COMPARATIVE EVALUATION OF FLUORIDE RELEASE AND COMPRESSIVE STRENGTH OF MODIFIED BIODENTINE USING 7 WT % SODIUM FLUOROSILICATE AND 1O W/V % OF 20 % HYDROFLUORIC ACID - AN INVITRO STUDY

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in partial fulfillment of the requirements
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BRANCH – IV

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2014 – 2017

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled "COMPARATIVE EVALUATION OF FLUORIDE RELEASE AND COMPRESSIVE STRENGTH OF MODIFIED BIODENTINE USING 7 WT % SODIUM FLUOROSILICATE AND 1O W/V % OF 20 % HYDROFLUORIC ACID - AN INVITRO STUDY" is a bonafide and genuine research work carried out by me under the guidance of Dr.M.KAVITHA M.D.S., Professor and HOD. Department Of Conservative Dentistry and Endodontics, TamilNadu Government Dental College and Hospital, Chennai -600003.

S. VELAYUDHAM

CERTIFICATE BY GUIDE

This is to certify that Dr S. VELAYUDHAM, 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 FLUORIDE RELEASE AND COMPRESSIVE STRENGTH OF MODIFIED BIODENTINE USING 7 WT % SODIUM FLUOROSILICATE AND 1O W/V % OF 20 % HYDROFLUORIC ACID - AN INVITRO 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.M.KAVITHA M.D.S.,
Professor & HOD.
GUIDE
Department of Conservative Dentistry and Endodontics.
TamilNadu Government Dental College and Hospital
Chennai- 600003

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

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

TamilNadu Government Dental College and Hospital.
Chennai- 600003.

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DECLARATION

TITLE OF DISSERTATION	**Comparative evaluation of fluoride release and compressive strength of modified Biodentine using 7 wt % sodium fluorosilicate and 10 w/v % of 20 % hydrofluoric acid - an invitro study**
PLACE OF STUDY	**TAMIL NADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL**
DURATION OF COURSE	**3 YEARS**
NAME OF THE GUIDE	**DR M.KAVITHA**
HEAD OF THE DEPARTMENT	**DR M. KAVITHA**

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This agreement herein after the "Agreement" is entered into on this day Dec 2016 between the Tamil Nadu Government Dental College and Hospital represented by its Principal having address at Tamil Nadu Government Dental College and Hospital, Chennai - 600 003, (hereafter referred to as, 'the college')

And

Mrs. Dr. M. Kavitha aged 46 years working as Professor& HOD in Department of Conservative Dentistry &Endodontics at the college, having residence address at 69/4, Mettu street, Ayanavaram, Chennai- 600 023 (herein after referred to as the Principal Investigator')

And

Mr.Dr.S.Velayudham aged 30 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').

Whereas the PG student as part of her curriculum undertakes to research on "COMPARATIVE EVALUATION OF FLUORIDE RELEASE AND COMPRESSIVE STRENGTH OF MODIFIED BIODENTINE USING 7 WT % SODIUM FLUOROSILICATE AND 1O W/V % OF 20 % HYDROFLUORIC ACID AN INVITRO STUDY"for which purpose the Principal Investigator shall act as principal investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co-investigator.

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Witnesses

Guide

1.

2.

ABSTRACT

AIM : The aim of this study is to evaluate the fluoride releasing properties and compressive strength of Biodentine modified with 7 wt% sodium fluorosilicate and with 10 w/v % of 20 % hydrofluoric acid using Spadns spectrophotometer &Instron Universal Testing machine respectively.

MATERIALS AND METHODS: The study comprised of a total of 80 samples divided into 4 groups of 20 samples in each group. Out of the 20 samples ,10 samples were allocated for fluoride analysis and 10 samples were destined for compressive strength analysis. Group A -Biodentine powder only modified with 7wt% $Na_2[SiF_6]$,Group B -Biodentine liquid only modified with 10 w/v % of 20% HF ,Group C - Biodentine powder modified with 7wt% $Na_2[SiF_6]$ & Biodentine liquid modified with 10 w/v % of 20% HF ,Group D - Glass Ionomer cement type II (positive control). Fluoride release was assessed at 24 hr ,3^{rd} day,7^{th} day and cumulatively thereafter on 2^{nd},3^{rd}& 4^{th} weeks. The 24 hr compressive strength was assessed by Instron Universal Testing machine.

RESULTS: At 24 hour, the fluoride release of Group A was higher than Group D which was statistically significant..On 3^{rd} day Group C showed higher fluoride release than Group D which was not statistically significant.On,7^{th} 14^{th}& 21^{st} days the Group C showed higher fluoride release than Group D .On 28^{th} day Group A had higher fluoride release followed by Group C & Group D which was not statistically significant. The 24 hr compressive strength found to be highest for Group Dfollowed by Group B ,Group A and the least compressive strength was for Group C .

CONCLUSION: The powder only modified Biodentine showed appreciable fluoride release without much compromise in the compressive strength.Hence the powder only modified Biodentine can be used as dentin substitute in posterior restorations tapping the fluoride release properties successfully.

KEY WORDS : Biodentine., dentin substitute, fluoriderelease, sodium fluorosilicate

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ABBREVIATIONS USED

MTA	**MINERAL TRIOXIDE AGGREGATE**
GIC	**GLASS IONOMER CEMENT**
SPADNS	**SODIUM 2-(PARASULFOPHENYLAZO)-1,8-DIHYDROXY-3,6-NAPHTHALENEDISULFONATE**
OFMSC	**OROFACIAL BONE MESENCHYMAL STEM CELLS**
PBS	**PHOSPHATE-BUFFERED SALINE**
HDPCS	**HUMAN DENTAL PULP CELLS**
DSP	**DENTIN SIALOPROTEIN**
CBCT	**CONE BEAM COMPUTED TOMOGRAPHY**
USPHS	**UNITED STATES PUBLIC HEALTH SERVICE**
MCPM	**MONOCALCIUM PHOSPHATE MONOHYDRATE**
APF	**ACIDULATED PHOSPHATE FLUORIDE**
GC	**GAS CHROMATOGRAPHY**
ISE	**ION SELECTIVE ELECTRODE**
EPA	**ENVIRONMENTAL PROTECTION AGENCY**
MEM/HF	**MORPHOLINOETHYL METHACRYLATE HYDROFLUORIDE**
TBATFB	**TETRABUTYLAMMONIUM TETRAFLUOROBORATE**
XDR	**X RAY DIFFRACTION**
FTIR	**FOURIER TRANSFORM INFRAREDSPECTROSCOPY**

Introduction

INTRODUCTION

The dental pulp is the neurovascular bundle contained within the pulp space that performs protective, formative, nutritive and sensory functions. But the prime function of the dental pulp is formation of dentine. The loss of dentin is inevitable in cavitated carious lesions. The dentin that is deposited in response to dental caries is called tertiary dentine secreted by differentiation of undifferentiated mesenchymal stem cells of the pulp. Vital pulp therapy procedures aim to achieve this regeneration of dentin by stimulating the undifferentiated stem cells.

An ideal dentin substitute should have good biocompatibility, long-term impermeability, antibacterial properties, ability to induce hard tissue regeneration, good stability, low solubility, non-absorbability, and ease of handling.[4]Many materials have been developed over the years for the replacement of dentin. Calcium hydroxide has been used widely and is one of the earliest introduced cements in the form of dentin substitutes.[43]For replacement of dentin in the coronal region, such as in case of deep carious lesions, materials such as glass ionomer cement were developed.[54]

It makes for an ideal dentin substitute as its physical properties such as coefficient of thermal expansion, dimensional changes, conductivity, opacity and hardness are very close to that of dentin and also its hydrophilicity helps it to bond and adapt well to the dentin surfaces it protects and covers. However it has its own limitation of inability to induce reparative dentin formation.[82]MTA (Mineral trioxide aggregate) another remineralizing dentin substitute with its limitations being difficulty in manipulation, longer setting time, and cost factor. Recently, bioceramics a group of biocompatible ceramic materials were introduced which have the ability to either function as human tissues or to encourage the regeneration of natural tissues.

. Biodentine, a tricalcium silicate based dental material was introduced by Septodont in the year 2010. The product was synthesized de novo and was free from the impurities present in the derivatives of portland cement like MTA.Biodentine allows a dentist to achieve biomimetic mineralisation within the depths of a carious cavity.[61]Biodentine known as "dentine in a capsule"possesses excellent handling[10] properties & better compressive strength. In vitro and short term clinical studies have established the superiority of Biodentine over MTA Biodentine had a similar efficacy in the clinical setting and may be considered an interesting alternative to MTA in pulp-capping treatment during vital pulp therapy.Dentin bridges with the highest average and maximum volumes were formed after the use of Biodentine followed by the use of MTA, $Ca(OH)_2$,. It can be used as dentine substitute [base] for posterior restorations.[57]

Although the properties such as compressive strength, flexural strength,modulus of elasticity, and micro hardness of Biodentine are comparable to glass ionomer which has been well established dentin substitute , the only property that biodentine lack is the fluoride release which is the desirable property of a dental restorative material.

Fluoride has anti cariogenic properties and improves the resistance of the enamel and dentine to acid mediated decalcification. Fluorides can be incorporated into dental materials in order to improve the anticariogenic and properties of the restorative material. Stannous fluoride and Hydrofluoric acid are two acidic fluorides which have the ability to improve the resistance of the dental hard tissues.

The various methods for incorporation of fluoride are 1) simple mixture of water soluble agents 2) dispersion of sparingly water soluble agents 3) use of matrix bound agents.

Hence in our study, we have tried an innovative approach of incorporating sodium fluorosilicate in powder component & HF in liquid component of Biodentine. There is no study in the literature published so far to study the fluoride incorporation and fluoride releasing properties of Biodentine modified with fluoride.

In our study, we have incorporated 10 w/v % of 20 % concentration of HF to the liquid component of Biodentine and 7wt % sodium fluorosilicate to powder component and fluoride release in distilled water was assessed periodically, at 24 hrs,3^{rd} ,7^{th} day and at weekly intervals upto 28 days..

The various methods available for fluoride estimation are 1) Titrimetry where fluoride ions are allowed to react with the titrant and then the solution is treated with an indicator dye, such as Alizarin Red S or SPADNS, 2) Direct potentiometric analysis using fluoride ion selective electrodes 3) Spectrophotometric method

We chose spectrophotometric method , a compound of a metal such as aluminium, iron, thorium, zirconium, lanthanum or cerium reacts with an indicator dye to form a complex of low dissociation constant. This complex reacts with fluoride to give a new complex (Jacobson et al. 1977). Due to the change in the structure of the complex, the absorption spectrum also shifts relative to the spectrum for the fluoride-free reagent solutions. This change can be detected by using a spectrophotometer. In the SPADNS method, zirconium reacts with SPADNS to form a red coloured complex. Fluoride bleaches the red colour of the complex and hence the change in

absorbance can be measured using a spectrophotometer.This method can detect the fluoride with accuracy of 0.1 ppm.

The 24 hr compressive strength of samples were tested using universal testing machine (Instron Corp Canton MA) at a cross head speed of 0.5 mm/min until the sample gets fractured & the compressive strength values were obtained in MPa.

So far, there is no study reported in the literature that has employed experimental modification of Biodentine with fluoride to assess its fluoride releasing properties. We have used one of the most versatile and biocompatible calcium trisilicate material in our study to infer the possibility of making Biodentine a holistic pulp capping material by incorporating fluoride and to confer anticariogenic property to this pulp capping and ideal dentine replacement material to prevent secondary caries which is one of the most commonest causes of restoration failure.[26,12,81]

Hence the purpose of this study is to evaluate fluoride release and compressive strength of modified Biodentine using spadns spectrophotometer & Instron universal testing machine respectively.

Aims & objectives

AIMS & OBJECTIVES

AIM :

The aim of this study is to evaluate the fluoride releasing properties and compressive strength of Biodentine modified with sodium fluorosilicate and hydrofluoric acid using Spadns spectrophotometer & Instron Universal Testing machine respectively.

OBJECTIVES :

1) To incorporate sparingly water soluble sodium fluorosilicate 7wt% $Na_2[SiF_6]$ into the powder component of Biodentine and to assess & quantify the fluoride release of thus modified Biodentine in distilled water using spadns spectrophotometer at 24 hrs, 3rd day, 7th day and at weekly intervals upto 28 days.

2) To admix 20% concentration of hydrofluoric acid (HF) to the liquid component of Biodentine in 10w/v % and to assess & quantify fluoride release in distilled water at 24 hrs, 3rd day ,7th day and at weekly intervals upto 28 days.

3) To modify both powder and liquid components simultaneously with 7wt% sodium fluorosilicate & 10w/v % of 20% HF respectively and to assess & quantify fluoride release in distilled water at 24 hrs, 3rd day, 7th day and at weekly intervals upto 28 days.

4) To evaluate the compressive strength of thus modified Biodentine at 24 hours.

Review of literature

REVIEW OF LITERATURE

BIODENTINE:

Laurent et al (2008)[51] tested a new tricalcium silicate (Ca_3SiO_5) - based material to evaluate its genotoxicity, cytoxicity and effects on the target cells specific function. The study concluded that the Biodentine material is biocompatible. The material was not found to affect the specific functions of the target cells and thus could safely be used.

Pradelle-Plasse N et al (2009)[60]conducted a study to evaluate the bioactivity of Biodentine and described the bioactivity of this material, demonstrating the formation of apatite when immersed in phosphate solution

About et al (2010)[1] investigated Biodentine activity by studying the effects on pulp progenitor cells activation, differentiation and dentine regeneration in the human teeth cultures. The study concluded that biodentine is stimulating dentine regeneration by inducing odontoblast differentiation from pulp progenitor cells.

Gandolfi MG et al (2011)[30]tested thehypothesis that material extracts ofcalcium-releasing calcium-silicate cements support biomimetic microenvironment for survival and differentiation of human orofacial bone mesenchymal stem cells (OFMSCs).Study results revealed that, extracts of calcium silicate cements sustained OFMSC survival, maintained steady state levels of vascular cell adhesion molecule-1, alkaline phosphatase, and bone sialoprotein while up regulating their respective gene transcripts. Thusit was concluded that, ion-releasing calcium silicate cements support a biomimetic microenvironment conducive to survival and differentiation of MSCs

and a combination of OFMSCs and calcium-silicate cement can potentially promote tissue regeneration in periapical bone defects

Han L and Okiji T (2011)[40] compared Biodentine and White Pro Root mineral trioxide aggregate (MTA) with regard to Calcium and Si uptake by adjacent root canal dentine in the presence of phosphate-buffered saline (PBS) &showed that along the material dentine interface, both materials formed a tag-like structure that was composed of either Ca- and P-rich crystalline deposits or the material itself. The Calcium and Si-rich layer width was significantly larger in Biodentine than MTA at 30 and 90 days and concluded that both Biodentine and MTA caused the uptake of Ca and Si in the adjacent root canal dentine in the presence of PBS. The dentine element uptake was more prominent for Biodentine than MTA

PengW et al (2011)[58]conducted an in vitro study to investigate the effects of tricalcium silicate (Ca_3SiO_5) on proliferation and odontogenic differentiation of human dental pulp cells (hDPCs). The MTT assay showed that hDPCs cultured with Ca_3SiO_5 extract proliferated more significantly as compared with Ca(OH)2 extract. Analysis of odontogenic marker genes indicated that Ca_3SiO_5 enhanced the expression of those genes

Atmeh AR et al (2012)[5]The interfacial properties of dentin-Biodentine interface were studied under microscope and tag-like microstructures were detected. They stated that flowable consistency of Biodentine penetrates dentinal tubules and help in the mechanical properties of the interface.

Laurent et al (2012)[52]Biodentine was applied directly onto the dental pulp in a human tooth culture model, resulting in a significant increase of TGF-β1 secretion from pulp cells and thus inducing an early form of dental pulp mineralization shortly after its application.

FirlaM (2012)[29]due to high alkaline pH Biodentine has inhibitory effect on the micro organisms. In addition, the alkaline change leads to the disinfection of surrounding hard and soft tissues.

Raskin A et al (2012)[64]in an invitro study evaluated the microleakage of Biodentine as a dentin substitute compared to Fuji II LC in cervical lining restorations. The study results concluded Biodentine as dentin substitute in cervical lining restorations or as a restorative material in proximal cavities when the cervical extent is under the CEJ.it seems to perform well without any conditioning treatment.

