT THE FILLING MATERIAL AND TOOTH WALL INTERFACE



SECTIONED TEETH WERE EXAMINED UNDER CONFOCAL MICROSCOPE AT 10X MAGNIFICATION IN FLUORESCENT MODE FOR EVALUATION OF MICROLEAKAGE

STUDY PARAMETER 2- CYTOTOXICITY

L929 MOUSE FIBROBLAST CELLS CULTURED IN DULBECCO'S MODIFIED EAGLES MEDIUM (DMEM)

SET TEST MATERIALS PREPARED AND PLACED IN DMEM-SERIALLLY DILUTED TO SIX CONCENTRATIONS

L929 CELLS SEEDED IN TISSUE CULTURE PLATES AND INCUBATED AT 37ºC FOR 24 HRS

200 µl OF ELUTE PLACED INTO THE WELLS

CELL VIABILITY CHECKED AFTER 48 HRS USING MTT ASSAY WHERE THE OPTICAL DENSITY WAS ABSORBED AT 540 nm AND EVALUATED UNDER INVERTED PHASE CONTRAST MICROSCOPE.

MATERIALS AND METHODS

STUDY MATERIALS

MATERIALS USED (Fig 1)

BONE CEMENT

MTA

BIODENTINE

METHODOLOGY

FIG 2 TOOTH SAMPLES DIVIDED INTO 5 GROUPS

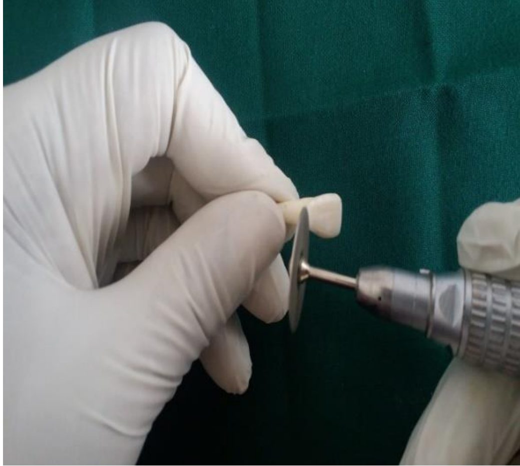


FIG 3 TEETH DECORONATED AT CEJ

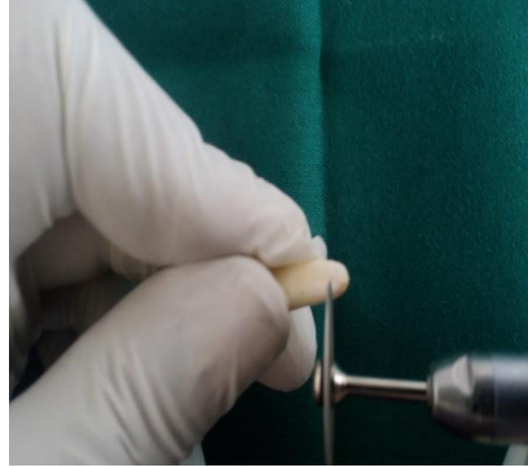


FIG 4 APICAL 3 MM OF ROOT RESECTED

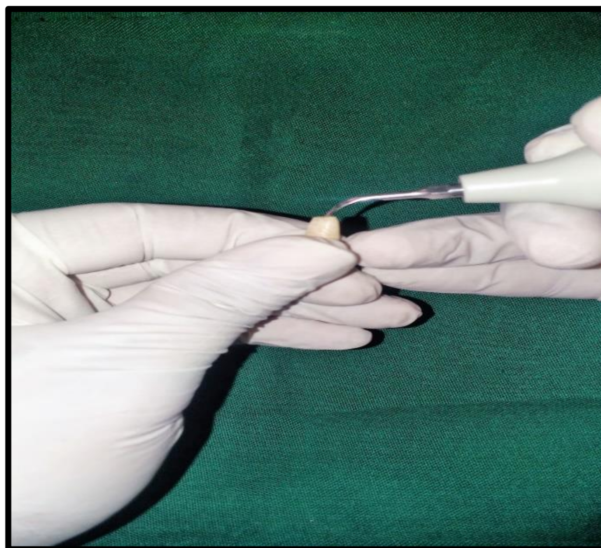


FIG 5 RETRO CAVITY PREPARED WITH ULTRASONIC TIPS

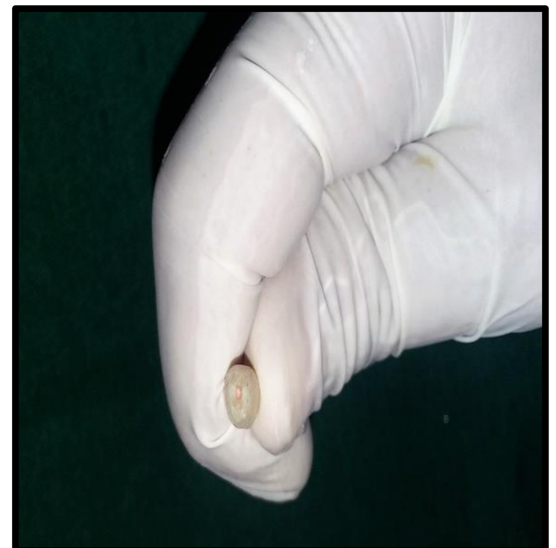


FIG 6 RETO CAVITY PREPARED

FIG 7 **SPECIMEN COATED WITH NAILVARNISH**

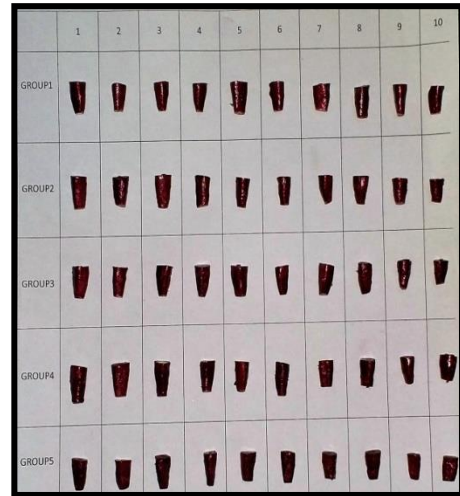