Tran et al (2012)[77] compared Biodentine to MTA and calcium hydroxide in terms of reparative dentine bridge formation by directly applying the materials to mechanically exposed rat pulps , they noted that the structure induced by $Ca(OH)_2$ contained several cell inclusions, also called tunnel defects. These defective regions were regarded as undesirable areas facilitating the migration of the microorganisms towards the pulp and predisposing the tooth to an endodontic infection. On the contrary, the dentine bridge formation induced by Biodentine showed a pattern well-localized at the injury site unlike that caused by calcium hydroxide that exhibited an expanding structure in the pulp chamber. The quality of the formed dentine was also much more favourable compared to calcium hydroxide and an orthodentin organization was noted in which dentine tubules could be clearly visualized.

Moreover, cells secreting the structure well exhibited DSP expression as well as osteopontin expression, which are critical regulators of reparative dentine formation.

Zanini et al (2012)[84] also evaluated the biological effect of Biodentine on immortalized murine pulp cells(OD-21) by analysing the expression of several biomolecular markers after culturing OD-21 cells with or without Biodentine. Their results, consistent with other studies, were in favour of Biodentine, which was found to be bioactive due to its ability to increase OD-21 cell proliferation and biomineralization.

Camilleri J (2013)[16]investigated the bioactivity of Biodentine ,MTA and a new tricalcium silicate cement . The study revealed that all three cements allowed the deposition of hydroxyapatite on the surface. This shows that all three materials arebioactive.

Han L &Okiji T (2013)[41]compared white Pro Root TA(WMTA),Endo Sequence BC sealer (BC sealer) and Biodentine, with regard to their ability to produce apatites and cause Ca- and Si incorporation in adjacent human rootcanaldentine after immersion inphosphate-buffered saline (PBS). All materials produced surface precipitates of a circular or lath-like morphology with Ca/P ratio of 1.6-2.0.Within dentinal tubules, the three materials formed tag-like structures that were frequently composed of Ca- and P-rich and Si-poor materials, suggesting intratubularprecipitation.Ca- and Si-incorporation depths were highest with Biodentine The concentration of released Ca was also found to be highest with Biodentine.

Nowicka et al (2013)[56] did a study on the response of human dental pulp capped with Biodentine and MTA and reported that the majority of specimens showed complete dentinal bridge formation and absence of inflammatory pulpal response. Layers of well arranged odontoblast and odontoblast –like cells were formed to tubular dentine under the osteodentin. Therefore he concluded that within the limitations of his study Biodentine had a good efficacy in the clinical setting and may be considered as an interesting alternative to MTA in pulp capping treatment during vital therapy.

Sanghavi T et al (2013)[68]The ability of mineral trioxide aggregate (MTA), Biodentine and Calcium phosphate cement to seal large furcation perforations were evaluated using a dye-extraction leakage method orthograde direction and dye extraction was performed using fullconcentration nitric acid. Dyeabsorbance was measured at 550 nm using spectrophotometer. Results showed that Pro Root MTA showed the least dye absorbance. Calciumphosphate showed the highest dye absorbance and Biodentin came at intermediate level then other groups

Zhou et al (2013)[85] performed a study , where Biodentine was compared with white MTA(ProRoot) and glass ionomer cement (FujiIX) using human fibroblasts, both white MTA and Biodentine were found to be less toxic compared to glass ionomer during the 1-and 7-day observation period. The authors commented that despite the uneven and crystalline surface topography of both Biodentine and MTA compared to the smooth surface texture of the glass ionomer, cell adhesion and growth were determined to be more favourable in the aforementioned materials compared to glass ionomer.

Butt et al (2014)[13] compared the mechanical and physical properties of MTA and Biodentine. According to this study the compressive strength of MTA in 24 hours was 41 MPa and after 28 days 76.8 MPa in comparison with Biodentine which demonstrated higher values of 170 MPa at 24 hrs and 304 MPa at 28 days respectively. The sealing ability of MTA at 4 and 24 hours was lesser than Biodentine but improved with time. The better handling properties of Biodentine makes it more convenient for clinical applications.

Camilleri et al (2014)[17]compared the hydration capabilities of Theracal LC, a light curable calcium trisilicate material, Biodentine and a prototype tri calcium silicate cement. Theracal LC exhibited incomplete hydration and had a heterogeneous structure because of inadequate moisture to allow hydration to proceed. The composition of Biodentine was optimized, and the environmental conditions did not affect material microstructure. Biodentine exhibited formation of calcium hydroxide and calcium ion leaching, which are beneficial to the dental pulp.

De Rossi & Silva (2014)[21]This study evaluated the pulpal and periapical responses of dogs' teeth after pulpotomy and pulp capping with a tricalcium silicate–based cement(Biodentine) when compared with mineral trioxide aggregate (MTA) by radiographic, histopathologic, and histomicrobiological analyses. They concluded that Biodentine presented tissue compatibility and allowed for mineralized tissue bridge formation after pulpotomy in all specimens withsimilar morphology and integrity to those formed with use of MTA.

Elnaghy AM (2014)[25] conducted the study to determine the influence of acidic environment on the properties of Biodentine and white Mineral TrioxideAggregate

(WMTA) . This study evaluated the microhardness, compressive strength, bond strength and morphologic microstructures of Biodentine and WMTA after exposure to a range of acidic pH levels Biodentine showed higher surface hardness , compressive strength andbond strength to root dentin compared with WMTA after exposure todifferent pH values . A substantial change in the microstructure of Biodentine and WMTA occurred afterexposure to different pH values.WMTA appeared to be more sensitiveto acidic pH environment than Biodentine .Hence Biodentine seems to be more appropriate for use when exposed to acidic environment compared with WMTA.

Hashem et al (2015)[42] performed a randomised controlled study comparing Biodentine and Glass Ionomer Cement (Fuji IX) as indirect pulp capping agents. The patients were followed up for a period of 12 months using baseline intra oral radiographs and CBCT with 12 months post operative intra oral radiographs and CBCT for comparison & concluded Biodentine showed better post operative healing in patients with reversible pulpitis than GIC when assessed clinically, radiographically and using CBCT.

Koruyucu M et al (2015)[49] evaluated the antibacterial properties of MTA, Biodentine and Dycal used as pulp capping materials and found that freshly mixed MTA had the best antibacterial property over time than the other two tested materials.

Simsek et al (2015)[69] compared the biocompatibility of MTA, Bioaggregate (BA) and Biodentine by implanting them in the sub cutaneous tissue of rats. The infiltration of lymphocytes and macrophages in the tissue was assessed histologically and it was found that MTA and BA exhibited more inflammatory reaction than Biodentine and inflammation declined more quickly in the case of Biodentine.

Kim et al (2016)[48] compared the reparative dentin formation of Proroot MTA, Biodentine and Bioaggregate using microcomputed tomography and immunohistochemistry. MicroCT showed that Proroot MTA and Biodentine formed thicker hard tissue barrier formation. Immunohistochemistry revealed that ProRoot MTA showed complete dentin bridge formation with normal pulpal histology. In the Biodentine and BioAggregate groups, a thick, homogeneous hard tissue barrier was observed. The ProRoot MTA specimens showed strong immunopositive reaction for DSP. The authors concluded by stating that calcium silicate-based pulp-capping materials induce favourable effects on reparative processes during vital pulp therapy and that both Biodentine and Bio Aggregate could be considered as alternatives to Pro Root MTA.

COMPRESSIVE STRENGTH:

Grech et al (2013)[36]stated that Biodentine exhibited high washout, low fluid uptake and sorption values, low setting time and superior mechanical properties when compared to bioaggregate, conventional radiopacified tricalcium silicate & intermediate restorative material .The authorsattributed this result to the enhanced strength due to the low water/cement ratio used in Biodentine. They further stated that this mode of the material is permissible as a water solublepolymer is added to the mixing liquid

Kayahan et al (2013)[47] evaluated the compressive strength from another perspective and drew conclusions specifically pertaining to clinical usage. Considering that acid etching is one of the steps following the application of Biodentine for the provision of mechanical adhesion, the authors aimed to assess

whether any alterations exist in terms of compressive strength following the etching procedure. They concluded that acid etching procedures after 7 days did not reduce the compressive strength of ProRoot MTA and Biodentine.

Koubi et al(2013)[50]Compared Biodentine to the composite Z100®, to evaluate whether and for how long it could be used as a posterior restoration according to selected United States Public Health Service (USPHS)' criteria. Secondly, when abrasion occurred, Biodentine was evaluated as a dentine substitute combined with Z100®.He concluded that Biodentine can be used as a dentine substitute under a composite for posterior restorations& also to restore posterior teeth for up to 6 months

FLUORIDE – ANTIBACTERIAL ACTION

Yaman et al (2004)[83]stated that fluoride-releasing restorative materials present the ability to inhibit enamel and dentin demineralization produced by acidic gels or demineralizing buffer solutions. This ability depends on the amount of fluoride ions released from the materials.

Subramani K & Ahmed W (2012)[71]The antibacterial action of fluoride is due to the acidification of the bacterial cytoplasm through the formation of H^+ and F^- ions from hydrogen fluoride and the disruption of thebacterial metabolism by inhibition of vital bacterial enzymes such as proton releasing adenosine triphosphatase and enolase..

SODIUM FLUOROSILICATE

Chow and Takagi (1991)[18] compared the fluoride depositing ability of two fluoride mouth rinses namely 228 ppm sodium fluoride and a two step rinse solution consisting of solution A (a soluble calcium salt and a buffer) and solution B (sodium fluorosilicate a complex fluoride salt). It was found that the amount of fluoride deposited with the two step solution was 19 times greater than sodium fluoride with the same fluoride content. Na_2SiF_6 on hydrolysis produced free fluoride caused the deposition of calcium fluoride. The authors concluded by saying that the two step solution could be more efficacious than sodium fluoride.

Eidelman and Chow (1991)[24] assessed the effects of pH and calcium on hydrolysis of Na_2SiF_6 and Na_2SnF_6. Under high concentrations of hydrogen ion (H+) and Ca2+, the promoting effect of Ca2+ on the hydrolysis of Na_2SiF_6 was stronger than the inhibition effect of H+. However, the inhibition effect of H+ on the hydrolysis of $Na_2SnF_6$was stronger than the promoting effect of Ca2+. Na_2SiF_6 and Na_2SnF_6 were found to have hydrolysis properties that may make them suitable for use with an acidic calcium phosphate solution in a topical fluoride treatment which forms dicalcium phosphate dihydrate as an intermediate.

Takagi et al (1992)[72] assessed the amounts of loosely bound fluoride (F) deposited on human enamel with either acidulated phosphate fluoride (APF) or a monocalcium phosphate monohydrate and sodium hexafluorosilicate (MCPM-SHFS)-containing gel. The results showed that the MCPM-SHFS treatments produced significantly more loosely bound F than did the APF treatments. The MCPM-SHFS

gel had the same F content as APF has the potential to be more efficacious than APF because it deposits greater amounts of both loosely bound and firmly bound F.

Vogel et al (1992)[79] assessed the in vivo fluoride concentrations measured for two hours after NaF or and a two solution mouth rinse comprising of solution A containing calcium chloride and sodium acetate and solution B containing a hydrolyzable source of fluoride (sodium hexafluorosilicate) and sodium phosphate. Results showed that, compared with NaF, the two-solution rinse produced significantly higher salivary fluoride concentrations and that the new rinse may provide a greater cariostatic effect at the same fluoride dosage than does a NaF rinse.

Appelbaum KS et al (2012)[2]conducted a study to determine the most appropriate amount of SF to add to Portland cement(PC)to decrease its setting time, 1%, 2%, 3%, 4%, 5%, 10%, and 15% SF by weight were added to PC and compared with PC without SF. Setting times were measured by using a Gilmore needle, and compressive strengths were determined by using a materials testing system at 24 hours and 21 days. concluded that , sodium fluorosilicate should not be used to decrease setting time and increase the compressive strength of Portland cement.

Weir et al (2012)[80]conducted a study to develop nanocomposite containing calcium fluoride nanoparticles (nCaF2), four composites were fabricated with fillers of: (1) 0% nCaF2 + 65% glass; (2) 10%nCaF2 + 55% glass; (3) 20% nCaF2 + 45% glass; (4) 30% nCaF2 + 35% glass and the long-term mechanical durabilityincluding wear, thermal-cycling and long-term water-aging behaviour were investigated and inferred that Combining nCaF2 with glass particles yielded nanocomposites with

long-term mechanical properties that were comparable to those of a commercial composite with little Frelease, and much better than those of RMGI controls.

HYDROFLUORIC ACID:

Pioch (2003)[59]assessed the effect of Hydrofluoric acid on the surface characteristics of dentin in vitro. According to this study, HF used after etching with orthophosphoric acid has the ability to seal the dentinal tubules that have been opened by etching and there is evidence of fluoride deposition in the tubules when HF was used.

Hjortsjo et al (2009)[44]studied the long term protective effects of 0.2% Hydrofluoric acid and 0.78% Stannous fluoride in reducing solubility of enamel. 0.1% citric acid challenge was used in this model and according to this study, HF reduced enamel solubility by 54% and 36 % at 1st and 7th day respectively. The authors concluded by saying that HF improves the protective ability of fluorides against acid erosion.

Hjortsjo et al (2014)[45] studied the etching effects of acidic fluorides namely 1.6% TiF4, 3.9% SnF2, 0.2% HF and 1.8% citric acid (CA) on enamel. The authors found that stannous fluoride and hydrofluoric acid were two acidic fluorides that had minimal erosive effect on enamel.

SPECTROPHOTOMETER

Zolgharnein et al (2009)[86] Spectrophotometric methods are widely used in the determination of fluoride because of advantages such as simplicity, convenience, accuracy and reproducibility

Barghouthi & Amereihm (2012)[7] New simple and sensitive spectrophotometric determination of fluoride in drinking groundwater has been developed using aluminium-resorcin blue complex. The method is based on the reaction of fluoride with the coloured complex to produce colourless aluminium fluoride complex and releasing of the free ligand. The relationship of the reaction of fluoride with the complex is sixth-order polynomial function. The reaction reaches equilibrium at fluoride concentration of 0.054 mM.

Materials & methods

MATERIALS & METHODS

ARMAMENTARIUM

1) Eppendorf tubes
2) Teflon moulds (5 mm X 5 mm)
3) Mylar strip
4) Milligram weighing machine
5) Micropipette
6) Watch glass
7) Amalgamator
8) Latex examination gloves
9) Plastic tweezers
10) Measuring caliper
11) Bard parker blade no 15
12) Plastic amalgam carrier
13) Metal amalgam condenser
14) Plastic instrument
15) Oil impervious paper
16) Agate mixing spatula
17) Plastic funnel
18) Wash bottle
19) 100ml polypropylene measuring cup
20) Cylindrical acrylic blocks (1.5cm x 2 cm)
21) Glass plates

22) Distilled water

23) Air tight Polypropylene containers

MATERIALS:

1) Biodentine (Septodont, Saint Maur des Fosses, France) (Fig.1)
2) Sodium fluorosilicate $Na_2[SiF_6]$(Loba Chemie, India) (Fig.2)
3) Hydrofluoric acid (HF) (Merck,India) (Fig.3)
4) Glass ionomer cement (Fuji II, GC, Tokyo, Japan).

COMPOSITION OF BIODENTINE

POWDER

Tricalcium silicate	Main core material
Dicalcium silicate	Second core material
Calcium carbonate & oxide	Filler
Iron oxide	Shade
Zirconium oxide	Radiopacifier

LIQUID

Calcium chloride	Accelerator
Hydrosoluble polymer	Water reducing agent

EQUIPMENTS:

1) Spadns spectrophotometer (UV-VIS spectrophotometer, made in SHIMADZU, model no. UV1601PC)
2) Universal testing machine (Instron Corp,Canton,MA)
3) Nessler tubes
4) Analytical balance

Reagents

Standard Fluoride Solution : NIST CRM for fluoride solution (1000 mg/L of F^-)

Working Solution:Dilute 1.0 ml of stock solution is made up to 100 ml (10 mg/ L F)

SPADNS Solution(Fig.14) : Dissolve 958 mg spadns in dissolved water and dilute to 500 ml.

Zirconyl Acid Reagent(Fig.15) : Dissolve 133 mg zirconyl chloride octahydrate (ZrOCl2) in about 25 ml distilled water. Add 350 ml conc. HCl and dilute to 500 ml with distilled water.

Reference reagent : Add 10 ml spadns solution to 100 ml of distilled water,dilute 7 ml conc. Hcl to 10 ml and add to the diluted spadns solution (Fig.16)the resulting solution used for setting the instrument reference point (zero)

Sodium arsenite solution : Dissolve 5.0 g $NaAsO_2$ and dilute to 10000 ml with distilled water

METHODOLOGY

The study was conducted in the Dept. of Conservative Dentistry And Endodontics., Tamil Nadu Govt. Dental College & Hospital Chennai., The fluoride analysis was done at Chennai Mettex Lab Pvt. Ltd, Chennai., The Compressive strength was evaluated at Dept. of Metallurgical and Materials Engineering, Indian Institute Of Technology Madras,Chennai.

Sample preparation

The study comprised of a total of 80 samples divided into 4 groups of 20 samples in each group. Out of the 20 samples ,10 samples were allocated for fluoride analysis and 10 samples were destined for compressive strength analysis. There are three test groups (GroupA,B,C) and one positive control group (Group D)

Group A - Biodentine powder only modified with 7wt% $Na_2[SiF_6]$

Group B - Biodentine liquid only modified with 10 w/v % of 20% HF

Group C - Biodentine powder modified with 7wt% $Na_2[SiF_6]$ & Biodentine liquid modified with 10 w/v % of 20% HF

Group D - Glass Ionomer cement type II (positive control) for fluoride release

Group D - Unmodified Biodentine (positive control) for compressive strength analysis

MODIFICATION OF BIODENTINE POWDER

The weight of powder contained in every capsule was measured as follows:

The gross weight of Biodentine was first obtained by weighing the powder along with the capsule in a milligram weighing machine then the weight of the powder was calculated by subtracting the weight of the empty capsule measured using same weighing machine.

This procedure was repeated for each and every capsule and the precise weight of $NaSiF_6$ to be added to each capsule was determined arithmetically.

The sample calculation is as follows

Gross weight of capsule with powder = 2.25 g

Weight of empty capsule = 1.53 g

Actual weight of Biodentine = Gross weight of capsule with powder ---Weight of empty capsule = 2.25- 1.53 = 0.7 g

Weight of 7wt% $Na_2[SiF_6]$to be added to Biodentine powder = 0.05 g= 50 mg

A pilot study was conducted in our Dept. with 5wt % ,7wt% and 10 wt % of $Na_2[SiF_6]$ to Biodentine powder component and compressive strength was assessed.

It was inferred that 7wt% can be incorporated to Biodentine without affecting the mechanical properties such as compressive strength and thus 7wt %$Na_2[SiF_6]$ was chosen for this study.

A clean disinfected watch glass was placed over the milligram weigh scale, the weight of which is subtracted. The arithmetically derived $Na_2[SiF_6]$was precisely weighed over it.(Fig.4)

The precisely measured quantity 50 mg of $Na_2[SiF_6]$is then added to Biodentine powder capsule and now it is placed in the amalgamator(Fig.5) at 300 oscillations for 3 minutes to ensure thorough mixing of $Na_2[SiF_6]$ particles with Biodentine powder. Thus the modified Biodentine powder is obtained

MODIFICATION OF BIODENTINE LIQUID

HF solution of 20 % was prepared by diluting 1 ml of commercially available 48 % HF (Merck, India) with 1.4 ml of deionised water. (Fig.6)

Liquid component of Biodentine is emptied into an eppendorf tube.

10 w/v % (20µl) of 20 % HF was pipetted using micropipette (Fig.7) and is transferred to the eppendorf tube containing the liquid component of Biodentine (Fig.8) .The modified Biodentine liquid is thus obtained.

Preparation of samples

The powder and liquid components of modified & unmodified Biodentine were manipulated (Fig.9) according to manufacturer's instructions in an amalgamator(Fig.10) and homogenous mix thus obtained is carried in a plastic instrument &compacted into the teflon moulds of dimensions 5mm diameter & 5mm height using an amalgam condenser.

The powder & liquid components of glass ionomer cement were hand mixed by folding method over an oil impervious paper , homogenous mix is then compacted into teflon moulds of similar dimensions. The top surface of the specimens were covered by mylar strip and allowed to set at room temperaturecompressed between two glass plates.(Fig.11)

Storage of samples

After the initial set of the materials, the cylindrical test specimens that were formed with the help of teflon moulds were safely retrieved from them cutting through the teflon moulds with no.15 BP blade and gently teasing out the samples.

100 ml of distilled water is measured using 100ml measuring beaker (Fig.12) and transferred to the corresponding air tight labelled polypropylene containers (Fig.13)

The retrieved samples that are destined for fluoride release were then transferred using a pair of plastic tweezers carefully to the polypropylene containers containing 100 ml distilled water. The samples that were destined for compressive strength analysis were stored in 100 % humidity at 37° C

FLUORIDE RELEASE ASSESSMENT

After the completion of 24 hours the distilled water was transferred to another corresponding labelled polypropylene containers analysed for fluoride release using Spadn's spectrophotometer (Chennai Mettex Lab Pvt Ltd, Chennai).

Principle for SPADNS calorimetric method :

This is based on the reaction between fluoride and zirconium dye lake, fluoride reacts with the dye lake dissociating a portion of it into colourless complex anion(ZrF_6^{2-})

As the amount of fluoride increases the colour produced becomes progressively lighter

$F^- +$ Zr dye lake $\longrightarrow$ ZrF_6^{2-} + dye

Procedure :

Preparation of standard curve :

1. Clean and dry all the apparatus thoroughly
2. Prepare fluoride standards in the range of 0 to 1.40 mg F /L diluting appropriate quantities of standard fluoride solution to 50 ml with distilled water .
3. Add 5.0 ml each of spadns solution & add 5.00 ml of zirconyl acid reagent
4. For each standard, mix well, avoid contamination & read absorbance at 570 nm.

Preparation of samples

1. If the sample contains residual chloride remove it by adding 1 drop of sodium arsenite /0.1 mg residual chloride
2. Use of 50ml sample or portion, diluted to 50 ml with distilled water
3. Adjust sample temperature to that used for standard curve
4. Add 5.00 ml of spadns solution & add 5 .00 ml of zirconyl- acid reagent
5. Mix well and read absorbance at 570 nm

Calculation

Fluoride mg/L = mg/L of Fluoride from calibration curve X dilution factor

Thus the fluoride is estimated in ppm

The containers were rinsed, washed and replenished with 100 ml of distilled water the same samples were immersed in the corresponding containers.

The same procedure was repeated at 24 hour intervals during the first 7 days. The fluoride release in distilled water was estimated on 3^{rd} and 7^{th} days.

From the 2^{nd} week onwards the fluoride release was assessed cumulatively on weekly basis , at 14^{th},21^{st} and 28^{th} days.

Conversion of parts per million (ppm) into microgram per square centimetre(μg/cm^2):-

After each reading was taken, the total fluoride released in micrograms was calculated by multiplying the parts per million (1 ppm = 1 μg/mL) by the water sample volume (100 mL). The total fluoride was then divided by the area of the sample disk to obtain the fluoride release in micrograms per square centimeter.

For example :

Take for instance the values of sample A1 , which was 1.51 ppm

It was multipled with the water sample volume (i.e) 100 mL which gives the total fluoride in solution == 151

When this is divided by the surface area of cylindrical sample = 1.17 cm^2

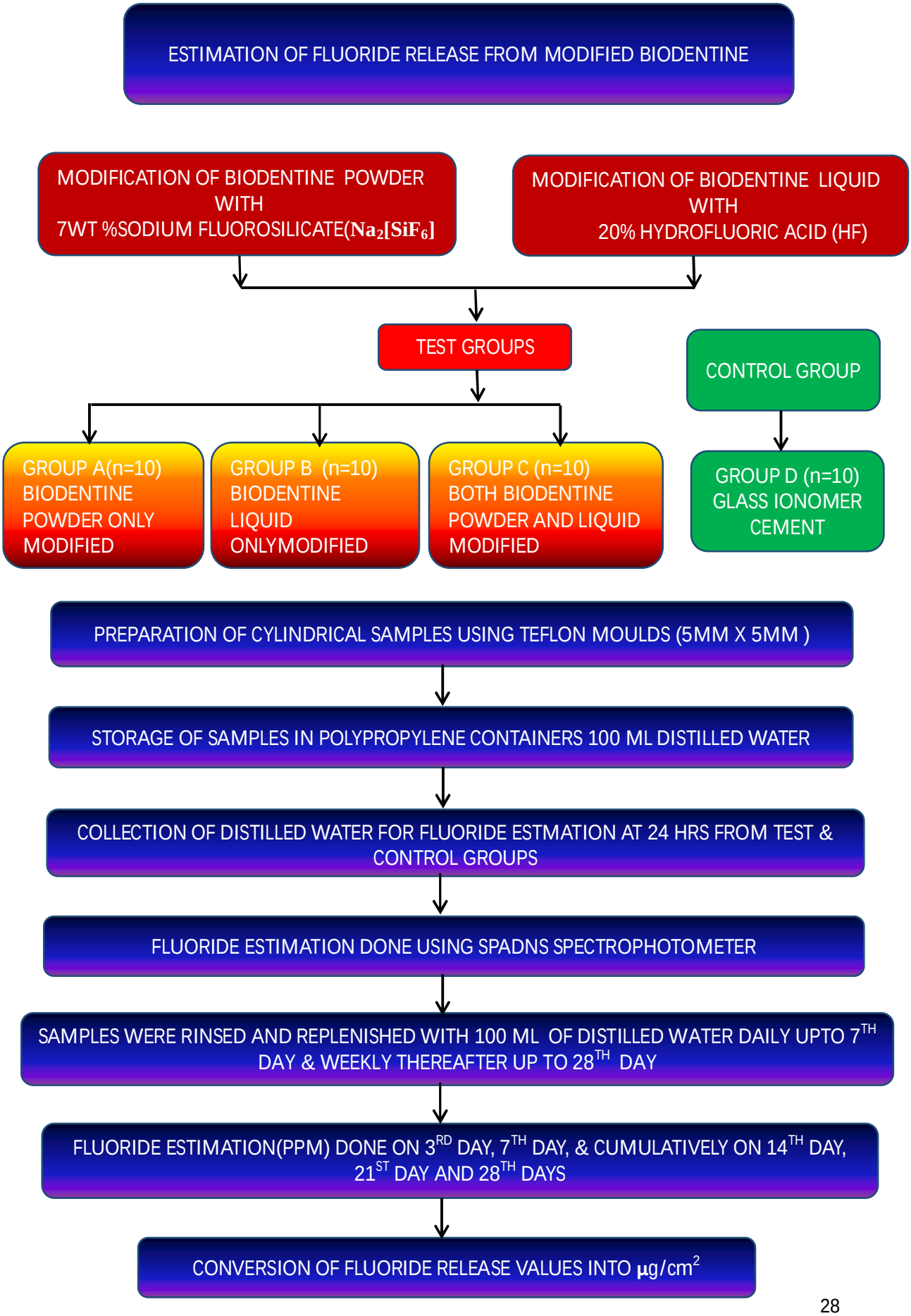
(2 л r^2 + 2л r h) = 2x 3.14x $(0.25)^2$+ 2x3.14x0.25 x 0.5 = 1.17 cm^2

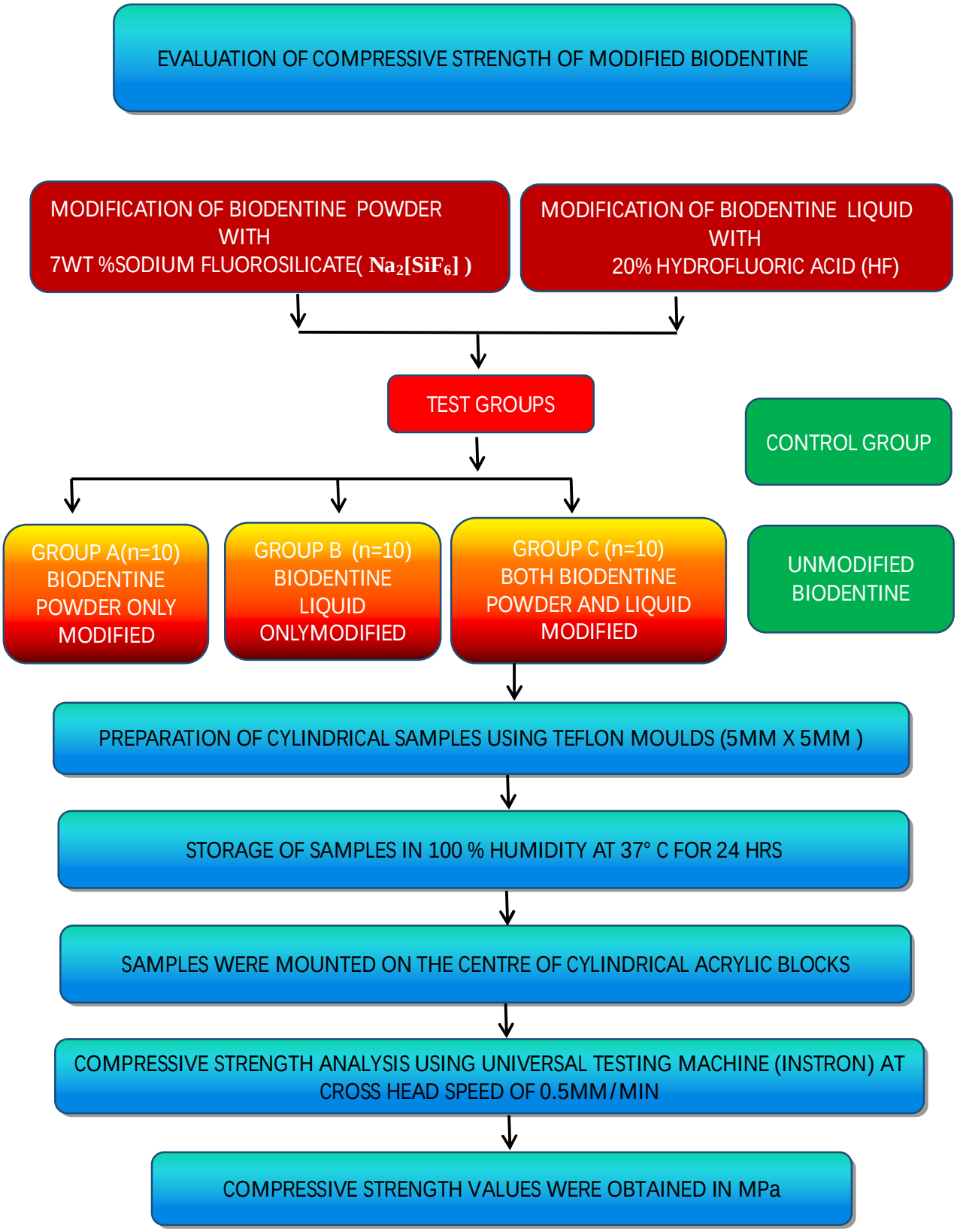
So the value is 151 ÷ 1.17 cm^2 = 129.05μg/cm^2

COMPRESSIVE STRENGTH ANALYSIS

After 24 hours being elapsed the test samples which were allotted for compressive strength evaluation were mounted on an cylindrical acrylic resin block using cyanoacrylate (Fig.19) and they are subjected to compressive strength analysis using Universal testing machine (Instron Corp Canton MA,IIT, Chennai) at a cross head speed of 0.5 mm/min until the sample gets fractured (Fig.20).The compressive strength values were obtained in MPa.