FIG 8 **NAIL VARNISH APPLIED**

FIG 9 **SPECIMEN PLACED IN RHODAMINE DYE SOLUTION**

FIG 10 SPECIMEN MOUNTED IN ACRYLIC BLOCKS

FIG 11 SPECIMEN PLACED IN JIG

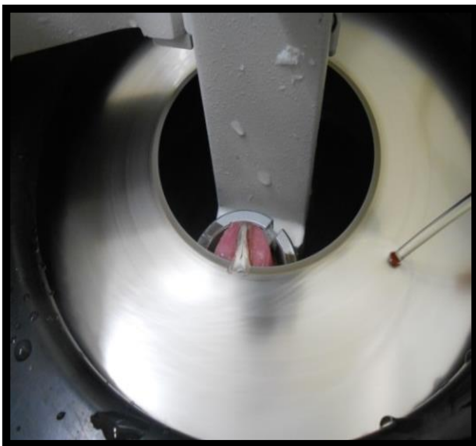


FIG 12 SPECIMEN SECTIONED IN MICROTOME

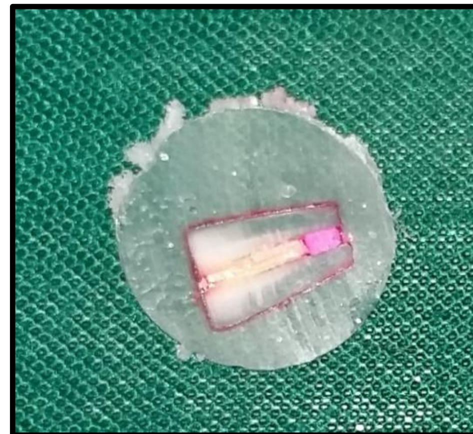


FIG 13 LONGITUDINAL TOOTH SECTION

FIG 14 HARD TISSUE MICROTOME

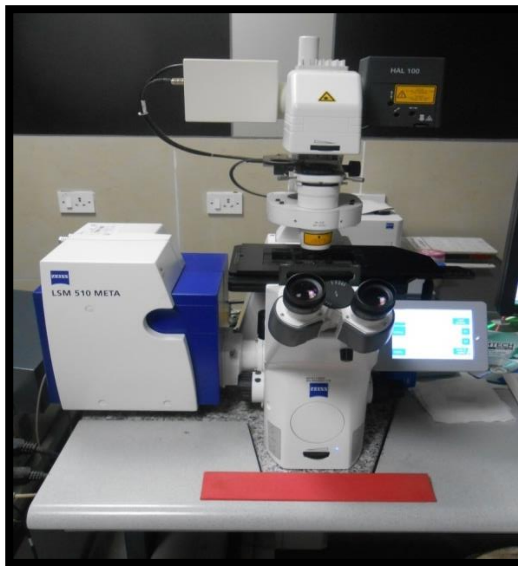


FIG 15 CONFOCAL MICROSCOPE

CYTOTOXICITY

Fig 16 SPECIMEN

Fig 17 FILTER STERILISED EXTRACTS OF TEST ITEM

Fig 18 DMEM CULTURE MEDIUM

Fig 19 L929 CELLS GROWN IN CULTURE FLASK

Fig 20 CO2 INCUBATOR FOR GROWING L929 CELLS

Fig 21 L929 CELL PELLET AFTER TRYPSINIZATION FROM CULTURE FLASK

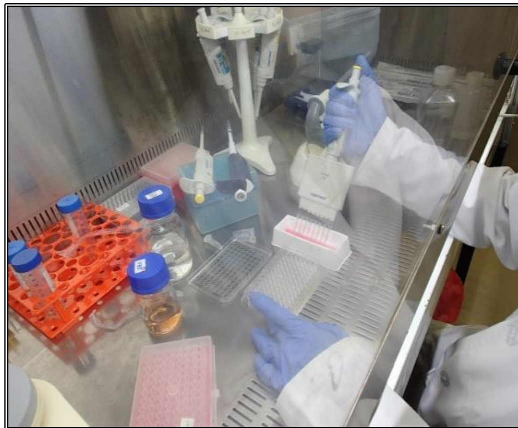


Fig 22 SEEDING OF L929 CELLS

Fig 23 L929 CELLS SEEDED IN 96 WELL CULTURE

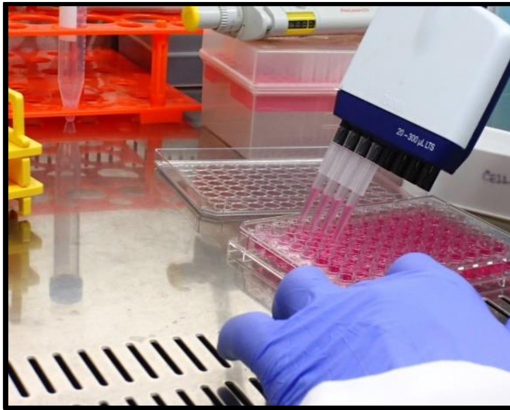


Fig 24 SERIAL DILUTION OF TEST ITEM 1

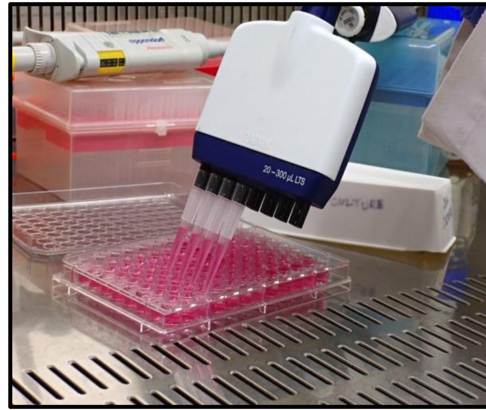


Fig 25 SERIAL DILUTION OF TEST ITEM 2

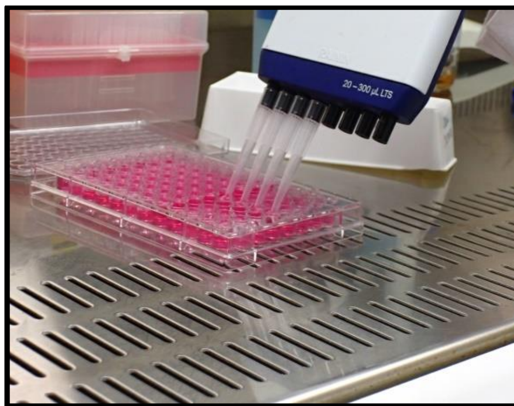


Fig 26 SERIAL DILUTION OF TEST ITEM 3

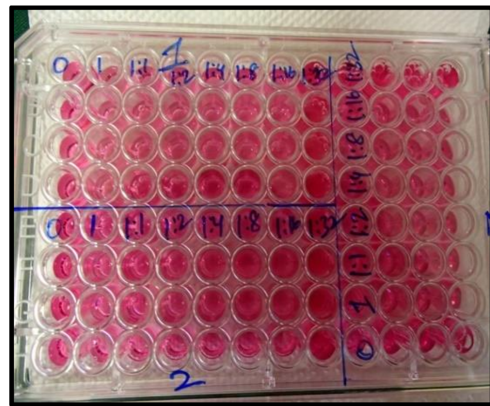


Fig 27 SERIAL DILUTION PLATE

Fig 28 ADDITION OF SPECIMENS TO L929 CELLS

Fig 29 INVERTED PHASE CONTRAST MICROSCOPE

Results

CONFOCAL IMAGES:

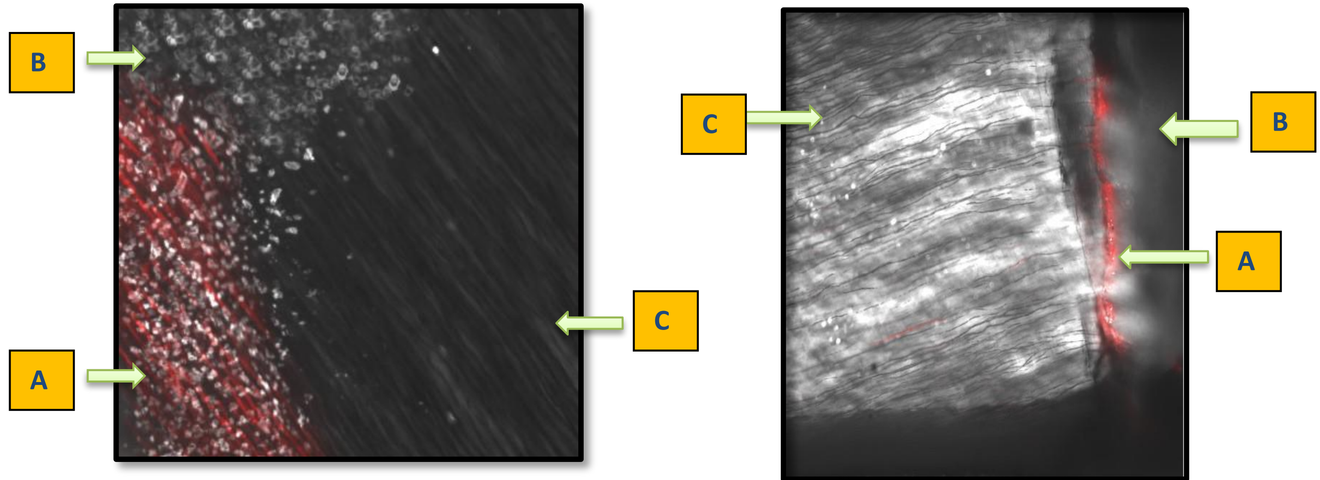


Fig 28 GROUP 1 -BONE CEMENT

A- EXPERIMENTAL CEMENT WITH DYE PENETRATION, B- EXPERIMENTAL CEMENT WITHOUT DYE PENETRATION, C- DENTINAL TUBULES

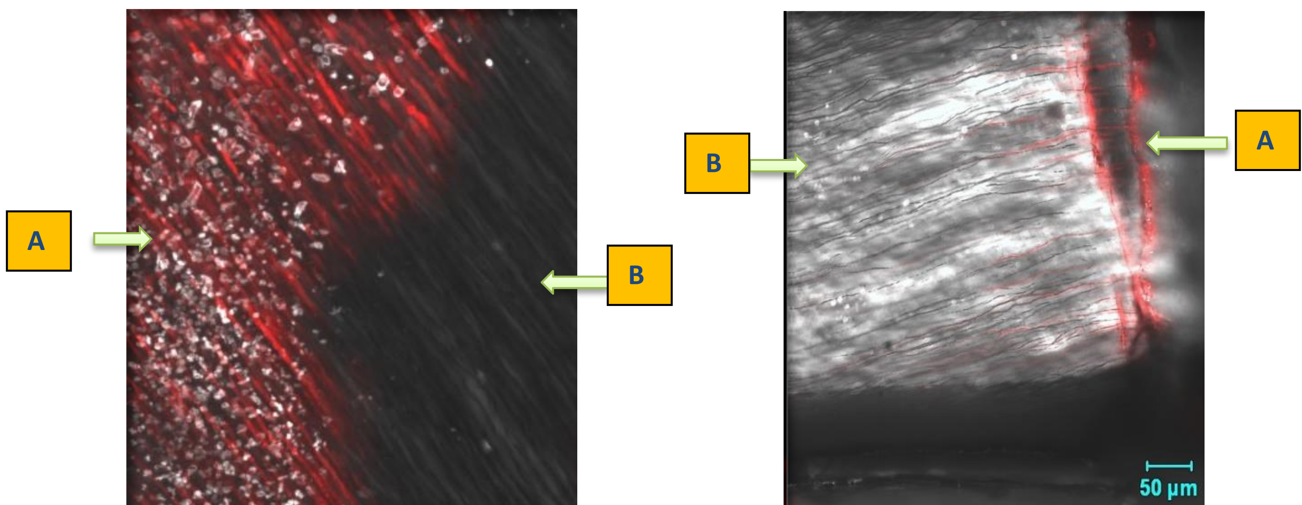


Fig 29 GROUP II- MTA

A- EXPERIMENTAL CEMENT WITH DYE PENETRATION, B- DENTINAL TUBULES

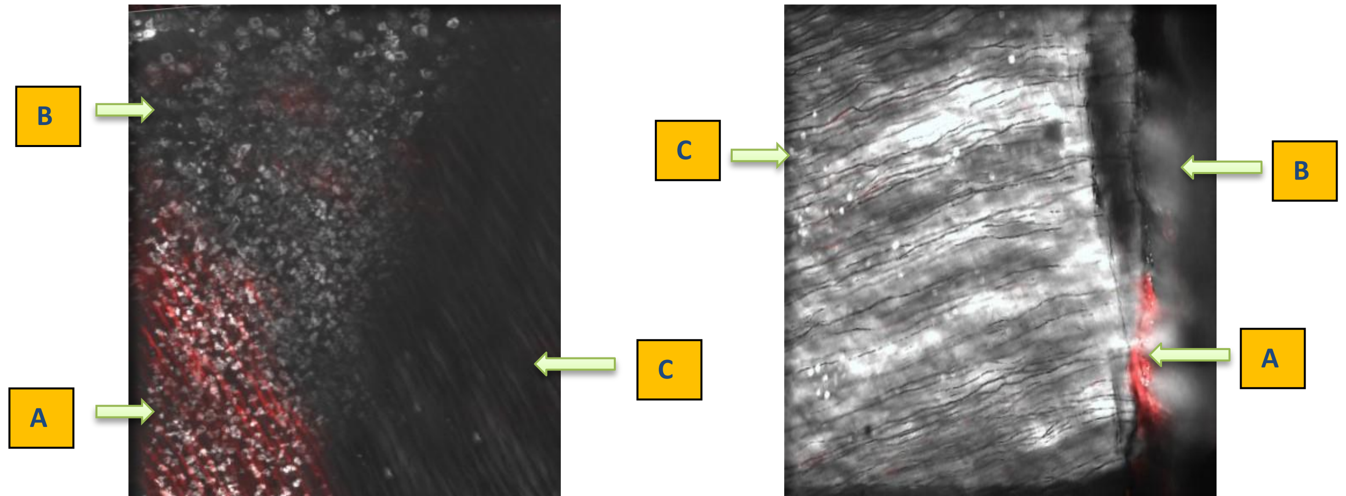


Fig 30 GROUP III BIODENTINE

A- EXPERIMENTAL CEMENT WITH DYE PENETRATION, B- EXPERIMENTAL CEMENT WITHOUT DYE PENETRATION, C- DENTINAL TUBULES

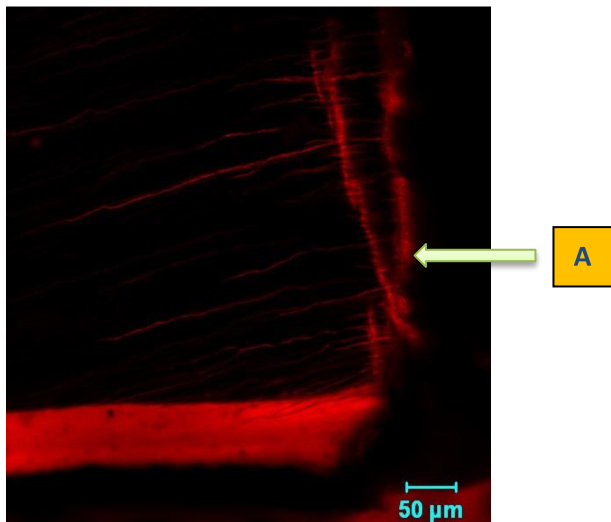


Fig 31 GROUP IV- POSITIVE CONTROL

A.COMPLETE DYE PENETRATION

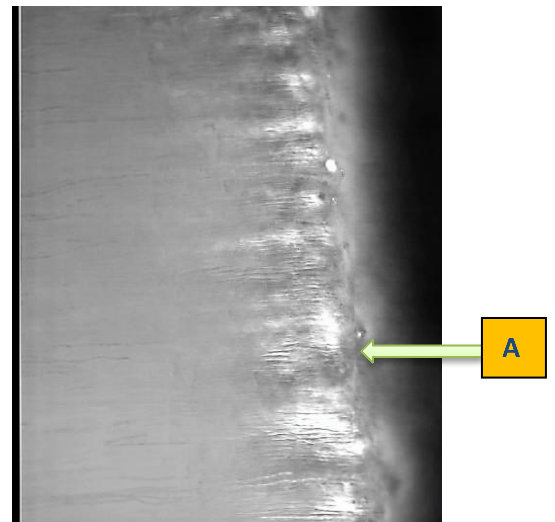


Fig 32 GROUP V-NEGATIVE CONTROL

A.NO DYE PENETRATION

RESULTS

Statistical analysis was carried out using statistical software SPSS version 16.The quantitative data obtained in the study was assessed for normality using Shapiro wilk test and was found to be parametric in nature. Inter group analysis of dye penetration levels were carried out using one way Anova (Table 4) and Paired wise comparison was conducted using Turkey post hoc test (Table 5). The intergroup comparison of micro leakage was assessed by Pearson's chi square test (Table 6). $P<0.05$ was considered statistically significant in this study.

1. SEALING ABILITY- DYE LEAKAGE VALUES (µm) (TABLE 2)

Sample no	Group 1	Group 2	Group 3	Group 4	Group 5
1	401 µm	435 µm	356 µm	3000 µm	0 µm
2	395 µm	425 µm	389 µm	3000 µm	0 µm
3	345 µm	426 µm	432 µm	3000 µm	0 µm
4	360 µm	453 µm	498 µm	3000 µm	0 µm
5	387 µm	420 µm	398 µm	3000 µm	0 µm
6	401 µm	410 µm	376 µm	3000 µm	0 µm
7	432 µm	426 µm	260 µm	3000 µm	0 µm
8	410 µm	402 µm	423 µm	3000 µm	0 µm
9	451 µm	478 µm	240 µm	3000 µm	0 µm
10	332 µm	423 µm	246 µm	3000 µm	0 µm

DESCRIPTIVES DYE DYE PENETRATION (TABLE 3)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A	10	3.9140E2	37.16390	11.75226	364.8145	417.9855	332.00	451.00
Group B	10	4.2980E2	21.72454	6.86990	414.2592	445.3408	402.00	478.00
Group C	10	3.6180E2	86.99783	27.51113	299.5655	424.0345	240.00	498.00
Total	30	3.9433E2	61.03833	11.14402	371.5412	417.1254	240.00	498.00

ANOVA (TABLE 4)

Dye Penetration	Sum of Squares	Df	Mean Square	F	**Sig.**
Between Groups	23249.067	2	11624.533	3.701	**.038**
Within Groups	84795.600	27	3140.578		
Total	108044.667	29			

Multiple Comparisons (TABLE 5)

Dye Penetration Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	**Sig.**	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	Group B	-38.40000	25.06223	**.292**	-100.5397	23.7397
	Group C	29.60000	25.06223	**.474**	-32.5397	91.7397
Group B	Group A	38.40000	25.06223	**.292**	-23.7397	100.5397
	Group C	68.00000*	25.06223	**.030**	5.8603	130.1397
Group C	Group A	-29.60000	25.06223	**.474**	-91.7397	32.5397
	Group B	-68.00000*	25.06223	**.030**	-130.1397	-5.8603

Dye Penetration Tukey HSD						
(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	**Sig.**	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	Group B	-38.40000	25.06223	**.292**	-100.5397	23.7397
	Group C	29.60000	25.06223	**.474**	-32.5397	91.7397
Group B	Group A	38.40000	25.06223	**.292**	-23.7397	100.5397
	Group C	68.00000*	25.06223	**.030**	5.8603	130.1397
Group C	Group A	-29.60000	25.06223	**.474**	-91.7397	32.5397
	Group B	-68.00000*	25.06223	**.030**	-130.1397	-5.8603
*. The mean difference is significant at the 0.05 level.						

Chi-Square Tests (TABLE 6)

	Value	**Df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	7.500[a]	2	**.024**
Likelihood Ratio	7.979	2	.019
Linear-by-Linear Association	.777	1	.378
N of Valid Cases	30		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 4.67.

INTERPRETATION OF RESULTS FOR DYE PENETRATION

The mean dye penetration values were found to be significantly higher for Group B (429.80± 21.72) followed by Group A (391.40± 37.16) and Group C (361.80 ± 86.99) and P= 0.038 was found statistically significant between the groups (Graph 1) (Fig 28, 29, 30). On individual comparison of the mean difference between all three groups using Tukey's post hoc test it was observed that a statistically significant difference in the dye penetration values was found between Groups B and C only i.e. P=0.030.

The order of dye penetration values were as follows

Group C ≤ Group A < Group B

There was a complete penetration of the dye in the Positive Group while in Negative Group there was no dye penetration (Fig 31, 32).

Normality of quantitative data collected was assured using Shapiro wilk tests and was found to be parametric system P<0.05.

2. CYTOTOXICITY

The comparison of optical density values between three different material (bone cement , MTA, Biodentine) were evaluated using one way Anova (Table 12) and individual comparison pairwise and was carried out using Tukey 's Post hoc test (Table 13) . $P<0.05$ was considered significant in this study.

PERCENTAGE (%) VIABILITY CALCULATION (TABLE 7)

Test Item 1 - 540 nm					
Rep. 1	Rep. 2	Rep. 3	Rep. 4	Avg. OD	% Viability
2.155	2.401	2.544	2.659	2.440	100
2.531	2.426	2.249	2.280	2.372	97
2.161	2.126	2.498	2.649	2.359	97
2.146	2.457	2.592	2.138	2.333	96
2.082	2.013	2.485	2.068	2.162	89
2.151	2.114	2.031	2.066	2.091	86
2.144	2.032	2.050	2.129	2.089	86
2.035	1.919	2.005	2.104	2.016	83

(TABLE 8)

Test Item 2 - 540 nm					
Rep. 1	Rep. 2	Rep. 3	Rep. 4	Avg. OD	% Viability
2.076	2.469	2.551	2.485	2.395	100
2.401	2.349	2.231	2.548	2.382	99
2.312	2.420	2.343	2.352	2.357	98
2.325	2.280	2.400	2.295	2.325	97
2.332	2.110	2.557	2.194	2.298	96
2.272	2.492	2.181	2.248	2.298	96
2.262	2.071	2.219	2.172	2.181	91
1.921	1.928	1.924	1.952	1.931	81

RESULTS

(TABLE 9)

Test Item 3 - OD540 nm					
Rep. 1	Rep. 2	Rep. 3	Rep. 4	Avg. OD	% Viability
2.543	2.473	2.416	2.410	2.461	100
2.573	2.522	2.237	2.220	2.388	97
2.299	2.490	2.405	2.314	2.377	97
2.327	2.102	2.334	2.449	2.303	94
2.319	2.506	2.179	2.199	2.301	94
2.145	2.120	2.125	2.185	2.144	87
2.225	1.806	2.315	2.140	2.122	86
1.972	2.104	2.096	2.299	2.118	86

(TABLE 10)

	Test Item 1				Test Item 2				Test Item 3				
0	2.155	2.401	2.544	2.659	2.076	2.469	2.551	2.485	2.543	2.473	2.416	2.410	540
1 in 32	2.531	2.426	2.249	2.280	2.401	2.349	2.231	2.548	2.573	2.522	2.237	2.220	540
1 in 16	2.161	2.126	2.498	2.649	2.312	2.420	2.343	2.352	2.299	2.490	2.405	2.314	540
1 in 8	2.146	2.457	2.592	2.138	2.325	2.280	2.400	2.295	2.327	2.102	2.334	2.449	540
1 in 4	2.082	2.013	2.485	2.068	2.332	2.110	2.557	2.194	2.319	2.506	2.179	2.199	540
1 in 2	2.151	2.114	2.031	2.066	2.272	2.492	2.181	2.248	2.145	2.120	2.125	2.185	540
1 in 1	2.144	2.032	2.050	2.129	2.262	2.071	2.219	2.172	2.225	1.806	2.315	2.140	540
1	2.035	1.919	2.005	2.104	1.921	1.928	1.924	1.952	1.972	2.104	2.096	2.299	540

SPSS (Statistical package for social sciences) version 16 (IBM Corp, IL, USA).

DESCRIPTIVES (TABLE 11)

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Zero	Group A	4	2.4398	.21720	.10860	2.0941	2.7854	2.16	2.66
	Group B	4	2.3952	.21577	.10789	2.0519	2.7386	2.08	2.55
	Group C	4	2.4605	.06190	.03095	2.3620	2.5590	2.41	2.54
	Total	12	2.4318	.16558	.04780	2.3266	2.5370	2.08	2.66
One in thiry two	Group A	4	2.3715	.13139	.06569	2.1624	2.5806	2.25	2.53
	Group B	4	2.3822	.13141	.06571	2.1731	2.5914	2.23	2.55
	Group C	4	2.3880	.18548	.09274	2.0929	2.6831	2.22	2.57
	Total	12	2.3806	.13730	.03963	2.2933	2.4678	2.22	2.57

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
One in16	Group A	4	2.3585	.25620	.12810	1.9508	2.7662	2.13	2.65
	Group B	4	2.3568	.04551	.02276	2.2843	2.4292	2.31	2.42
	Group C	4	2.3770	.08871	.04435	2.2358	2.5182	2.30	2.49
	Total	12	2.3641	.14389	.04154	2.2727	2.4555	2.13	2.65
One in8	Group A	4	2.3332	.22763	.11382	1.9710	2.6955	2.14	2.59
	Group B	4	2.3250	.05339	.02669	2.2401	2.4099	2.28	2.40
	Group C	4	2.3030	.14521	.07260	2.0719	2.5341	2.10	2.45
	Total	12	2.3204	.14435	.04167	2.2287	2.4121	2.10	2.59
One in4	Group A	4	2.1620	.21738	.10869	1.8161	2.5079	2.01	2.48
	Group B	4	2.2983	.19527	.09764	1.9875	2.6090	2.11	2.56
	Group C	4	2.3008	.15015	.07508	2.0618	2.5397	2.18	2.51
	Total	12	2.2537	.18445	.05325	2.1365	2.3709	2.01	2.56
One in2	Group A	4	2.0905	.05277	.02638	2.0065	2.1745	2.03	2.15
	Group B	4	2.2982	.13478	.06739	2.0838	2.5127	2.18	2.49
	Group C	4	2.1438	.02955	.01477	2.0967	2.1908	2.12	2.18
	Total	12	2.1775	.12009	.03467	2.1012	2.2538	2.03	2.49
One in1	Group A	4	2.0888	.05596	.02798	1.9997	2.1778	2.03	2.14
	Group B	4	2.1810	.08203	.04101	2.0505	2.3115	2.07	2.26
	Group C	4	2.1215	.22214	.11107	1.7680	2.4750	1.81	2.32
	Total	12	2.1304	.13318	.03845	2.0458	2.2150	1.81	2.32
No dilution	Group A	4	2.0157	.07667	.03833	1.8938	2.1377	1.92	2.10
	Group B	4	1.9312	.01413	.00706	1.9088	1.9537	1.92	1.95
	Group C	4	2.1178	.13510	.06755	1.9028	2.3327	1.97	2.30
	Total	12	2.0216	.11392	.03289	1.9492	2.0940	1.92	2.30