The fluoride release values that were obtained in μg/$cm^2$for the various time periods and the 24 hr compressive strength values that were obtained in MPa were tabulated and statistically analysed using SPSS software version 16.0


ESTIMATION OF FLUORIDE RELEASE FROM MODIFIED BIODENTINE
MODIFICATION OF BIODENTINE POWDER WITH 7WT %SODIUM FLUOROSILICATE($Na_2[SiF_6]$
MODIFICATION OF BIODENTINE LIQUID WITH 20% HYDROFLUORIC ACID (HF)
TEST GROUPS
CONTROL GROUP
GROUP A(n=10) BIODENTINE POWDER ONLY MODIFIED
GROUP B (n=10) BIODENTINE LIQUID ONLYMODIFIED
GROUP C (n=10) BOTH BIODENTINE POWDER AND LIQUID MODIFIED
GROUP D (n=10) GLASS IONOMER CEMENT
PREPARATION OF CYLINDRICAL SAMPLES USING TEFLON MOULDS (5MM X 5MM)
STORAGE OF SAMPLES IN POLYPROPYLENE CONTAINERS 100 ML DISTILLED WATER
COLLECTION OF DISTILLED WATER FOR FLUORIDE ESTMATION AT 24 HRS FROM TEST & CONTROL GROUPS
FLUORIDE ESTIMATION DONE USING SPADNS SPECTROPHOTOMETER
SAMPLES WERE RINSED AND REPLENISHED WITH 100 ML OF DISTILLED WATER DAILY UPTO 7TH DAY & WEEKLY THEREAFTER UP TO 28TH DAY
FLUORIDE ESTIMATION(PPM) DONE ON 3RD DAY, 7TH DAY, & CUMULATIVELY ON 14TH DAY, 21ST DAY AND 28TH DAYS
CONVERSION OF FLUORIDE RELEASE VALUES INTO µg/cm2


EVALUATION OF COMPRESSIVE STRENGTH OF MODIFIED BIODENTINE
MODIFICATION OF BIODENTINE POWDER WITH 7WT %SODIUM FLUOROSILICATE($Na_2[SiF_6]$)
MODIFICATION OF BIODENTINE LIQUID WITH 20% HYDROFLUORIC ACID (HF)
TEST GROUPS
CONTROL GROUP
GROUP A(n=10) BIODENTINE POWDER ONLY MODIFIED
GROUP B (n=10) BIODENTINE LIQUID ONLYMODIFIED
GROUP C (n=10) BOTH BIODENTINE POWDER AND LIQUID MODIFIED
UNMODIFIED BIODENTINE
PREPARATION OF CYLINDRICAL SAMPLES USING TEFLON MOULDS (5MM X 5MM)
STORAGE OF SAMPLES IN 100 % HUMIDITY AT 37° C FOR 24 HRS
SAMPLES WERE MOUNTED ON THE CENTRE OF CYLINDRICAL ACRYLIC BLOCKS
COMPRESSIVE STRENGTH ANALYSIS USING UNIVERSAL TESTING MACHINE (INSTRON) AT CROSS HEAD SPEED OF 0.5MM/MIN
COMPRESSIVE STRENGTH VALUES WERE OBTAINED IN MPa

MATERIALS USED IN THIS STUDY

FIG. 1.BIODENTINE

FIG.2.SODIUM FLUOROSILICATE

FIG.3.HYDROFLUORIC ACID

MODIFICATION OF POWDER COMPONENT

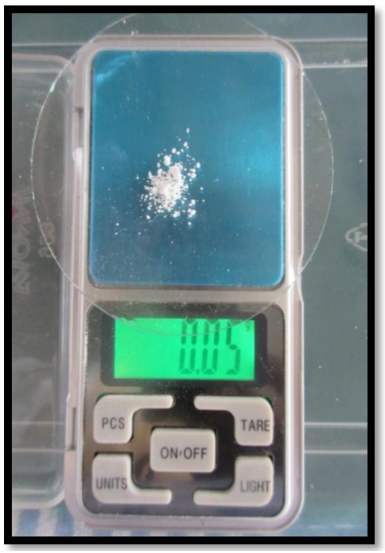


FIG.4.MILLIGRAM WEIGHING MACHINE

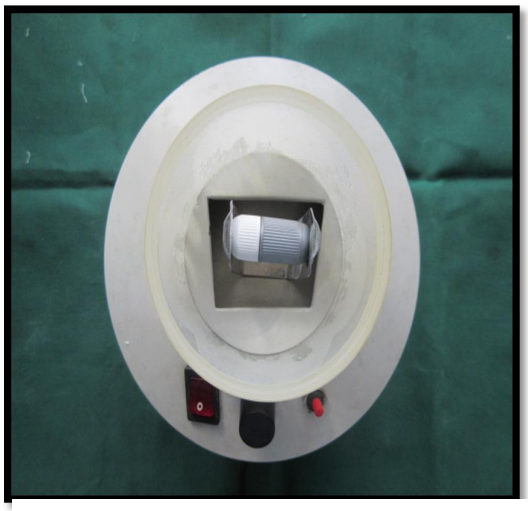


FIG.5.MIXING OF $Na_2[SiF_6]$ WITH POWDER COMPONENT

MODIFICATION OF LIQUID COMPONENT

FIG.6. 20 % HYDROFLUORIC ACID

FIG 7.MICROPIPETTE

FIG.8.ADDING HF TO LIQUID COMPONENT INEPPENDORF TUBE

PREPARATION OF SAMPLES

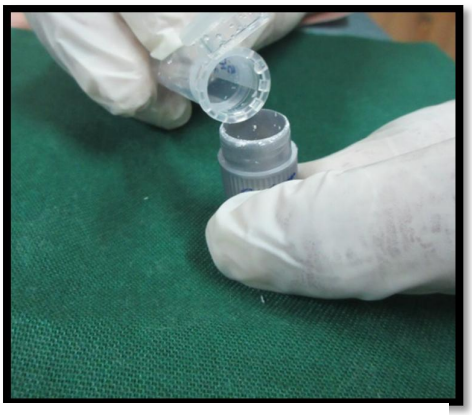


FIG.9.DISPENSING LIQUID INTO CAPSULE

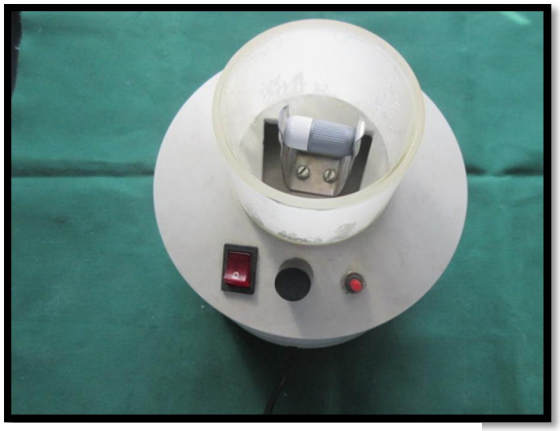


FIG.10.MIXING IN AMALGAMATOR

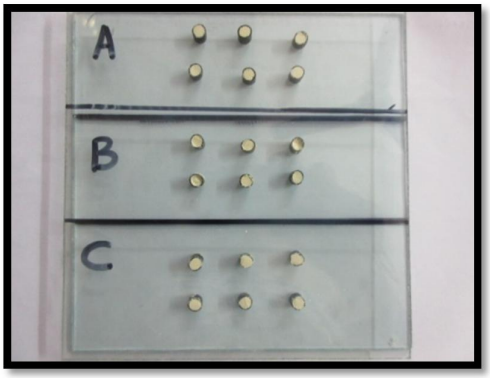


FIG.11.SETTING OF SAMPLES BETWEEN GLASS PLATES

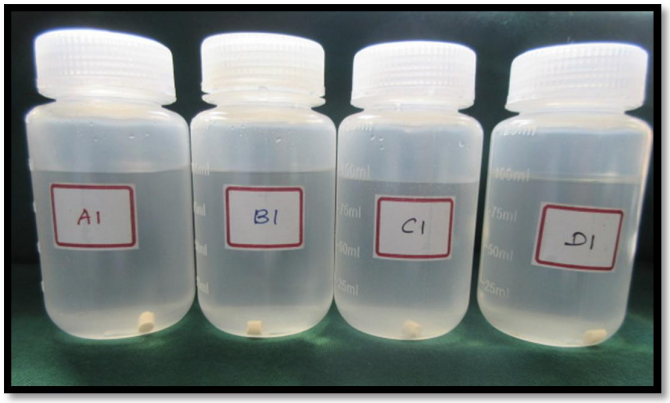


FIG.12.STORAGE OF SAMPLES

FLUORIDE ESTIMATION

FIG 13.SAMPLES FOR ESTIMATION OF FLUORIDE

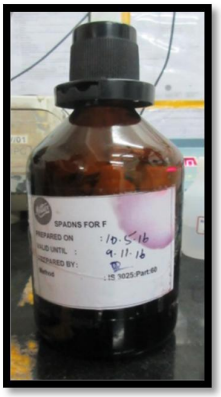


FIG.14.SPADNS REAGENT

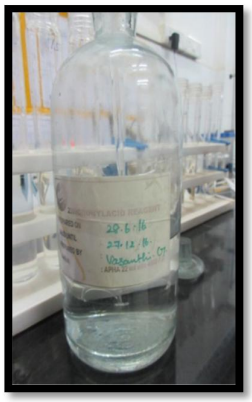


FIG .15.ZIRCONYL REAGENT

FIG.16.PREPARATION OF SAMPLES

FIG.17.SPECTROPHOTOMETER

FIG.18.CUVETTES WITH TEST SAMPLES

COMPRESSIVE STRENGTH EVALUATION

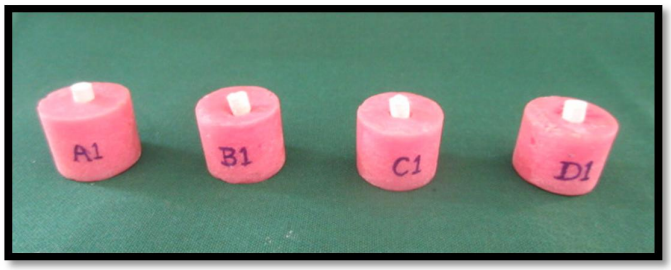


FIG.19.SAMPLES FOR COMPRESSIVE STRENGTH

FIG 20.UNIVERSAL TESTING MACHINE WITH SAMPLE

Results

RESULTS

I . EVALUATION OF FLUORIDE RELEASE

The values of fluoride release in µg/cm^{2}for the groups A,B,C,D that were obtained were tabulated as below

Table 1.Fluoride release in µg/cm^{2}of GROUP A at various time intervals

Sample	24 hours	3rd day	7th day	2nd week	3rd week	4th week
A1	129.05	68.37	64.10	164.10	153.85	121.36
A2	136.75	54.70	47.00	162.39	162.39	119.65
A3	148.72	58.12	45.30	153.85	170.94	111.11
A4	147.86	64.96	54.70	170.94	153.85	116.23
A5	135.65	56.76	48.23	169.32	146.75	102.56
A6	134.33	54.78	49.83	166.67	170.94	105.98
A7	137.12	53.33	43.12	161.11	165.81	112.82
A8	142.46	61.12	45.87	172.12	156.41	109.40
A9	137.45	59.64	46.62	163.24	164.96	113.68
A10	141.12	55.77	48.34	158.36	152.99	105.98

GROUP A - POWDER ONLY MODIFIED BIODENTINE

Table 2.Fluoride release in µg/cm^{2}of GROUP B at various time intervals

Sample	24 hours	3rd day	7th day	2nd week	3rd week	4th week
B1	11.11	17.95	19.65	26.49	23.93	14.53
B2	14.52	16.24	19.65	23.07	21.36	12.82
B3	10.26	17.95	21.36	21.36	24.78	13.67
B4	13.67	17.09	23.07	23.08	25.64	10.26
B5	11.97	16.24	16.24	19.65	22.22	13.68
B6	11.11	19.65	21.36	24.78	23.93	12.82
B7	13.67	18.80	22.22	25.64	19.66	15.38
B8	11.97	16.24	11.97	22.22	25.64	15.38
B9	13.67	17.95	9.40	17.95	25.64	14.53
B10	21.37	17.09	17.09	23.07	23.93	17.09

GROUP B - LIQUID ONLY MODIFIED BIODENTINE

Table 3.Fluoride release in µg/cm^{2}of GROUP C at various time intervals

Sample	24 hours	3rd day	7th day	2nd week	3rd week	4th week
C1	111.11	81.20	102.56	183.76	256.41	136.75
C2	106.83	73.50	94.02	196.58	250.42	111.11
C3	102.56	70.09	111.11	205.12	256.41	145.30
C4	102.56	94.02	111.11	205.12	266.67	145.30
C5	94.02	78.63	81.20	170.94	270.09	102.56
C6	83.76	82.05	83.76	179.49	250.42	94.02
C7	111.11	94.02	78.63	188.03	250.42	94.02
C8	95.72	76.92	102.56	196.58	271.79	82.90
C9	85.47	77.77	111.11	183.76	261.53	83.76
C10	82.91	76.92	94.02	179.49	248.71	82.90

GROUP C- BOTH POWDER AND LIQUID MODIFIED BIODENTINE

Table 4.Fluoride release in µg/cm^{2}of GROUP D at various time intervals

Sample	24 hours	3rd day	7th day	2nd week	3rd week	4th week
D1	118.62	72.82	49.16	89.27	108.46	88.57
D2	112.83	68.46	45.27	87.16	102.63	93.62
D3	122.83	79.12	52.82	96.34	110.22	95.44
D4	109.63	68.65	46.66	90.52	101.26	87.82
D5	115.32	75.58	53.71	96.34	106.38	94.63
D6	123.17	79.19	54.43	101.68	111.36	99.26
D7	120.06	78.62	50.17	103.27	114.74	94.63
D8	119.11	67.28	47.83	96.83	106.52	88.57
D9	121.66	79.26	55.36	98.66	112.12	87.82
D10	122.33	75.22	52.63	96.34	109.92	98.18

GROUP D- GLASS IONOMER CEMENT TYPE II

INTERGROUP ANALYSIS

Table 5 .Descriptives&One Way ANOVA – statistical analysis at various time intervals

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
24 hours	Group A	10	139.051	6.07775	1.88790	134.8403	143.3817	129.05	148.72
	Group B	10	13.3320	3.15146	0.99658	11.0776	15.5864	10.26	21.37
	Group C	10	97.6050	10.90497	3.44845	89.8041	105.4059	82.91	111.11
	Group D	10	118.56	4.58184	1.44890	115.2783	121.8337	109.63	123.17
	Total	40	92.1510	48.86292	7.72591	76.5239	107.7781	10.26	148.72
3rd day	Group A	10	58.7550	4.85678	1.53585	55.2807	62.2293	53.33	68.37
	Group B	10	17.5200	1.15542	0.36538	16.6935	18.3465	16.24	19.65
	Group C	10	80.5120	7.90515	2.49983	74.8570	86.1670	70.09	94.02
	Group D	10	74.4200	4.83331	1.52843	70.9625	77.8775	67.28	79.26
	Total	40	57.8017	25.39507	4.01531	49.6800	65.9235	16.24	94.02
7th day	Group A	10	49.3110	6.04590	1.91188	44.9860	53.6360	43.12	64.10
	Group B	10	18.2010	4.53848	1.43519	14.9544	21.4476	9.40	23.07
	Group C	10	97.0080	12.64585	3.99897	87.9617	106.0543	78.63	111.11
	Group D	10	50.8040	3.48801	1.10300	48.3088	53.2992	45.27	55.36
	Total	40	53.8310	29.39411	4.64762	44.4303	63.2317	9.40	111.11
2nd week	Group A	10	164.21	5.72950	1.81183	160.1154	168.3126	153.85	172.12
	Group B	10	22.7310	2.61690	.82754	20.8590	24.6030	17.95	26.49
	Group C	10	188.89	11.53446	3.64752	180.6357	197.1383	170.94	205.12
	Group D	10	95.6410	5.22182	1.65128	91.9055	99.3765	87.16	103.27
	Total	40	117.87	65.86063	10.41348	96.8050	138.9315	17.95	205.12
3rd week	Group A	10	159.89	8.27680	2.61736	153.9681	165.8099	146.75	170.94
	Group B	10	23.6730	2.01649	.63767	22.2305	25.1155	19.66	25.64
	Group C	10	258.29	8.73605	2.76258	252.0376	264.5364	248.71	271.79
	Group D	10	108.36	4.22024	1.33456	105.3420	111.3800	101.26	114.74
	Total	40	137.55	86.30069	13.64534	109.9522	165.1528	19.66	271.79
4th week	Group A	10	111.88	6.11478	1.93366	107.5027	116.2513	102.56	121.36
	Group B	10	14.0160	1.85292	.58595	12.6905	15.3415	10.26	17.09
	Group C	10	107.86	25.57494	8.08750	89.5668	126.1572	82.90	145.30
	Group D	10	92.8540	4.35090	1.37588	89.7416	95.9664	87.82	99.26
	Total	40	81.6522	42.19332	6.67135	68.1582	95.1463	10.26	145.30