ANOVA (TABLE 12)

		Sum of Squares	Df	Mean Square	F	Sig.
Zero	Between Groups	.009	2	.004	.137	**.874**
	Within Groups	.293	9	.033		
	Total	.302	11			
One in thiry two	Between Groups	.001	2	.000	.012	**.988**
	Within Groups	.207	9	.023		
	Total	.207	11			
One in16	Between Groups	.001	2	.001	.020	**.980**
	Within Groups	.227	9	.025		
	Total	.228	11			
One in 8	Between Groups	.002	2	.001	.039	**.962**
	Within Groups	.227	9	.025		
	Total	.229	11			
One in 4	Between Groups	.050	2	.025	.701	**.521**
	Within Groups	.324	9	.036		
	Total	.374	11			
One in 2	Between Groups	.093	2	.047	6.403	**.019**
	Within Groups	.065	9	.007		
	Total	.159	11			
One in1	Between Groups	.017	2	.009	.443	**.655**
	Within Groups	.178	9	.020		
	Total	.195	11			
No dilution	Between Groups	.070	2	.035	4.301	**.049**
	Within Groups	.073	9	.008		
	Total	.143	11			

Multiple Comparisons (TABLE 13)

Tukey HSD							
Dependent Variable	**(I) Groups1**	**(J) Groups1**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
Zero	Group A	Group B	.04450	.12752	.936	-.3115	.4005
		Group C	-.02075	.12752	.986	-.3768	.3353
	Group B	Group A	-.04450	.12752	.936	-.4005	.3115
		Group C	-.06525	.12752	.868	-.4213	.2908
	Group C	Group A	.02075	.12752	.986	-.3353	.3768
		Group B	.06525	.12752	.868	-.2908	.4213
One in thirty two	Group A	Group B	-.01075	.10719	.994	-.3100	.2885
		Group C	-.01650	.10719	.987	-.3158	.2828
	Group B	Group A	.01075	.10719	.994	-.2885	.3100
		Group C	-.00575	.10719	.998	-.3050	.2935
	Group C	Group A	.01650	.10719	.987	-.2828	.3158
		Group B	.00575	.10719	.998	-.2935	.3050
One in16	Group A	Group B	.00175	.11223	1.000	-.3116	.3151
		Group C	-.01850	.11223	.985	-.3319	.2949
	Group B	Group A	-.00175	.11223	1.000	-.3151	.3116
		Group C	-.02025	.11223	.982	-.3336	.2931
	Group C	Group A	.01850	.11223	.985	-.2949	.3319
		Group B	.02025	.11223	.982	-.2931	.3336
One in 8	Group A	Group B	.00825	.11236	.997	-.3055	.3220
		Group C	.03025	.11236	.961	-.2835	.3440
	Group B	Group A	-.00825	.11236	.997	-.3220	.3055
		Group C	.02200	.11236	.979	-.2917	.3357
	Group C	Group A	-.03025	.11236	.961	-.3440	.2835
		Group B	-.02200	.11236	.979	-.3357	.2917

Tukey HSD							
Dependent Variable	(I) Groups1	(J) Groups1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
One in 4	Group A	Group B	-.13625	.13412	.586	-.5107	.2382
		Group C	-.13875	.13412	.575	-.5132	.2357
	Group B	Group A	.13625	.13412	.586	-.2382	.5107
		Group C	-.00250	.13412	1.000	-.3770	.3720
One in 2	Group A	Group B	-.20775*	.06031	**.018**	-.3761	-.0394
		Group C	-.05325	.06031	.664	-.2216	.1151
	Group B	Group A	.20775*	.06031	**.018**	.0394	.3761
		Group C	.15450	.06031	**.072**	-.0139	.3229
	Group C	Group A	.05325	.06031	**.664**	-.1151	.2216
		Group B	-.15450	.06031	**.072**	-.3229	.0139
One in1	Group A	Group B	-.09225	.09934	**.637**	-.3696	.1851
		Group C	-.03275	.09934	**.942**	-.3101	.2446
	Group B	Group A	.09225	.09934	**.637**	-.1851	.3696
		Group C	.05950	.09934	**.824**	-.2178	.3368
	Group C	Group A	.03275	.09934	**.942**	-.2446	.3101
		Group B	-.05950	.09934	**.824**	-.3368	.2178
No dilution	Group A	Group B	.08450	.06368	**.416**	-.0933	.2623
		Group C	-.10200	.06368	**.294**	-.2798	.0758
	Group B	Group A	-.08450	.06368	**.416**	-.2623	.0933
		Group C	-.18650*	.06368	**.040**	-.3643	-.0087
	Group C	Group A	.10200	.06368	**.294**	-.0758	.2798
		Group B	.18650*	.06368	**.040**	.0087	.3643
*. The mean difference is significant at the 0.05 level.							

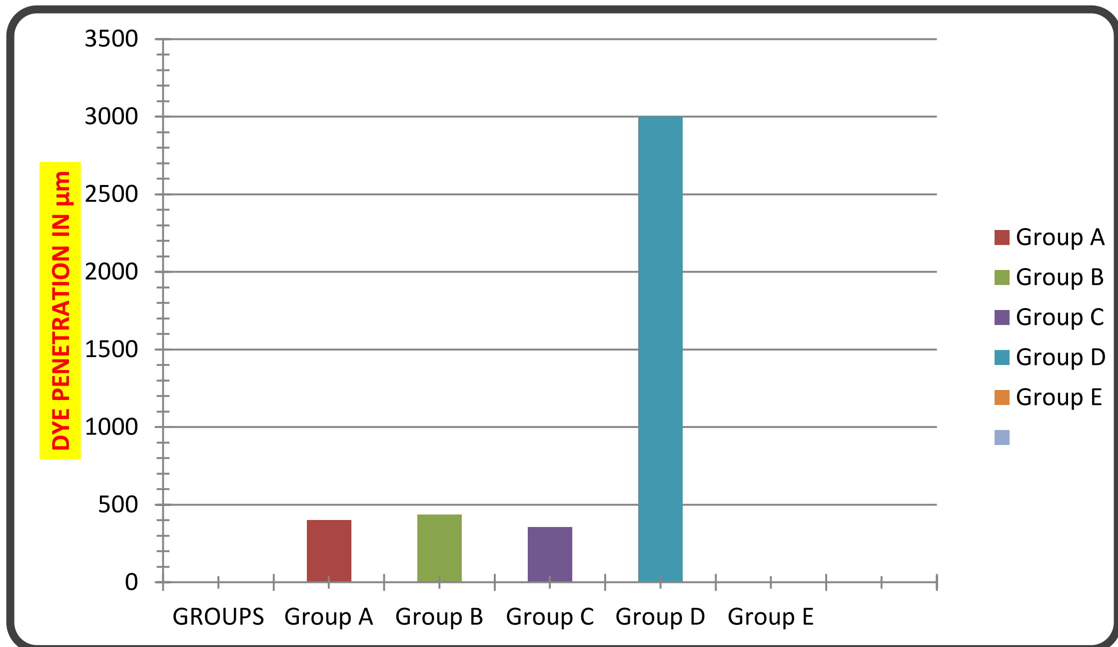
INTERPRETATION OF RESULTS FOR CYTOTOXICITY

Pair wise comparison using Anova revealed that the optical density among the three cements was statistically significant i.e. P= 0.049 (Graph 2). On individual comparison of the mean difference between all three Groups using Tukey's post hoc test it was observed that:

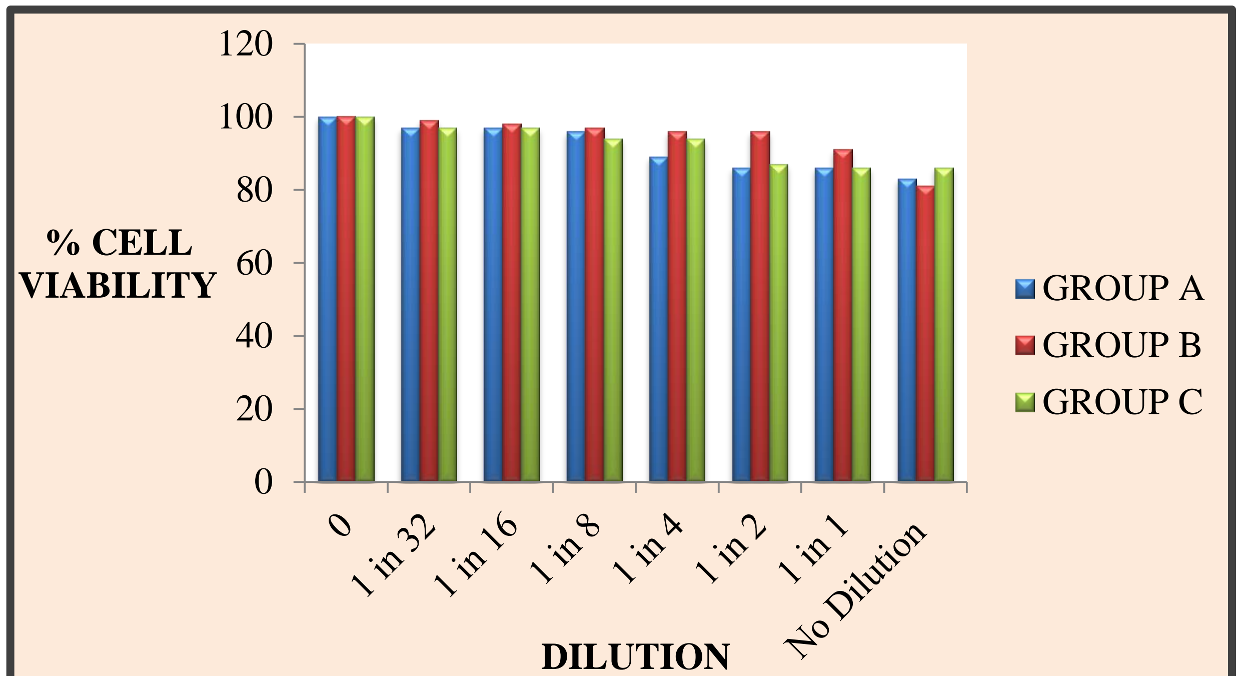
1. The mean difference of optical density between Group A and Group B was statistically significant i.e. P=0.018 when the extracts from the Groups have been diluted at a concentration of 1:2 which means that the cytotoxicity is less in Group B than Group C and Group A.
2. The mean difference of optical density between Group B and Group C was statistically significant i.e. P=0.040 when the extracts from the Groups have not been diluted which means the cytotoxicity is less in Group C than Group A and Group B.

All the three dental cements (Group A, B & C) exhibited cytotoxicity, which does not exceed 20% when compared to L929 mouse fibroblast cells.

GRAPH 1- COMPARISON OF DYE PENETRATION AMONG THE GROUPS



GRAPH 2- COMPARISON OF CYTOTOXICITY AMONG THE GROUPS



Discussion

The outcome and success of root canal treatment depends upon thorough debridement of infected canal contents with aid of cleaning and shaping followed by obturation in order to obtain fluid tight seal between pulp space and periradicular area.[69] Due to failure of conventional endodontic treatment the role of surgical approach to salvage the teeth comes into play[25], which involves root end resection and retro preparation followed by filling of root end cavity with a retrograde material in order to completely seal the root end against microleakage.[68,26]

Root end resection helps in elimination of ledges, perforation defects, anatomical variations e.g. apical delta (branching pattern of small accessory canals and minor foramina seen at the tip or apex of some tooth roots), resorptive defects, and canal obstruction and separated instruments that may be present in the area of root.

An ideal retrograde filling material should provide a perfect apical seal that prevent microleakage, should stimulate the formation of tissues, should be biocompatible with the periradicular tissues, should be insoluble to tissue fluids, non- resorbable, radiopaque, dimensionally stable and should be easy to handle and manipulate.[14, 48, 65].

Also the root end filling material must set soon as it comes in contact with oral hard tissues to allow dimensional stability of the filling material and to confer adequate strength to avoid displacement during its placement [27]. Materials that have been used as root end filling materials include amalgam, Gutta percha , cavit , intermediate restorative

material (IRM), Super EBA, glass ionomer cement (GIC), composite resins , carboxylate cement , zinc phosphate cement , zinc oxide eugenol cement , MTA and Biodentine [14, 48].

Initially amalgam was widely used as a root end filling material but due to its dis advantages, modified zinc oxide eugenol compounds like IRM and Super EBA cements were used[20]. Since .both Super EBA and IRM contain eugenol, concern has been expressed about possible harmful effects on the periapical tissues[20]. Later glass ionomer cement was taken due to its chemical adhesion to tooth structure[16.] However the sealing ability of glass ionomer cement gets severely affected due to contamination of it during placement in the root end cavity[16].

Torabinejad at Loma Linda University in 1993 introduced MTA, whose major constituents are tricalcium silicate, tricalcium aluminate, tricalcium oxide, bismuth oxide, silicate oxide . MTA is used in variety of endodontic applications such as pulp capping , perforation repair , apexification , apexogenesis, root end filling material and pulpotomy due to its unique feature of antibacterial nature , good compressive strength (67 Mpa) , biocompatibility , more radiopacity then conventional gutta-percha and dentin and thus easily distinguishable on radiographs, better sealing ability due to its slight expansion upon setting, has ability to set in presence of moisture and blood and its capacity to promote hard tissue formation including deposition of cementum[74,34]. When exposed to physiologic fluid there is formation of hydroxyapatite

like structure which can release calcium and phosphorous continuously thus promoting remineralization of hard tissues and forms chemical bond between MTA and dentin[55].

However, MTA is difficult to handle due to its granular consistency , slow setting time (2 hr 45 mins), initial looseness resulting in possible displacement out of the retrograde cavity[74,34.]once the mixture starts to dry its loses its cohesiveness and becomes hard to handle[13]. Besides, MTA has an alkaline pH. During setting, MTA changes from pH 10.2 to pH 12.5 in 3 h. Because of the interaction between MTA and the organic phase of dentin, this result in a degradation of type 1 collagen and the micro hardness of dentin is altered.

New experimental active Ca_3SiO_5 based restorative cement is introduced by name of Biodentine™ (Septodont , Saint – Maurdes –Fosses, France). It is available in form of powder and liquid. Powder is composed of tricalcium, dicalcium silicate, calcium carbonate, zirconium dioxide. In liquid calcium chloride is added in aqueous solution in increase its setting time. This Biodentine has to be triturated for 30 s prior to insertion. The setting time is about 12 min. Consistency of Biodentine is similar to that of phosphate cement. The interfacial properties of dentine Biodentine interface were studied under microscope and tag like microstructures were detected. The flowable consistency of Biodentine penetrates dentinal tubules and helps in mechanical properties of interface[9]. Being osteoinductive in nature Biodentine helps in stimulation of immature cells to develop into preosteoblast. There are various applications of Biodentine in field of

endodontics like perforation repairs, crown and root dentine repair material, repair of resorptive defects, apexifications. With the addition of setting accelerators and softeners made its manipulation easy.

It has also certain advantages over MTA i.e. short setting time (12 min), easy manipulation , better compressive strength , no effect of blood contamination on its physical properties and cost effective and hence can be suggested as an alternative to MTA as a root end filling material[84].

One of the new materials that might potentially provide the necessary properties of
a root-end filling material is polymethylmethacrylate (PMMA) bone cement. It is widely used in orthopedic surgery, mainly for fixation of the prosthesis but also for stabilizing compressive vertebral fracture or filling bone defects[77].Commercial acrylic bone cements are supplied as 2 components, a powder polymer and a liquid monomer that are mixed at the time of application. PMMA bone cement has excellent adaptation to the cavity margins, in spite of the well-known polymerization shrinkage of acrylics. This is because the volume of cement increases to a maximum during polymerization before shrinking slightly, although not to its initial volume. The cement also tolerates a moist environment very well and is not affected by blood contamination[77].