Table 6 .ANOVA between the groups at various time intervals

		Sum of Squares	df	Mean Square	F	Sig.
24 hours	Between Groups	91446.465	3	30482.155	657.351	.000
	Within Groups	1669.364	36	46.371		
	Total	93115.829	39			
3rd day	Between Groups	24154.498	3	8051.499	290.732	.000
	Within Groups	996.980	36	27.694		
	Total	25151.478	39			
7th day	Between Groups	31633.434	3	10544.478	183.995	.000
	Within Groups	2063.110	36	57.309		
	Total	33696.543	39			
2nd week	Between Groups	167367.384	3	55789.128	1.116E3	.000
	Within Groups	1799.879	36	49.997		
	Total	169167.263	39			
3rd week	Between Groups	288964.229	3	96321.410	2.311E3	.000
	Within Groups	1500.308	36	41.675		
	Total	290464.537	39			
4th week	Between Groups	63006.280	3	21002.093	117.687	.000
	Within Groups	6424.484	36	178.458		
	Total	69430.764	39			

Table 7. Multiple Comparisons using Tukey'spost hoc test between the groups

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
24 hours	Group A	Group B	125.77900*	3.04536	.000	117.5771	133.9809
		Group C	41.50600*	3.04536	.000	33.3041	49.7079
		Group D	20.55500*	3.04536	.000	12.3531	28.7569
	Group B	Group A	-125.77900*	3.04536	.000	-133.9809	-117.5771
		Group C	-84.27300*	3.04536	.000	-92.4749	-76.0711
		Group D	-105.22400*	3.04536	.000	-113.4259	-97.0221
	Group C	Group A	-41.50600*	3.04536	.000	-49.7079	-33.3041
		Group B	84.27300*	3.04536	.000	76.0711	92.4749
		Group D	-20.95100*	3.04536	.000	-29.1529	-12.7491
	Group D	Group A	-20.55500*	3.04536	.000	-28.7569	-12.3531
		Group B	105.22400*	3.04536	.000	97.0221	113.4259
		Group C	20.95100*	3.04536	.000	12.7491	29.1529
3rd day	Group A	Group B	41.23500*	2.35346	.000	34.8966	47.5734
		Group C	-21.75700*	2.35346	.000	-28.0954	-15.4186
		Group D	-15.66500*	2.35346	.000	-22.0034	-9.3266
	Group B	Group A	-41.23500*	2.35346	.000	-47.5734	-34.8966
		Group C	-62.99200*	2.35346	.000	-69.3304	-56.6536
		Group D	-56.90000*	2.35346	.000	-63.2384	-50.5616
	Group C	Group A	21.75700*	2.35346	.000	15.4186	28.0954
		Group B	62.99200*	2.35346	.000	56.6536	69.3304
		Group D	6.09200	2.35346	**.063**	-.2464	12.4304
	Group D	Group A	15.66500*	2.35346	.000	9.3266	22.0034
		Group B	56.90000*	2.35346	.000	50.5616	63.2384
		Group C	-6.09200	2.35346	**.063**	-12.4304	.2464
7thday	Group A	Group B	31.11000*	3.38552	.000	21.9920	40.2280
		Group C	-47.69700*	3.38552	.000	-56.8150	-38.5790
		Group D	-1.49300	3.38552	**.971**	-10.6110	7.6250
	Group B	Group A	-31.11000*	3.38552	.000	-40.2280	-21.9920
		Group C	-78.80700*	3.38552	.000	-87.9250	-69.6890
		Group D	-32.60300*	3.38552	.000	-41.7210	-23.4850
	Group C	Group A	47.69700*	3.38552	.000	38.5790	56.8150
		Group B	78.80700*	3.38552	.000	69.6890	87.9250
		Group D	46.20400*	3.38552	.000	37.0860	55.3220
	Group D	Group A	1.49300	3.38552	**.971**	-7.6250	10.6110
		Group B	32.60300*	3.38552	.000	23.4850	41.7210
		Group C	-46.20400*	3.38552	.000	-55.3220	-37.0860

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
2nd week	Group A	Group B	141.48300*	3.16217	.000	132.9666	149.9994
		Group C	-24.67300*	3.16217	.000	-33.1894	-16.1566
		Group D	68.57300*	3.16217	.000	60.0566	77.0894
	Group B	Group A	-141.48300*	3.16217	.000	-149.9994	-132.9666
		Group C	-166.15600*	3.16217	.000	-174.6724	-157.6396
		Group D	-72.91000*	3.16217	.000	-81.4264	-64.3936
	Group C	Group A	24.67300*	3.16217	.000	16.1566	33.1894
		Group B	166.15600*	3.16217	.000	157.6396	174.6724
		Group D	93.24600*	3.16217	.000	84.7296	101.7624
	Group D	Group A	-68.57300*	3.16217	.000	-77.0894	-60.0566
		Group B	72.91000*	3.16217	.000	64.3936	81.4264
		Group C	-93.24600*	3.16217	.000	-101.7624	-84.7296
3rd week	Group A	Group B	136.21600*	2.88705	.000	128.4405	143.9915
		Group C	-98.39800*	2.88705	.000	-106.1735	-90.6225
		Group D	51.52800*	2.88705	.000	43.7525	59.3035
	Group B	Group A	-136.21600*	2.88705	.000	-143.9915	-128.4405
		Group C	-234.61400*	2.88705	.000	-242.3895	-226.8385
		Group D	-84.68800*	2.88705	.000	-92.4635	-76.9125
	Group C	Group A	98.39800*	2.88705	.000	90.6225	106.1735
		Group B	234.61400*	2.88705	.000	226.8385	242.3895
		Group D	149.92600*	2.88705	.000	142.1505	157.7015
	Group D	Group A	-51.52800*	2.88705	.000	-59.3035	-43.7525
		Group B	84.68800*	2.88705	.000	76.9125	92.4635
		Group C	-149.92600*	2.88705	.000	-157.7015	-142.1505
4th week	Group A	Group B	97.86100*	5.97424	.000	81.7710	113.9510
		Group C	4.01500	5.97424	.907	-12.0750	20.1050
		Group D	19.02300*	5.97424	.015	2.9330	35.1130
	Group B	Group A	-97.86100*	5.97424	.000	-113.9510	-81.7710
		Group C	-93.84600*	5.97424	.000	-109.9360	-77.7560
		Group D	-78.83800*	5.97424	.000	-94.9280	-62.7480
	Group C	Group A	-4.01500	5.97424	.907	-20.1050	12.0750
		Group B	93.84600*	5.97424	.000	77.7560	109.9360
		Group D	15.00800	5.97424	.075	-1.0820	31.0980
	Group D	Group A	-19.02300*	5.97424	.015	-35.1130	-2.9330
		Group B	78.83800*	5.97424	.000	62.7480	94.9280
		Group C	-15.00800	5.97424	.075	-31.0980	1.0820

INTRAGROUP ANALYSIS

REPEATED MEASURES ANOVA

GROUP A

Table 8.Descriptive Statistics& repeated measures ANOVA for Group A

	Mean	Std. Deviation	N
24 HOURS	139.05	6.07775	10
3RD DAY	58.7550	4.85678	10
7TH DAY	49.3110	6.04590	10
2ND WEEK	164.21	5.72957	10
3RD WEEK	159.89	8.27680	10
4TH WEEK	111.88	6.11478	10

Table 9. Bonferroni'sPairwise Comparisons for Group A

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
24 hr	3rdday	80.296*	2.468	.000	70.535	90.057
	7thday	89.740*	3.200	.000	77.086	102.394
	2ndwk	-25.159*	2.813	.000	-36.283	-14.035
	3rdwk	-20.838*	3.053	.001	-32.909	-8.767
	4thwk	27.174*	2.914	.000	15.652	38.696
3rd day	24 HR	-80.296*	2.468	.000	-90.057	-70.535
	7thday	9.444*	1.126	.000	4.993	13.895
	2ndwk	-105.455*	1.927	.000	-113.074	-97.836
	3rdwk	-101.134*	3.528	.000	-115.084	-87.184
	4thwk	-53.122*	1.745	.000	-60.021	-46.223
7thday	24HR	-89.740*	3.200	.000	-102.394	-77.086
	3rdday	-9.444*	1.126	.000	-13.895	-4.993
	2ndwk	-114.899*	2.289	.000	-123.951	-105.847
	3rdwk	-110.578*	3.819	.000	-125.680	-95.476
	4thwk	-62.566*	1.963	.000	-70.327	-54.805

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
2nd wk	24HR	25.159*	2.813	.000	14.035	36.283
	3rd day	105.455*	1.927	.000	97.836	113.074
	7th day	114.899*	2.289	.000	105.847	123.951
	3rd wk	4.321	3.845	1.000	-10.884	19.526
	4th wk	52.333*	2.772	.000	41.371	63.295
3rd wk	24HR	20.838*	3.053	.001	8.767	32.909
	3rd day	101.134*	3.528	.000	87.184	115.084
	7th day	110.578*	3.819	.000	95.476	125.680
	2nd wk	-4.321	3.845	1.000	-19.526	10.884
	4th wk	48.012*	3.083	.000	35.819	60.205
4th wk	24HR	-27.174*	2.914	.000	-38.696	-15.652
	3rd day	53.122*	1.745	.000	46.223	60.021
	7th day	62.566*	1.963	.000	54.805	70.327
	2nd wk	-52.333*	2.772	.000	-63.295	-41.371
	3rd wk	-48.012*	3.083	.000	-60.205	-35.819

Based on estimated marginal means

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

a. Adjustment for multiple comparisons: Bonferroni.

GROUP B

Table 10.Descriptive Statistics& repeated measures ANOVA for group B

	Mean	Std. Deviation	N
24 HOURS	13.3320	3.15146	10
3RD DAY	17.5200	1.15542	10
7TH DAY	18.2010	4.53848	10
2ND WEEK	22.7310	2.61690	10
3RD WEEK	23.6730	2.01649	10
4TH WEEK	14.0160	1.85292	10

Table 11. Bonferroni's pairwise comparisons for Group B

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
24 hr	3rd day	-4.188	1.143	.078	-8.707	.331
	7thday	-4.869	1.846	.405	-12.169	2.431
	2ndwk	-9.399*	1.306	.001	-14.561	-4.237
	3rdwk	-10.341*	1.238	.000	-15.235	-5.447
	4thwk	-.684	.898	1.000	-4.236	2.868
3rd day	24 HR	4.188	1.143	.078	-.331	8.707
	7thday	-.681	1.347	1.000	-6.006	4.644
	2ndwk	-5.211*	.759	.001	-8.211	-2.211
	3rdwk	-6.153*	.762	.000	-9.165	-3.141
	4thwk	3.504*	.691	.010	.773	6.235
7thday	24HR	4.869	1.846	.405	-2.431	12.169
	3rd day	.681	1.347	1.000	-4.644	6.006
	2ndwk	-4.530*	1.066	.032	-8.744	-.316
	3rdwk	-5.472	1.784	.201	-12.526	1.582
	4thwk	4.185	1.780	.648	-2.854	11.224
2ndwk	24HR	9.399*	1.306	.001	4.237	14.561
	3rd day	5.211*	.759	.001	2.211	8.211
	7th day	4.530*	1.066	.032	.316	8.744
	3rdwk	-.942	1.218	1.000	-5.758	3.874
	4thwk	8.715*	1.002	.000	4.755	12.675
3rdwk	24HR	10.341*	1.238	.000	5.447	15.235
	3rdday	6.153*	.762	.000	3.141	9.165
	7thday	5.472	1.784	.201	-1.582	12.526
	2ndwk	.942	1.218	1.000	-3.874	5.758
	4thwk	9.657*	.936	.000	5.956	13.358
4thwk	24HR	.684	.898	1.000	-2.868	4.236
	3rd day	-3.504*	.691	.010	-6.235	-.773
	7thday	-4.185	1.780	.648	-11.224	2.854
	2ndwk	-8.715*	1.002	.000	-12.675	-4.755
	3rdwk	-9.657*	.936	.000	-13.358	-5.956

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

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

GROUP C

Table 12.descriptive statistics & repeated measures ANOVA for group C

	Mean	Std. Deviation	N
24 HOURS	97.6050	10.90497	10
3RD DAY	80.5120	7.90515	10
7TH DAY	97.0080	12.64585	10
2ND WEEK	188.89	11.53446	10
3RD WEEK	258.29	8.73605	10
4TH WEEK	107.86	25.57494	10

Table 13.Bonferroni's Pairwise Comparisons for Group C

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
24 hr	3rdday	17.093*	3.638	.017	2.706	31.480
	7thday	.597	5.193	1.000	-19.936	21.130
	2ndwk	-91.282*	3.659	.000	-105.752	-76.812
	3rdwk	-160.682*	4.492	.000	-178.444	-142.920
	4thwk	-10.257	6.595	1.000	-36.336	15.822
3rd day	24 HR	-17.093*	3.638	.017	-31.480	-2.706
	7th day	-16.496	5.205	.171	-37.079	4.087
	2ndwk	-108.375*	4.370	.000	-125.655	-91.095
	3rdwk	-177.775*	3.656	.000	-192.232	-163.318
	4thwk	-27.350	8.253	.135	-59.983	5.283
7th day	24HR	-.597	5.193	1.000	-21.130	19.936
	3rd day	16.496	5.205	.171	-4.087	37.079
	2ndwk	-91.879*	3.298	.000	-104.921	-78.837
	3rdwk	-161.279*	4.073	.000	-177.386	-145.172
	4thwk	-10.854	7.209	1.000	-39.360	17.652
2ndwk	24HR	91.282*	3.659	.000	76.812	105.752
	3rd day	108.375*	4.370	.000	91.095	125.655
	7th day	91.879*	3.298	.000	78.837	104.921
	3rdwk	-69.400*	4.324	.000	-86.498	-52.302
	4thwk	81.025*	6.744	.000	54.359	107.691

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
3rd wk	24HR	160.682*	4.492	.000	142.920	178.444
	3rd day	177.775*	3.656	.000	163.318	192.232
	7th day	161.279*	4.073	.000	145.172	177.386
	2nd wk	69.400*	4.324	.000	52.302	86.498
	4th wk	150.425*	8.359	.000	117.373	183.477
4th wk	24HR	10.257	6.595	1.000	-15.822	36.336
	3rd day	27.350	8.253	.135	-5.283	59.983
	7th day	10.854	7.209	1.000	-17.652	39.360
	2nd wk	-81.025*	6.744	.000	-107.691	-54.359
	3rd wk	-150.425*	8.359	.000	-183.477	-117.373

Based on estimated marginal means

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

a. Adjustment for multiple comparisons: Bonferroni.

GROUP D

Table 14.Descriptive Statistics& repeated measures for group D

	Mean	Std. Deviation	N
24 HOURS	118.56	4.58184	10
3RD DAY	74.4200	4.83331	10
7TH DAY	50.8040	3.48801	10
2ND WEEK	95.6410	5.22182	10
3RD WEEK	108.36	4.22024	10
4TH WEEK	92.8540	4.35090	10

Table 15.Bonferroni's Pairwise Comparisons for group D

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
24 hr	3rd day	44.136*	1.109	.000	39.752	48.520
	7th day	67.752*	1.014	.000	63.742	71.762
	2nd wk	22.915*	1.252	.000	17.963	27.867
	3rd wk	10.195*	.732	.000	7.302	13.088
	4th wk	25.702*	1.489	.000	19.815	31.589
3rd day	24 HR	-44.136*	1.109	.000	-48.520	-39.752
	7th day	23.616*	.791	.000	20.490	26.742
	2nd wk	-21.221*	1.210	.000	-26.007	-16.435
	3rd wk	-33.941*	.819	.000	-37.179	-30.703
	4th wk	-18.434*	1.463	.000	-24.217	-12.651
7th day	24HR	-67.752*	1.014	.000	-71.762	-63.742
	3rd day	-23.616*	.791	.000	-26.742	-20.490
	2nd wk	-44.837*	1.219	.000	-49.656	-40.018
	3rd wk	-57.557*	.985	.000	-61.451	-53.663
	4th wk	-42.050*	1.368	.000	-47.461	-36.639
2nd wk	24HR	-22.915*	1.252	.000	-27.867	-17.963
	3RD	21.221*	1.210	.000	16.435	26.007
	7TH	44.837*	1.219	.000	40.018	49.656
	21ST	-12.720*	.961	.000	-16.521	-8.919
	28TH	2.787	1.651	1.000	-3.740	9.314
3rd wk	24HR	-10.195*	.732	.000	-13.088	-7.302
	3rd day	33.941*	.819	.000	30.703	37.179
	7th day	57.557*	.985	.000	53.663	61.451
	3rd wk	12.720*	.961	.000	8.919	16.521
	4th wk	15.507*	1.530	.000	9.458	21.556
4th wk	24HR	-25.702*	1.489	.000	-31.589	-19.815
	3rd day	18.434*	1.463	.000	12.651	24.217
	7th day	42.050*	1.368	.000	36.639	47.461
	2nd wk	-2.787	1.651	1.000	-9.314	3.740
	3rd wk	-15.507*	1.530	.000	-21.556	-9.458

Based on estimated marginal means

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

a. Adjustment for multiple comparisons: Bonferroni.

INTRAGROUP COMPARISON – REPEATED MEASURES ANOVA

Table 16.Bonferroni's post hoc test for Group A showing the p values

Time intervals	24 hours	3rd day	7th day	2nd week	3rd week	4th week
24 hours	-	0.0001	0.0001	0.0001	0.001	0.0001
3rd day	0.0001	-	0.0001	0.0001	0.0001	0.0001
7th day	0.0001	0.0001	-	0.0001	0.0001	0.0001
2nd week	0.0001	0.0001	0.0001	-	**1.000**	0.0001
3rd week	0.001	0.0001	0.0001	**1.000**	-	0.0001
4th week	0.0001	0.0001	0.0001	0.0001	0.0001	-

P value is significant at the 0.05 level

Table 17.Bonferroni's post hoc test for GroupB showing the p values

Time intervals	24 hours	3rd day	7th day	2nd week	3rd week	4th week
24 hours	-	**0.078**	**0.405**	0.001	0.0001	**1.000**
3rd day	**0.078**	-	1.000	0.001	0.0001	0.010
7th day	**0.405**	**1.000**	-	0.032	**0.201**	**0.648**
2nd week	0.001	0.001	0.032	-	**1.000**	0.0001
3rd week	0.0001	0.0001	**0.201**	**1.000**	-	0.0001
4th week	**1.000**	0.010	**0.648**	0.0001	0.0001	-

P value is significant at the 0.05 level

Table 18.Bonferroni's post hoc test for Group C showing the p values

Time intervals	24 hours	3rd day	7th day	2nd week	3rd week	4th week
24 hours	-	0.017	**1.000**	0.0001	0.0001	**1.000**
3rd day	0.017	-	**0.171**	0.0001	0.0001	**0.135**
7th day	**1.000**	**0.171**	-	0.0001	0.0001	**1.000**
2nd week	0.0001	0.0001	0.0001	-	0.0001	0.0001
3rd week	0.0001	0.0001	0.0001	0.0001	-	0.0001
4th week	**1.000**	**0.135**	**1.000**	0.0001	0.0001	-

P value is significant at the 0.05 level

Table 19.Bonferroni's post hoc test for GroupD showing p values

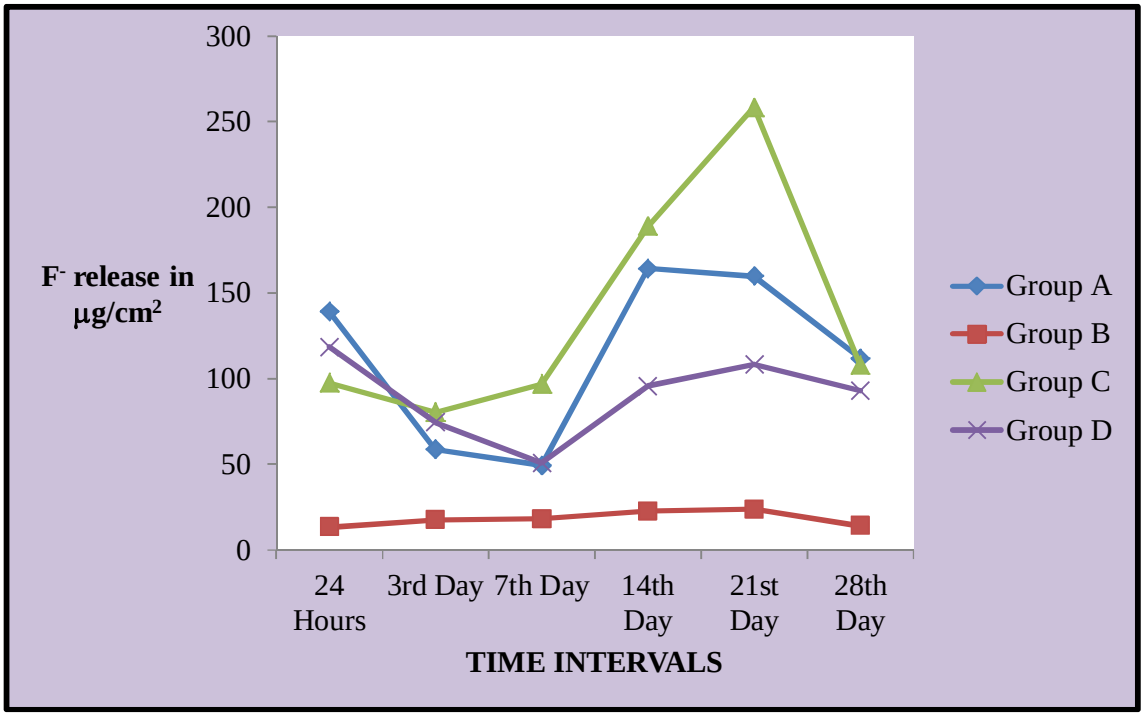
Time intervals	24 hours	3rd day	7th day	2nd week	3rd week	4th week
24 hours	-	0.0001	0.0001	0.0001	0.0001	0.0001
3rd day	0.0001	-	0.0001	0.0001	0.0001	0.0001
7th day	0.0001	0.0001	-	0.0001	0.0001	0.0001
2nd week	0.0001	0.0001	0.0001	-	0.0001	**1.000**
3rd week	0.0001	0.0001	0.0001	0.0001	-	0.0001
4th week	0.0001	0.0001	0.0001	**1.000**	0.0001	-

P value is significant at the 0.05 level

Table 20.Inter group &Intragroup comparison of F release at respective time intervals

TIME INTERVAL	MEAN F⁻RELEASE IN µg/cm^2				P VALUE
	GROUP A	GROUP B	GROUP C	GROUP D	
24 HOUR	139.05±6.07	13.33 ± 3.15	97.61 ± 10.90	118.56 ± 4.58	0.0001
3RD DAY	58.75 ± 4.85	17.52 ± 1.15	80.51 ±7.90	74.42 ± 4.83	0.0001
7TH DAY	49.31 ± 6.04	18.20 ±4.53	97.00 ± 12.64	50.80 ±3.48	0.0001
2ND WEEK	164.21 ±5.72	22.73 ± 2.61	188.89 ±11.53	95.64 ± 5.22	0.0001
3RD WEEK	159.89 ± 8.27	23.67 ± 2.01	258.29 ± 8.73	108.36 ± 4.22	0.0001
4TH WEEK	111.88 ± 6.11	14.01 ± 1.85	107.86 ± 5.27	92.85 ± 4.35	0.0001
P VALUE	0.0001	0.001	0.0001	0.0001	

P value is significant at the 0.05 level



Graph 1.Line Diagram showing Fluoride release at various time intervals in µg/cm^2

INFERENCE

INTERGROUP COMPARISON

One way ANOVA and Tukey'spost hoc statistical tests were done by using SPSS software version 16.0

In **24 hours** the mean fluoride release was found to be highest for the group A(139.05±6.07µg/cm^2) followed by group D(118.56 ± 4.58µg/cm^2), group C(97.61 ± 10.90µg/cm^2) and group B(13.33± 3.15µg/cm^2) respectively.This difference was found to be statistically significant (p=0.0001)

On the **7th day**the mean fluoride release was found to be highest for the group C(97.00± 12.64 μg/cm^2)followed by group D(50.80 ±3.48μg/cm^2) followed by groupA(49.31 ±6.04 μg/cm^2)and group B(18.20 ±4.53 μg/cm^2)

On the **2nd week** where the fluoride release was measured cumulatively from 8th day to 14th day the mean fluoride release was found to be highest for the group C(188.89±11.53μg/cm^2) followed by group A(164.21±5.72μg/cm^2) followed by group D(95.64±5.22μg/cm^2)and groupB(22.73± 2.61μg/cm^2)

On the **3rd week** the cumulative mean fluoride release of fluoride from 15th to 21st day was found to be highest for group C (258.29 ± 8.73μg/cm^2) followed by group A (159.89 ± 8.27μg/cm^2), group D (108.36± 4.22 μg/cm^2) and group B (23.67 ± 2.01μg/cm^2)

On the **4th week** the cumulative mean fluoride release of fluoride from 22nd to 28th day was found to be highest for group A (111.88 ± 6.11 μg/cm^2) followed by group C (107.86± 25.27μg/cm^2) ,group D (92.85± 4.35μg/cm^2) and group B (14.01 ± 1.85μg/cm^2)

With respect to intergroup comparison

24 hour mean fluoride release between the groups were statistically significant (p=0.0001)

GROUP A> GROUP D> GROUP C> GROUP B

On the 3rd day the mean fluoride release values of group C and group D were not statistically significant(p=0.063)

GROUP C ≥GROUP D> GROUP A> GROUP B

On the 7th day the mean fluoride release values of group A and group D were not statistically significant(p=0.971)

GROUP C> GROUP D≥GROUP A > GROUP B

On 2nd& 3rd weeks the mean cumulative values of fluoride release between the groups were statistically significant (p=0.0001)

14TH DAY -- GROUP C> GROUP A> GROUP D> GROUP B

21STDAY --GROUP C> GROUP A> GROUP D> GROUP B

On 4th week the mean fluoride release values of group A and group Cand as well as the mean fluoride release values of group C and group D were not statistically significant(p=0.907)

GROUP A≥GROUP C≥GROUP D> GROUP B

INTRAGROUP COMPARISON

For Group A, The mean fluoride release values at 24 hr, 3rd day, 7th day were 139.05 ± 6.07 μg/cm^2,58.75± 4.85μg/cm^2,49.31 ± 6.04μg/cm^{2}respectively. The fluoride release values showed a drastic decrease on 3rd day and an appreciable decrease from 3rd to 7th day ,the difference of which was found to be statistically significant (p=0.0001).

24 hr>3rd day >7th day

The mean cumulative fluoride release for 2nd week, 3rd week, 4th week were found to be 168.21 ± 5.72 μg/cm^2 **,**159.89 ± 8.27 μg/cm^2, 111.88 ± 6.11 μg/cm^{2}respectively. There was a minimal decrease in fluoride release from 2nd week to 3rd week which was not statistically significant (p = 1). The cumulative release finally dropped during 4th week which was statistically significant (p=0.0001) when compared to both 2nd& 3rd weeks.

2nd week ≥ 3rd week > 4th week

For Group B, the mean fluoride release at 24 hr,3rd day,7th day were found to be 13.33 ± 3.15μg/cm^2 **,** 17.52 ± 1.15μg/cm^{2}18.20 ± 4.53 μg/cm^{2}respectively. There was a steady

increase in the fluoride release from the 24 hr to the 7th day but this difference was not found to be statistically significant (P=0.405)

24 hr ≤ 3rd day ≤ 7th day

The mean cumulative fluoride release for 2nd week, 3rd week,4th week were found to be 22.73 ± 2.61μg/cm^2 **,**23.67 ± 2.01μg/cm^2,14.01 ± 1.85 μg/ cm^{2}respectively. There was a minimal increase in the cumulative fluoride release from 2nd week to 3rd week which was not statistically significant (p= 1). There was a dip in the release during 4thweek which was statistically significant (p=0.0001) when compared to 2nd week & 3rd weeks.

3rd week≥ 2nd week > 4th week

For Group C,The mean fluoride release at 24 hr ,3rd day ,7th day were 97.60 ± 10.90 μg/cm^2 80.51 ± 7.90 μg/cm^2 **,** 97.00 ± 12.64 μg/cm^{2}respectively. There was a dip in fluoride release from 24 hr to 3rd day which was statistically significant(p = 0.017) which raised again to reach 24hr values on 7th day which was not statistically significant (p= 1)

24 hr ≥ 7th day > 3rd day

The mean cumulative fluoride release for 2nd week, 3rd week, 4th week were found to be 188.89 ± 11.53 μg/cm^2 **,** 258.29 ± 8.73μg/cm^2 **,** 107.86 ± 25.57μg/cm^{2}respectively. There was a steep raise in fluoride release from 2nd week to 3rd week which was statistically significant (p = 0.0001) and showed a drastic decline during 4th week which was also statistically significant (p = 0.0001)

3rd week > 2nd week > 4th week

For Group D, the mean fluoride release at 24 hr, 3rd day ,7th day were 118.56 ± 4.58 μg/cm^{2}74.42 ± 4.83μg/cm^2 **,** 50.80 ± 3.48μg/cm^2 respectively. There was a prominent

decrease in fluoride release from 24 hr to 3rd day and a notable dip from 3rd day to 7th day both of which were statistically significant (p = 0.0001) .

24 hr> 3rd day > 7th day

The mean cumulative fluoride release on 2nd week, 3rd week,4th week were found to be 95.64 ± 5.22 μg/cm^2,108.36 ± 4.22μg/cm^2, 92.85 ± 4.35 μg/cm^2 respectively. There was a increase in cumulative fluoride release values from 2nd week to 3rd week which was statistically significant (p = 0.0001).The cumulative values dropped finally during the 4th week which was not statistically significant (p = 1) when compared to 2nd week.

3rd week > 2nd week ≥ 4th week

COMPRESSIVE STRENGTH EVALUATION

The compressive strength values of the specimens that were obtained in MPa after subjecting the samples to universal testing machine were tabulated as below.

Table 21. 24 hour compressive strength of groups in MPa

Sample no.	GROUP A	GROUP B	GROUP C	GROUP D
1	75.34	81.19	34.23	92.32
2	69.45	85.56	35.56	95.16
3	72.87	89.76	30.17	97.22
4	69.21	85.44	28.64	102.62
5	68.62	82.26	32.65	98.46
6	74.26	81.78	35.12	94.38
7	76.66	90.21	29.93	99.83
8	72.97	91.71	36.31	101.36
9	77.34	82.01	34.23	98.46
10	68.87	83.16	30.17	95.16

Table 22 .Descriptive &One Way ANOVA – statistical analysis at various time intervals

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Upper	Lower		
Compressive Strength	Group A	10	72.5590	3.34291	1.05712	70.1676	74.9504	68.62	77.34
	Group B	10	85.3080	3.93139	1.24321	82.4957	88.1203	81.19	91.71
	Group C	10	32.7010	2.76306	.87376	30.7244	34.6776	28.64	36.31
	Group D	10	97.4970	3.26569	1.03270	95.1609	99.8331	92.32	102.62
	Total	40	72.0162	24.87074	3.93241	64.0622	79.9703	28.64	102.62

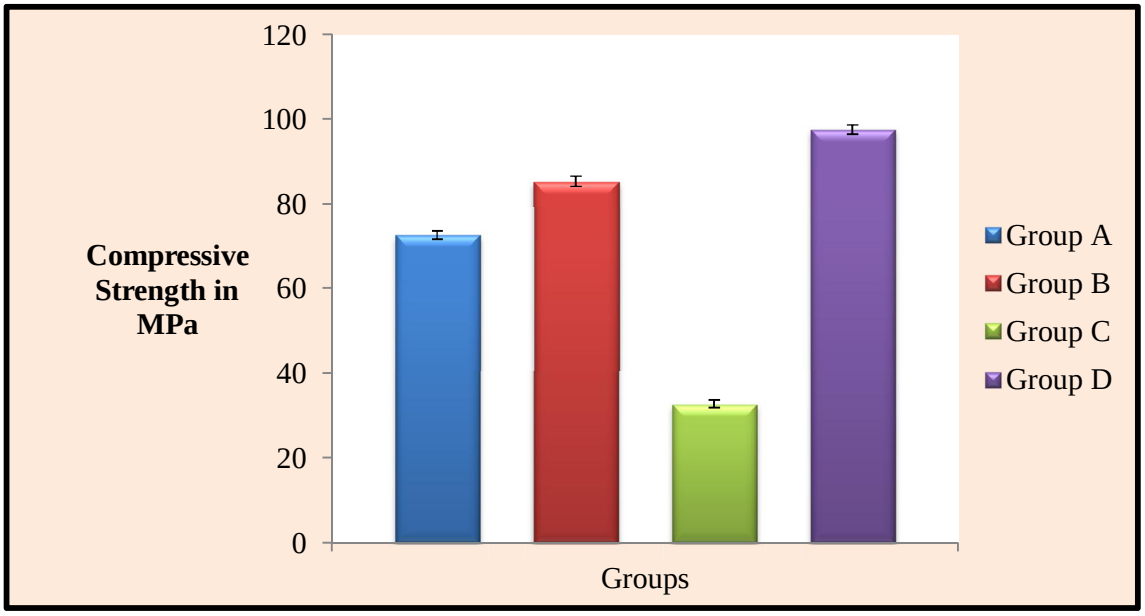
Table 23.ANOVA between the groups for compressive strength

		Sum of squares	df	Mean Square	F	Sig.
Compressive Strength	Between Groups	23719.227	3	7906.409	703.886	.000
	Within Groups	404.371	36	11.233		
	Total	24123.598	39			

Table 24. Multiple Comparisons using Tukey'spost hoc test between the groups

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Compressive Strength	Group A	Group B	-12.74900*	1.49883	.000	-16.7857	-8.7123
		Group C	39.85800*	1.49883	.000	35.8213	43.8947
		Group D	-24.93800*	1.49883	.000	-28.9747	-20.9013
	Group B	Group A	12.74900*	1.49883	.000	8.7123	16.7857
		Group C	52.60700*	1.49883	.000	48.5703	56.6437
		Group D	-12.18900*	1.49883	.000	-16.2257	-8.1523
	Group C	Group A	-39.85800*	1.49883	.000	-43.8947	-35.8213
		Group B	-52.60700*	1.49883	.000	-56.6437	-48.5703
		Group D	-64.79600*	1.49883	.000	-68.8327	-60.7593
	Group D	Group A	24.93800*	1.49883	.000	20.9013	28.9747
		Group B	12.18900*	1.49883	.000	8.1523	16.2257
		Group C	64.79600*	1.49883	.000	60.7593	68.8327

*. The mean difference is significant at the 0.05 level



Graph 2.Bar diagram showing 24 hour compressive strength of groups in MPa

INFERENCE

One way ANOVA and Tukey's post hoc statistical tests were done by using SPSS software version 16.0

The mean 24 hour compressive strength was found to be highest for group D (97.49±3.26MPa) followed by group B (85.30 ± 3.93MPa) ,group A (72.55 ± 3.34MPa) and the least compressive strength was for group C (32.70 ± 2.76MPa)

Group D > GROUP B > GROUP A > GROUP C

This difference was found to be statistically significant (p=0.0001)

Discussion

DISCUSSION

The advent of calcium silicate technology has transformed the entire face of restorative dentistry. Mineral Trioxide Aggregate (MTA) is a calcium silicate based biomaterial that has been investigated for endodontic and regenerative applications since the early 1990s.Originally developed by Torabinejad at Loma Linda University, MTA was first described in the dental scientific literature in 1993 and was approved approval for endodontic use by the U.S. Food and Drug Administration in 1998.