However in the present study the bone cement which has been used is bioactive (Surgiwear) which is non ceramic Hydroxyapatite bone material which has two

components Calcium phosphate powder and a setting liquid. The powder is made of tri-calcium phosphate and tetra calcium phosphate. The fluid has different sodium and calcium salts. The two parts are mixed and putty like material is obtained. It can be used to fill the defects. Putty can be moulded to any shape by hands. Being putty and easy mouldability, it provides very good contact with bony tissues, resulting in better healing.

In the present study the objective was to compare the sealing ability and cytotoxicity of Bone cement, MTA, Biodentine when it is used as a root end filling material.

SEALING ABILITY

In our study root end resection was done by resecting 3 mm of root end perpendicular to long axis[8] of the root to remove all the apical anatomic variations like accessory canals, apical delta, and isthmus etc[42]. Resection done at an angle exposes greater surface area of dentinal tubules and increases its permeability leading to apical leakage[23] and hence resection at 90º angle was done for this study.

In this study, ultrasonic retro tip (Satelec P14D) was used to prepare the retro cavity as it produces cleaner, conservative and deeper cavity centered in root canal[63]. It has been confirmed by the studies that use of ultrasonic instruments create fewer micro fractures than burs during root end cavity preparations[48]. Also depth of root end cavity preparation plays a significant role in achieving hermetic apical seal. Studies have demonstrated that a

3 mm deep class I cavity for root end filling reduced apical leakage[23] and therefore a 3mm conventional class I cavity was prepared in this study.

Several methods are available to evaluate leakage studies of root end filling materials; they include dye penetration, bacterial leakage, electrochemical means radioisotope or fluid filtration method[48, 31]. Despite criticism, dye leakage tests are still in practice as they are cheaper, simpler, safer and easier to handle than radioisotopes[23]. This consists of semi quantitative analysis, often involving only one plane of view[48]. Torabinejad et al. (1993) stated that a material that is able to prevent the penetration of small molecules (dye) should be able to prevent larger substances like bacteria and their byproducts[20, 31]. In this study Rhodamine B was used as a dye where the specimens were immersed in 0.5% aqueous solution. Rhodamine B which is water soluble fluorescent dye which is easily detectable , even in low concentrations , moves freely along the interface , low toxicity and are stable in aqueous environment , stable in varying pH, nondestructive to the substrate or material in contact[72]. Rhodamine B can be applied in studies of dye penetration because it has smaller particles, presenting a great diffusibility in dentinal tubules, which is easily visualized under confocal microscope[11].

Confocal laser scanning microscope is a non-destructive technique of visualizing the extent of dye penetration. Certain advantage of it includes visualizing subsurface tissue features indicating the clear indication of leakage limits, due to lens focus that can occur

some microns beneath the observed surface. This also helps in stain spread caused by specimen sectioning and reduces polishing artifacts that can increase dye penetration depths[86]. It also eliminates the scattered, reflected and fluorescent light from various other planes, increased clarity in focal plane[43]. It does not require any specific sectioning technique thus decreasing the possibility of artifacts produced during preparation of specimens as compared to SEM which requires dehydration and sputter-coating procedure.

The mean dye penetration values were found to be significantly higher for MTA i.e. Group B (429.80± 21.72) followed by Bone cement i.e. Group A (391.40± 37.16) and Biodentine i.e. Group C (361.80± 86.99).

The probable reasons could be:

1. Biodentine forms tag like structures when it comes in contact with dentine as an interfacial layer called the "mineral infiltration zone", where the alkaline caustic effect of calcium silicate cements hydration products degrades the collagenous component of interfacial dentine [59].

2. The sealing ability of Biodentine is most likely due to formation of tags. Han and Okiji showed that there is calcium and silicon ion uptake in dentine which leads to formation of tag like structures in Biodentine which was higher than MTA[30].

3. Better sealing ability of Biodentine can be credited because of addition of setting accelerators and softeners; a new pre-dosed capsule formulation for use in mixing device improves its sealing ability.

4. Biodentine has better handling properties thus adaptation to cavity walls is better which improves its sealing ability.

5. It has fast setting time (12min) thereby sealing the interface earlier to increase the amount of micro leakage and bacterial contamination.

6. It has smaller particle size which adapts to cavity surface sealing its interface.

7. Porosity and pore volume in set Biodentine material is also less than MTA that could be one of the reasons for better sealing ability.

Ankita Khandelwal et al[6] compared the micro leakage using MTA and Biodentine as root end filling material using Rhodamine B dye and concluded that Biodentine showed significantly less micro leakage than MTA.

There was no significant difference between Group A (Bone Cement) and Group B (MTA) as MTA is a well-tested material and is used in various endodontic application. It contains principal ions of hard tissue i.e. calcium and phosphorous which makes it biocompatible with cells and tissues. However MTA has a few drawbacks namely difficulty in handling, slow setting, surface disintegration which may lead to micro leakage, loss of marginal adaptation.

While bone cement has many characteristics, that make it potential root end filling material. This is because it has excellent interlocking of cement of soft and hard tissues of bone without cell necrosis[32]. Also Bone cement on exposure to stimulated tissue fluid (physiologic fluid), it gets covered with layer of apatite crystals which nucleate and grow, filling the microscopic spaces between bone cement and dentinal wall. Another property which makes bone cement as a substitute in retrograde filling is its osteoinductive in nature, thus acts as a medium for crystal growth and nucleation. This bone cement has better handling properties as it can be manipulated to dough form and easily placed in root end filling cavity and shorter setting time as after mixing the powder and liquid it is usable for 3-5 minutes and unaffected by presence of moisture, thus having no tendency to be out in presence of oral fluids.

All the positive controls showed dye penetrations throughout the cavities thus confirming that root end filling material was necessary to prevent micro leakage.

All the negative controls showed no dye penetration.

CYTOTOXICITY

It is utmost necessary to know the toxic effects of root end filling material, as they are in direct contact with periradicular tissue and any damage or irritation could cause degeneration of periapical tissues and can cause delay in wound healing[29]. Fibroblast are the cells which are associated with healing process[38,49] and thus it is important to evaluate the cytotoxic nature of the cements which are placed in direct contact with the tissues. To check the biocompatibility of root end filling material an in vitro cytotoxicity is the first test to be done. In vitro test are simple, inexpensive to perform, provide significant amount of information, can be conducted under controlled conditions and may elucidate the mechanism of cellular toxicity[62]. The cell lines which are used in this study are established L929 cell lines which are mouse fibroblast commonly used in biocompatibility[49] studies as they are easy to prepare and culture and provide more reproducible results[2].

Cytotoxicity of materials can be checked in cultured mammalian cells by variety of test systems. Permeability assays monitor the integrity of cell membranes by inclusion or exclusion of vital dyes, or by release of radiolabeled chromium. Replication assays indirectly assess the ability of the cells to proliferate by measuring the incorporation of nucleotide analogues that have been radiolabeled or are detectable by immunoassay during DNA synthesis. Morphological studies evaluate the changes in cellular

cytoskeleton or at cell surface. Functional assays typically evaluate the cells ability to provide energy necessary for anabolic activities, or the end products of such additives[29, 40]

The assay used in this study was MTT assay which uses tetrazolium salt MTT to measure mitochondrial dehydrogenase activity. It is pale yellow substrate that produces a dark blue formazan product when cleaved by active mitochondria, and so the reaction only occurs in living, metabolically active cells[29, 40]. It is important to use a particular test system which is in consonance with the chemical nature of the material being tested. Therefore if a test system is less likely to cause change in cell membrane permeability, then it is less apt to determine cytotoxicity in a valid manner[40].
Since MTA is hydrophilic substance it is likely that it will release ionic components which would interfere with the intracellular enzyme activities of the cell rather than influence its membrane permeability[29, 40]. Therefore MTT assay was chosen for the present study as it is not only quantitative and reproducible, but also can test materials in a fresh and set state[2].

It is the amount of viable cells on which cytotoxicity of material depends. More the cells are viable less is the cytotoxicity of material. Elutes (extracts) of the test materials were used in the present investigation as they can be easily sterilized by filtration, as direct sterilization of the test materials introduces the possibility of changing the properties of material . Also the ability of examining the effect of materials on cells that are both

distant and in contact with them can also is evaluated[29, 40]. It also stimulates the immediate postsurgical root end environment in which toxic elements of retro – filling materials leach into the surrounding fluids in bony crypt[29, 40]. A series of extracts of different concentrations were made to observe a possible dose response relationship and to determine the most ideal concentration for sensitivity of cells tested[45].