MTA is a bioactive, Portland cement based, tricalcium silicate cement that has been shown to be a successful pulp-capping agent based on recent in vitro, animal and in vivo studies . The success of MTA can be attributed to its small particle size, sealing ability that is possibly credited to the synthesis of an interface with dentine that resembles hydroxyapatite in composition, alkaline pH and slow discharge of calcium ions. Investigators have reported that MTA induces the proliferation of pulp cells, releases cytokine, and promotes hard tissue formation. The material is non-absorbable, sets in the presence of moisture, has a relatively high compressive strength and has a sustained high alkaline pH.[57]

The main drawbacks of MTA include its potency to cause tooth discoloration, presence of toxic elements in it's composition, difficult handling properties, long setting time, cost ineffectiveness, the absence of a known solvent to retrieve the material and the difficulty faced during it's removal post hardening.[57]

Calcium silicates set in the presence of moisture, are highly bioactive and biocompatible but these materials possess less reactivity, have long setting times, low mechanical properties and difficult handling properties.[9]

Biodentine™ was developed as a dentine replacement material, a novel clinical application of this family of materials, intending it to function as a coronal restoration. The relatively short setting time of 12 min, enabled the use of this cement for restorative procedures; impossible with MTAs that achieve an initial setting 3–4 h.[76]Biodentine™ is principally composed of a highly purified tri-calcium silicate powder that is prepared synthetically in the lab de novo, rather than derived from a clinker product of cement manufacture. The components of Biodentine™ are di-calcium silicate, calcium carbonate and zirconium dioxide as a radiopacifer. The di-calcium and tri-calcium silicate phases comprised about 70% of the weight of Biodentine's de-hydrated powder, which is close to that of white MTA and white Portland cement.[8,15]

Biodentine is a versatile material and can be used both in the coronal and radicular parts of the tooth. Its uses include pulp protection, temporary enamel replacement and permanent dentine substitute, deep caries management, direct and indirect pulp capping and pulpotomy in coronal part & resorption repair, perforation repair and as a root end filling material in radicular part.[62,64, 37,61] Thus Biodentine is used primarily as a dentine replacement material.[15]The release of calcium hydroxide from the set material makes calcium trisilicates the ideal material for dentine replacement as it has both the beneficial effects of a liner material while the calcium silicate matrix will act as a rigid structure replacing the dentine in bulk.

Glass ionomer is a well established dentine substitute which served successfully dentistry for years together , now the other promising dentine substitute is Biodentine. The flexural strength ,modulus of elasticity,24 hr compressive strength and microhardness of Biodentine are, 24.0 MPa,22.0GPa,213.7 MPa and 60.9 HVN respectively whereas for glass ionomer the values were 26.6MPa, 14.9GPa, 124.7 MPa and 77.8HVN respectively.[9] The physical properties are almost comparable between Biodentine and glass ionomer, **but the only lacunae in properties of Biodentine that hinders it from replacing glass ionomer as a dentine substitute is the fluoride releasing property . So we have undertaken an innovative approach of modifying Biodentine by incorporating fluoride both in the powder and liquid components of Biodentine & also assessed the fluoride release at various time intervals in this study. We have also assessed the alteration in the physical property(compressive strength) and setting time of this experimental material.**

It was over 70 years ago, the fluoride was introduced into dentistry, and it is already recognized as the main factor responsible for the dramatic decline in caries prevalence worldwide.[14]The widespread use of fluoride has contributed to improve the remineralization of the enamel and dentine exposed to acid challenge in the oral environment.[46]

The effect of fluoride on demineralization & remineralization of incipient caries lesions in enamel and dentine is the most important mechanism of fluoride action. It has been recognized that the initial carious lesion should be exposed to fluoride in the aqueous phase for a prolonged period of time to achieve the cariostatic effect.[75,27]

Fluoride present in low, sustained concentrations in the oral fluids during an acidic challenge is able to absorb to the surface of the apatite crystals, inhibiting demineralization. When the pH is re-established, traces of fluoride in solution will make it highly supersaturated with respect to fluorhydroxyapatite, which will speedup the process of remineralization. The mineral formed under the nucleating action of the partially dissolved minerals will then preferentially include fluoride and exclude carbonate, rendering the enamel more resistant to future acidic challenges.[14]

In the last two decades, the addition of fluoride to restorative materials has attracted the attention of dental researchers and clinicians as for the possibility of using these materials as a source of low fluoride release to the teeth, within long periods. These so-called "intelligent" dental materials have been elaborated with the purpose of reducing secondary caries and neutralizing the pH decrease, especially in high-caries risk patients. Their mechanical and esthetic properties have been improved and most of them can now be used to restore posterior teeth.[31]

The principal reason for restoration failure is secondary caries in both permanent and primary dentition.[12,26,81]Recommendations have been made to aid in the prevention of secondary caries, includes tooth brushing, topical fluoride therapy, sealing restoration margins, and utilization of antimicrobial agents.[39]The idea of incorporating fluoride into dental material conceived principally to tap this anticariogenic property of fluoride in preventing secondary caries and to prevent restoration failure.

In vitro studies have shown that fluoride released from fluoride-containing restorative materials effectively protected the tooth tissues from demineralization in the region near to the restorative materials.[74,34]

Fluoride that is in an aqueous phase surrounding dental tissues inhibits demineralization much more effectively than fluoride incorporated into crystals of apatite. Moreover, fluoride precipitated onto tooth surfaces in the form of CaF_2 serves as a reservoir of fluoride when pH drops.[23]

For fluoride to be successfully incorporated into a dental material and have a positive effect, three conditions must be met, namely:

1) The material must be chemically capable of accepting the fluoride
2) The fluoride must be released from the material in sufficient quantities and at a constant rate over the lifespan of the dental material.
3) The fluoride must not adversely affect the properties of the dental material.[6]

Only restorative materials that release high amounts of fluoride ions such as GICs, have been shown to effectively inhibit the demineralization of tooth structures adjacent to restorative margins.[34]Tantbirojn et al. found that under an *in vitro* demineralization challenge, glass-ionomer liners in an open-sandwich restoration exhibited pronounced inhibition zones at the dentine margin and lowered the amount of mineral loss in the vicinity of 0.25 mm from the restoration interface.[73]Another study reported that the degree of protection was highest in the closest vicinity of the restorations and the depth of lesions increased with the distance in an inverse relationship to fluoride released.[28]

The various fluoride releasing dental materials are glass ionomer cements, RMGIC, composites, polyacid modified composites, giomers and glass carbomers. Glass ionomer cements and their modifications are inherently fluoride releasing materials.[5]

Gjorgievska et al.found that glass-ionomers, both conventional or resin-modified, are more effective at protecting the tooth against further decay than either compomers or fluoride-releasing composites, with the best protection of all being provided by conventional glass-ionomers. The nature of the tooth had no influence on these outcomes.[32]**Thus in our study we have used conventional Glass Ionomer Cement as the positive control to compare the release of fluoride from the fluoride modified Biodentine.**

Fluoride modifications of composite

Appropriate fluoride containing compounds are added to composites which include inorganic salts (e/g. NaF or SrF_2), fluoridated glasses or organic fluoride compounds. Longer-term sustained release requires only sparingly soluble fluoride salts, such as SrF_2 orYbF_3, or leachable glass fillers.[67]

Composite resins are not inherently fluoride-releasing, but can be made so by adding fluoride compounds.[35]

There are at least three methods used to combine therapeutic agents such as fluoride into dental materials. These are simple mixture of water-soluble agents, dispersion of sparingly water-soluble agents and use of matrix-bound agents**.**[66]

The new techniques have been employed to incorporate fluoride ion in the composite resins. One approach was to incorporate inorganic fluoride, such as NaF, into the monomer system.[20]A second approach is that of dispersion agents which are sparingly soluble in water into a polymer matrix, water diffuse into the polymer which results in release of some amount of fluoride ion,[19,70] or sparingly soluble salt such as

YbF_3.[3,70] A similar approach was tried to encapsulate in an insoluble material as filler in a polymer matrix.[65]

Rawls and Zimmerman[65] have described a fluoride releasing monomer based on acrylic-amine-HF salt from which fluoride can be released by replacement by another negatively charged ion from surrounding fluids. Some have extended work of Rawls to make a fluoride exchange material using morpholinoethyl methacrylate hydrofluoride (MEM/HF) as the fluoride releasing co-monomer, it was reported that rate of fluoride release was almost double as previously reported by others.[34]Other materials such as tetrabutyl ammonium tetrafluoroborate (TBATFB) had been used along with composite and fluoride release were found to be comparable with GIC materials.

FLUORIDE AGENTS ADDED IN THIS STUDY:

i) Sodium fluorosilicate/ Sodium hexafluorosilicate/ Sodium silico fluoride ($Na_2[SiF_6]$):

Applebaum et al[2] conducted a study in which Sodium fluorosilicate $Na_2[SiF_6]$ was added to MTA and the properties of the resultant material was assessed. In our study we have used the same agent with Biodentine and assessed the fluoride released from this modified material. Sodium fluorosilicate is a complex fluoride salt.

Chow and Takagi[18]have used $Na_2[SiF_6]$ as a mouth rinse and found that the fluoride uptake and cariostatic effects were efficacious with $Na_2[SiF_6]$. Eidelman and Chow[24]assessed the effects of Ca^{++} ions and pH on the hydrolysis of $Na_2[SiF_6]$. According to this study the promoting effect of Ca^{++} ions was stronger than the

inhibitory effect of H^{++} ions. This hydrolysis property of $Na_2[SiF_6]$made it suitable to be used with an acidic calcium phosphate solution for remineralisation.

Takagi et al[72] assessed the content of loosely bound and firmly bound fluoride comparing acidulated phosphate fluoride (APF) and monocalcium phosphate monohydrate-sodium fluorosilicate (MCPM-$Na_2[SiF_6]$) applied to enamel assessed after 4 minutes and 2 hours and they found that the degree of loosely bound and firmly bound fluoride was much greater with MCPM-$Na_2[SiF_6]$ than APF and could be a potential topical fluoride agent. **In our study, we have taken this sparingly soluble complex agent as one of the modification agents by adding it to the powder component of Biodentine**

A pilot study was conducted in our department by admixing 5wt % ,7wt% and 10 wt % of $Na_2[SiF_6]$ to Biodentine powder component, fluoride releasing properties & compressive strength were assessed, we found that F^- release was highest for 10wt % followed by 7 wt%& 5 wt %. But the difference in F^- release was not statistically significant between 7 wt%& 10wt %. The compressive strength was poor for 10 wt % $Na_2[SiF_6]$ modified Biodentine whereas 7 wt%$Na_2[SiF_6]$ modified Biodentine had only minimal reduction in compressive strength. Hence we chose 7 wt%$Na_2[SiF_6]$ for modifying powder component of Biodentine in our study.

ii) Hydrofluoric acid (HF):

Hydrofluoric acid is a water soluble agent that is a good source of fluoride and has been tested as an agent to protect enamel against erosion. Hjortsjo et al[44] used HF and Stannous fluoride (SnF_2) on enamel which was priorly exposed to an acid challenge of citric acid to assess the protective effect of these agents on enamel after

erosion. The authors concluded that the repeated effect of HF and SnF_2 could be helpful in increasing the protective effects of these fluorides against erosion on enamel.

Pioch et al[59]assessed the effect of HF on dentine after exposing dentine to orthophosphoric acid (H_3PO_4). They found that HF has the ability to seal the dentinal tubules and protects the smear layer thereby exhibiting a protective effect on the dentine.

Thus in our study we chose a water soluble agent already tried on enamel and dentine namely HF as the second fluoridating agent. Not only is HF water soluble, it has made the modification of the liquid component of Biodentine possible.

A pilot study was conducted in our department modifying the liquid component of Biodentine with various conc. of HF such as 10 w/v % of 5 %,10% & 20 % hydrofluoric acid. The study showed that 10 w/v% of 20 % HF showed appreciable fluoride release .Hence we chose 10 w/v% of 20 % HF for modifying liquid component in our study

FLUORIDE ANALYSING METHODS

Fluoride is an element found in biologic tissues and the environment and there are various methods to detect its presence and assess the content present. The assessment of fluoride content in biological tissues like saliva, bones, teeth etc. can be estimated primarily by potentiometric methods (ion selective electrode [ISE]) and gas chromatographic (GC) methods.[78] Colorimetric methods are available, but are more time consuming and lack the sensitivity of the other methods. Fluoride detection from environmental media such as air, water or soil requires techniques like ion selective

electrode, chromatography and spectrophotometry. Particularly to detect fluoride from water, colorimetric analysis is predominantly used.[78]

Spadns spectrophotometry is a technique employed specifically to detect and quantify fluoride content of water samples. In this method a compound of a metal such as aluminium, iron, thorium, zirconium, lanthanum or cerium reacts with an indicator dye to form a complex of low dissociation constant. This complex reacts with fluoride to give a new complex (Jacobson et al. 1977). In environmental protection agency (EPA) Method 340.1, **the sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonate (SPADNS) reagent** is used, and the color loss is measured at 570 nm (EPA 1998c). Due to the change in the structure of the complex, the absorption spectrum also shifts relative to the spectrum for the fluoride-free reagent solutions. This change can be detected by using a spectrophotometer.

We used the SPADNS method, as it does not involve sophisticated instruments and expensive chemicals. Interference from other ions such as aluminium, iron, hexametaphosphate and orthophosphate which are commonly found in raw water samples, is less for the SPADNS method compared to other methods such as the Cerium (III)- alizarin complex which is one of the methods in analysing the fluoride in drinking water.

Brossoket al[11] compared the accuracy and interference factors associated with colorimetric analysis. According to this study, the accuracy of colorimetric techniques differed only in the presence of sulphates. In our study we are not using biological medium like saliva or artificial saliva but distilled water instead, so the presence of extraneous impurities and the resultant influence on fluoride values because of this is rare. In our study the fluoride release was estimated among the study groups in the

distilled water which can considered as partial simulation of dentinal fluid & other media like artificial saliva or phosphate buffer solution were not used, since this modified Biodentine is not expected to come into contact with the oral fluids .