According to the results of this study, Group C (Biodentine) showed increased percentage of cell viability when compared to Group A (Bone Cement) and Group B (MTA).

The comparison of optical density values between three different materials (Bone cement, MTA, Biodentine) was carried out and it was found that the Group C (Biodentine) is more biocompatible or less cytotoxic when compared to Group A (Bone cement) and Group B (MTA) when the experimental groups are not diluted. To observe a possible dose response relationship between the material and the cells, a series of extracts of various concentrations were made in the present study, extracts derived from all the test materials were examined on L929 mouse fibroblast cells for their viability and it's clearly brought to notice that cell motility and survival were highly dependent on extract concentration and in our present study it was observed that at no dilution , the cytotoxic effect is more in MTA than Bone Cement and least with Biodentine (Graph 2).

On review of literature, many studies have investigated the cytotoxicity of MTA and found it to be biocompatible and non – genotoxic[29, 36]. According to the degree of cytotoxicity based on cell viability, more than 90% cell viability was considered non cytotoxic, 60-90 % as mildly cytotoxic, 30-59% as moderately cytotoxic and less than 30% was considered as strongly cytotoxic[29]. In this study all the Groups (A, B, C) falls in range of mild cytotoxicity.

Based on the findings of Biodentine and MTA, Balto et al[89] reported good spread and high density of attached human periodontal ligament fibroblast to the surface of set specimens of MTA. Recently it was reported that one of major leached components of MTA and Biodentine were calcium ions. Since calcium plays a potential role in fibroblast adhesion, the constant release of calcium ions is essential regarding the attachment of cells to the surface of the material[3]. When exposed to physiologic solutions there is hydration of tri calcium silicate which produces a calcium silicate gel and crystalline calcium hydroxide which later precipitates to form hydroxyapatite layer. A suitable platform for cell adhesion is created due to formation of calcium induced uneven crystalline surface matrix on the test materials, which strongly indicates the enhanced biological performance of the bioactive Biodentine material[44].

The purpose of choosing Bone cement in this study is because it is comparatively a newer repair material in the field of dentistry, but it has been already in use in field of

maxillofacial and orthopaedic surgery for past 40 years. It has various characteristics like good strength and load bearing capacity, faster setting time of around 15 mins, tolerates moist environment very well and low cytotoxicity comparable to MTA[85], can be used both as retrograde and graft material .Also Bone cement is already being used in field of oral surgery in injectable form for filling of post traumatic bone defects in orbital, periorbital and malar regions. The Bone cement which was chosen was already bioactive consisting of hydroxyapatite, calcium phosphate powder and in liquid it has various sodium and calcium salts. These calcium salts on exposure to tissue fluids forms a layer of apatite crystals and provide a suitable platform for cell adhesion. Also Bone cement being osteoinductive in nature acts as a medium for crystal growth and nucleation[89].

In this study, viabilities of cells exposed to extracts from Group A, B, C were dependent on extract concentrations (dilution). As the dilution of the extracts increases the amount of cell viability also increases thereby stating the fact that diluted extract concentrations proved to be less cytotoxic.

Biodentine performed superior in terms of both sealing ability and cytotoxicity however in this study, it was an innovative approach of using Bone cement as it has both sealing ability and cytotoxicity better than MTA.

Summary

SUMMARY

The present study was undertaken for comparative evaluation of the sealing ability and cytotoxicity of Bone cement, MTA and Biodentine as retro filling material.

Fifty maxillary central incisors which were caries free and extracted due to periodontal reasons were chosen for this study. The teeth were cleaned with ultrasonic scaler and disinfected in 0.5% chloramine solution for 2 weeks. Then they were stored in saline until use. The teeth were randomly divided into five groups of 10 teeth each.

Group A, B, C were the experimental groups and Group D was Positive Control (No cements placed as retrograde filling material) and Group E was Negative Control (The teeth were coated with nail varnish at the root apex).

The above mentioned groups were evaluated for the following study parameter.

<u>SEALING ABILITY</u>– The teeth were decoronated at the cementoenamel junction using a diamond disc to obtain a standard root length of 14 mm. A round bur was used to gain access and straight line entry to the root canal was obtained. Cleaning and shaping was done with manual K files and nickel titanium rotary system (Protaper rotary system Dentsply / Maillefer, Tulsa, OK, USA) upto F5. After each instrumentation the canal was irrigated with 2 ml of 2.5% sodium hypochlorite solution and 2 ml of normal saline with a 27 – gauge needle. . Then the canals were obturated with Gutta- percha Protaper F5 and AH plus sealer. Then the apical third of each root was resected at 3 mm level perpendicular to the long axis of the root using a high speed hand piece with a diamond disk. Retrograde preparation of 1.5 mm diameter and 3 mm depth was prepared using an

ultrasonic tip (Satelec P14D). The retro cavities were filled with test materials. The apical part of tooth specimens were immersed in 0.5% aqueous rhodamine dye for 48 hours. The teeth were then mounted in acrylic block of 18mm X 30mm dimensions and then sectioned along the long axis of tooth to get section of 1mm thickness using a hard tissue microtome (Leica SP 1600 Leica Biosystems, Melbourne, Australia) and then the depth of dye penetration were observed in the section of 1 mm under LSM 510 Meta Confocal Laser Scanning Microscope (Carl Zeiss).

All the results were statistically analysed using One way Anova and Post hoc tukey test. Based on the results obtained and the statistical analysis, the following conclusion were drawn that Group C (Biodentine) (361.80 ± 86.99) showed the least microleakage followed by Group A (Bone cement) (391.40± 37.16) and Group B (MTA) (429.80± 21.72). Hence this suggest that the sealing ability of Biodentine is comparatively better than the Bone cement and MTA and also Bone cement being a newer material and better than MTA, can be used as retrograde filing material in future.

Cytotoxicity- L929 mouse fibroblast cells were used and were grown in DMEM culture media in order to check for cell viability of the experimental groups after a suitable amount of L929 cells are acquired. The cements were mixed according to manufacturer's instruction and were placed in individual wells in a 6-well culture plate. In order to prepare extracts of the experimental groups this 6-well culture plate was incubated at

37ºC under control humidified atmosphere. Serial dilution was performed by transferring one volume of the concentrated extract to the well containing an equal volume of media to prepare 1:1 dilution of the dental cement extract and subsequent dilutions were prepared by the transfer and mixing of an equal volume of the previous dilution with an equal volume of culture media .Cell viability was checked using MTT assay.

The intensity of the colour i.e. OD (optical density) developed was absorbed at 540 nm in a multimode microplate reader and checked under inverted phase contrast microscope. All the results were statistically analysed using One way Anova and Post hoc tukey test. Based on the results obtained and the statistical analysis the following conclusion were drawn, Group C (Biodentine) showed increased percentage of cell viability when compared to Group A (Bone Cement) and Group B (MTA).

The degree of cytotoxicity depends on the serial dilution of the extracts i.e. in state of no dilution Group C was least cytotoxic than Group A and Group B, whereas when serial dilution of extract was 1:2 Group B was least cytotoxic than Group C and Group A. However all the cements which were tested showed mild degree of cytotoxicity and are comparable.

Within the limitations of the study, it can be concluded that Biodentine has better sealing ability and cell viability as compared to MTA and Bone cement .However the seal and cell viability provided by Bone cement was better than MTA and thus seems to be an excellent and promising material which can be used as retro filling material in future.

Conclusion

CONCLUSION

From the present study it can be concluded that:

1. Group C (Biodentine) had better sealing ability than Group A (Bone cement) and Group B (MTA).
2. Sealing ability of Bone cement is on par with Biodentine as a retrofilling material.
3. Cytotoxic evaluation of all three groups revealed Biodentine the most biocompatible material.
4. All the three cements exhibited mild cytotoxicity within the acceptable limits.
5. Cells exposed to extracts from Biodentine, Bone Cement, MTA showed that the concentration of extracts plays important role in determining the cell viability of the tissues which are in direct contact with the cements.
6. Since Bone cement is comparable with Biodentine and better in terms of sealing ability and cytotoxicity than MTA, it makes it a possible material for retrofilling procedure.

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