Since the fluoride estimation was expected to be high on the initial days of the first week, the estimation was carried on specific days i.e 24 hr, 3rd and 7th day as individual day fluoride release. But thereafter from 8th day onwards the cumulative fluoride release was assessed on weekly basis at 14th, 21st 28th days.

At 24 hour, the fluoride release of Group A (powder only modified) was higher than Group D (glass ionomer) which was statistically significant.

On 3rd day Group C (both component modified) showed higher fluoride release than Group D (glass ionomer) which was not statistically significant

On,7th 14th& 21st days the Group C (both component modified) showed higher fluoride release than Group D (glass ionomer)

On 28th day group A had higher fluoride release followed byGroup C & Group D which was not statistically significant.

At all estimation time periods , the powder only modified & both component modified Biodentine groups showed higher fluoride values when compared to positive control group (glass ionomer) whereas on 28th day the fluoride release declines and stays on par with glass ionomer cement.

The mechanism by which fluoride has released from modified Biodentine could be due to the burst effect of F^- ions [66] similar to GIC , surface wash off, release through pores and cracks & bulk release .

All the three groups of modified Biodentine showed fluoride release .The powder only modified Biodentine showed better fluoride release than glass ionomer

which could be attributed to the sparingly water soluble nature of sodium fluorosilicate which was used in our study.

The liquid only modified Biodentine showed minimal fluoride release in comparison to other modified Biodentine groups & also glass ionomer cement ,which could be attributed to partial entrapment of fluoride ion in the set Biodentine in the form of calcium fluoride (CaF_2) which could be probably due to the reaction with calcium chloride ($CaCl_2$)present in the liquid component of Biodentineas an accelerator. The both component modified group showed a much more better fluoride release than the powder only modified Biodentine conveying that the synergistic effect of sodium fluorosilicate and hydrofluoric acid could be the probable reason.

SETTING TIME & WORKABILITY

It was inferred from this study that the workability of this modified Biodentine was not much affected in powder only modified group with the setting time of 11 min which is on par with the setting time of unmodified Biodentine (12 min) which is claimed by the manufacturer. The liquid only modified group was less viscous to manipulate with a prolonged setting time of 15 minutes & finally the both powder and liquid modified Biodentine had mild effervescence on manipulation, less workablity and exhibited a setting time of 9 minutes.

COMPRESSIVE STRENGTH ANALYSIS

With regards to the physical properties of modified Biodentine, where we have assessed the compressive strength at 24 hour period, the compressive strength of the modified Biodentine groups were lower than the unmodified Biodentine which was statistically significant. Among the modified groups of Biodentine, the Group B (liquid only modified) showed higher compressive strength followed by Group A

(powder only modified) and Group C (both components modified) showing the least.

Dijkman & Arends[22] reported that a monthly cumulative fluoride release of 200–300 μg/cm^2 is sufficient to completely inhibit enamel demineralization. In the present study both the powder only modified as well as the both component modified groups showed monthly cumulative fluoride release of around 600 μg/cm^2 & 700 μg/cm^2 respectively which is nearly 2 to 3 times the expected levels of fluoride to inhibit demineralisation.

Taking into consideration the compressive strength of the modified Biodentine, the compressive strength achieved by modifying powder component alone (i.e. around 70 MPa)seems to be appropriate and comparable to other intermediate restorative materials. The simultaneous modification of both components severely affects the compressive strength. The amount of fluoride release values of both component modified were almost comparable to powder only modified group.

Within the limitations of this study ,it can be concluded that modification of Biodentine can be done judiciously to achieve desirable fluoride release not compromising the compressive strength when the powder component alone is modified. Hence it is better to restrict ourselves with the modification of powder alone

The future perspectives of this study are to evaluate localisation of the fluoride ion in the three dimensional set structure of this modified Biodentine using X ray diffraction (XDR) and Fourier transform infrared spectroscopy (FTIR), long term evaluation of compressive strength, flexural strength, fluoride recharge properties & biocompatibility of thus modified Biodentine.

Summary

SUMMARY

The aim of this study was to evaluate the fluoride releasing properties & compressive strength of Biodentine modified with sodium fluorosilicate and hydrofluoric acid using Spadns spectrophotometer.

The modification of powder component of Biodentine was done using 7 wt% of sodium fluorosilicate and liquid component was modified by incorporating 10 w/v % of 20 % hydrofluoric acid.

The study comprised of a total of 80 samples divided into 4 groups of 20 samples in each group among which 10 samples were allocated for fluoride analysis and 10 samples were destined for compressive strength analysis in each group

The cylindrical samples were made using teflon moulds and the sample groups are as follows:

Group A : Biodentine powder only modified with 7wt%$Na_2[SiF_6]$

Group B : Biodentine liquid only modified with 10 w/v % of 20% HF

Group C : Biodentine powder modified with 7wt%$Na_2[SiF_6]$& Biodentine liquid modified with 10 w/v % of 20% HF

Group D : Glass Ionomer cement type II (positive control)

The samples were immersed in polypropylene containers containing distilled water. the samples were rinsed and replenished with distilled water every 24 hours during the first 7 days and weekly thereafter upto a period of 28 days. The fluoride estimation was done at 24 hours, 3^{rd} day 7^{th} day to assess the fluoride release of the corresponding days and cumulative fluoride release was assessed during the 14^{th} $21^{st}$and 28^{th} days using spadns spectrophotometer and the values were obtained in

ppm which were later converted into micrograms/square centimeter ($\mu g/cm^2$)

The cylindrical samples of similar dimensions which were stored in 100 % humidity at 37℃ were mounted on acrylic blocks and subjected to compressive strength analysis by universal testing machine at cross head speed of 0.5mm/min and the compressive strength values were obtained in MPa

It was inferred from this study that the workability of this modified Biodentine was not much affected in powder only modified group with the setting time of 11 min which is on par with the setting time of unmodified Biodentine, the liquid only modified group was a less viscous to manipulate with a prolonged setting time of 15 minutes & finally the both powder and liquid modified Biodentine had mild effervescence on manipulation, less workablity and exhibited a setting time of 9 minutes

Fluoride estimation by spadns spectrophometer showed that all the three modified groups of Biodentine released fluoride in distilled water. At 24 hr the mean average fluoride release of powder only modified was the highest followed by glass ionomer, both modified group and liquid only modified group in decreasing order. On the 3^{rd} day and 7^{th} days fluoride release was highest for the both components modified group and the least for liquid only modified group with no statistical difference between both components modified Biodentine and glass ionomer on 3^{rd} day and between glass ionomer and powder only modified Biodentine on 7^{th} day.

The cumulative release of fluoride which was estimated on 14^{th}&21^{st}days showed that the fluoride release was maximum for both components modified Biodentine followed by powder only modified Biodentine, glass ionomer and liquid only modified Biodentine.

On 28th day the fluoride release of both modified group dropped down, with powder only modified Biodentine showing higher fluoride release followed by both component modified Biodentine, glass ionomer and liquid only modified Biodentine showing the least fluoride release.

With regards to the 24 hours compressive strength, unmodified Biodentine group had the highest compressive strength followed by the liquid only modified Biodentine which were almost comparable to it. The powder only modified Biodentine showed minimal decrease in the compressive strength whereas the both component modified Biodentine exhibited poor compressive strength values

The future perspectives of this study are to evaluate localisation of the fluoride ion in the three dimensional set structure of this modified Biodentine using X ray diffraction (XDR) and Fourier transform infrared spectroscopy (FTIR) ,long term evaluation of compressive strength, flexural strength, fluoride recharge properties & biocompatibility of thus modified Biodentine.

Conclusion

CONCLUSION

Within the limitations of this study, the following conclusions can be drawn,

1. The fluoridation of Biodentine is achievable by incorporating sodium fluorosilicate in the powder component and hydrofluoric acid in the liquid component
2. Simultaneous modification of both components of Biodentine showed better fluoride release than the powder only modification
3. On 28th day, the fluoride release of powder only modified Biodentine & both components modified Biodentine were comparable with glass ionomer cement
4. Simultaneous modification of both components of Biodentine showed 67% reduction in 24hr compressive strength whereas the powder only modified group showed only 25 % reduction in the 24 hr compressive strength
5. The powder only modified Biodentine showed appreciable fluoride release without much compromise in the compressive strength.
6. Hence the powder only modified Biodentine can be used as a dentin substitute in posterior restorations utilising the fluoride release properties successfully.

Bibliography

BIBLIOGRAPHY

1. **About I, Laurent P, Tecles.** Bioactivity of Biodentine a Ca_3SiO_5-based Dentine Substitute. Oral session. IADR Congress July 2010,Barcelona,Spain.
2. **Appelbaum KS, Stewart JT, Hartwell GR.** Effect of sodium fluorosilicate on the properties of Portland cement. J Endod 2012 ; 38(7):1001-1003.
3. **Arends J and van der Zee Y .** Fluoride uptake in bovine enamel and dentine from a fluoride releasing composite resin. Quintessence Int 1990; 21: 541 – 544.
4. **Arora V, Nikhil N, Sharma N, Arora P.** Bioactive dentin replacement. IOSR- JDMS 2013;2(4): 51-57.
5. **Atmeh AR, Chong EZ, Richard G, Festy F, Watson TF.** Dentin - cement interfacial interaction: calcium silicates and polyalkenoates. J Dent Res 2012;91(5):454-459.
6. **Babar MG & Lin SL.** Cariostatic Effect of Fluoride-containing Restorative Materials: A Review. Malays Dent J 2009; 30(2): 130-136.
7. **Barghouthi Z, Amereihm S.** Spectrophotometric Determination of Fluoride in Groundwater Using Resorcin Blue Complexes.American Journal of Analytical Chemistry 2012; 3: 651-655.
8. **Belío-Reyes I.A, Bucio L, Cruz-Chavez E.** Phase composition of ProRoot mineral trioxide aggregate by X-ray powder diffraction. J Endod 2009; 35 (6):875–878.
9. Biodentine-active biosilicate technology , scientific file, septodont (www.septodont.fr/fichiers_upload/biodentinescientificfile.pdf)

10. **Briso AL, Mestrener SR, Delico G, Sunfeld RH et al.** Clinical Assessment of postoperative sensitivity in posterior composite restorations. Oper Dent 2007; 32:421-426.
11. **Brossok GE, McTigue DJ, Kuthy RA.** The use of a colorimeter in analyzing the fluoride content of public well water. Pediatr Dent 1987; 9 (3):204-207.
12. **Burke FJT, Cheung SW, Mjor IA, Wilson NHF.** Restoration longevity and analysis of reasons for the placement and replacement of restorations provided by vocational dental practitioners and their trainees in the United Kingdom. Quintessence Int 1999;30(4):234-242.
13. **Butt N, Talwar S, Chaudhry S, Nawal RR, Yadav S, Bali A.** Comparison of physical and mechanical properties of mineral trioxide aggregate and Biodentine. Indian J Dent Res 2014;25:692-697.
14. **Buzalaf MA, Passan JP, Honorio HM, Ten Cate JM.** Mechanisms of action of fluoride for caries control. Monogr Oral Sci 2011;22:97-114.
15. **Camilleri J.** Characterization and hydration kinetics of tricalcium silicate cement for use as a dental biomaterial. Dent Mater 2011 ;27 (8):836–844.
16. **Camilleri J**. Investigation of Biodentine as dentine replacement material. J Dent. 2013 ; 41(7):600-610.
17. **Camilleri J, Laurent P, About I.** Hydration of Biodentine, Theracal LC, and a prototype tricalcium silicate-based dentin replacement material after pulp capping in entire tooth cultures. J Endod 2014 ;40(11):1846-1854.
18. **Chow LC, Takagi S.** Deposition of fluoride on tooth surfaces by a two-solution mouthrinse in vitro. Caries Res 1991;25(6):397-401.

19. **Cook PA & Youngson CC.** A fluoride containing composite resin – An in-vitro study of a new material for orthodontic bonding. Br J Orthodont 1989;16: 207 – 212.
20. **Cooley RL, McCourt RL, Huddleston AM**. Evaluation of a fluoride-containing sealant by SEM, microleakage and fluoride release. Pediatric Dent 1990 12: 38 – 42.
21. **De Rossi A & Silva LA.** Comparison of pulpal responses to pulpotomy and pulp capping with biodentine and mineral trioxide aggregate in dogs. J Endod 2014; 40:1362–1369.
22. **Dijkman GE & Arends J**. Secondary caries in situ around fluoride-releasing light-curing composites: a quantitative model investigation on four materials with fluoride content between 0 and 26 vol%. Caries Res 1992; 26(5) : 351-7.
23. **Dionysopoulos D.** The effect of fluoride-releasing restorative materials on inhibition of secondary caries formation. Research review Fluoride 2014 ;47(3):258–265.
24. **Eidelman N & Chow LC.** Effects of pH and calcium on hydrolysis of Na_2SiF_6 and Na_2SnF_6. A quasi-constant c omposition titration study. Caries Res. 1991;25(2):1
25. **Elnaghy AM.** Influence of Acidic Environment on Properties of Biodentine and White Mineral Trioxide Aggregate: A Comparative Study. J Endod 2014; 40(7) 953–957.
26. **Featherstone JDB**. The science and practice of caries prevention. J Am Dent Assoc 2000;131(7):887-899.

27. **Featherstone JDB, Glena R, Shariati M, Shields CP.** Dependence of *in vitro* demineralization of apatite and remineralization of dental enamel on fluoride concentration. J Dent Res 1990;69 :620-625.
28. **Ferracane JL, Mitchem JC, Adey JD.** Fluoride penetration into the hybrid layer from a dentin adhesive. Am J Dent 1998;11(1):23-28.
29. **Firla M.** Dentine-Ersatzmaterial auf der Basis der Active Biosilicate Technology. DZW Kompakt 2011; 14(1) : 11-14.
30. **Gandolfi MG , Shah SN, Feng R, Prati C, and Akintoye SO.** Biomimetic Calcium-Silicate Cements Support Differentiation of Human Orofacial Mesenchymal Stem Cells. J Endod 2011;37(8): 1102-1108.
31. **Garcez RMV, Buzalaf MAR, Araujo PA.** Fluoride Release Of Six Restorative Materials In Water And pH-Cycling Solutions. J Appl Oral Sci 2007;15(5):406-411.
32. **Gjorgievska E, Nicholson WJ, Iljovska S, Slipper I**. The potential of fluoride-releasing dental restoratives to inhibit enamel demineralization: an SEM study. Prilozi 2009;30(1):191-204.
33. **Glasspoole EA, Erickson RL, Davidson CL.** Demineralization of enamel in relation to the fluoride release of materials. Am J Dent 2001;14(1):8-12.
34. **Glasspoole EA, Erickson RL, Davidson CL.** A fluoride-releasing composite for dental applications. Dent Mater2001 ; 17(2): 127-133.
35. **Gomwe T, Booth SE, Nicholson JW.** The kinetics of fluoride uptake by synthetic hydroxyapatite. Ceramics-Silikaty,2012; 56:85-88.

36. **Grech L, Mallia B, Camilleri J.** Investigation of the physical properties of tricalcium silicate cement-based root-end filling materials. Dent Mater 2013;29(2): e20–e28.
37. **Guneser MB, Akbulut MB, Eldeniz AU.** Effect of various endodontic irrigants on the push out bond strength of biodentine and conventional root perforation repair materials. J Endod 2013;39:380-384.
38. **Hamer M & Suh BI** (1991) Fluoride release agent copolymer prepared using morpholinoethyl methacrylate hydrofluoride comonomer, US Patent 5,037,638. (http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.827.5185&rep=rep1&type=pdf).
39. **Han L, Cv E, Li M, Niwano K et al.** Effect of fluoride mouth rinse on fluoride releasing and recharging from aesthetic dental materials. Dent Mater J 2002;21(4):285-295.
40. **Han L & Okiji T.** Uptake of calcium and silicon released from calcium silicate-based endodontic materials in root canal dentine. Int Endod J 2011; 44:1081-1087.
41. **Han L & Okiji T.** Bioactivity evaluation of three calcium silicate based endodontic materials. Int Endod J 2013;46:808–814.
42. **Hashem D, Mannocci F, Patel S, Manoharan A et al.** Clinical and Radiographic Assessment of the Efficacy of Calcium Silicate Indirect Pulp Capping: A Randomized Controlled Clinical Trial. J Dent Res 2015;94(4): 562 –568.
43. **Hilton TJ**, Keys to clinical success with pulp capping: A review of the literature. Oper Dent 2009;34: 615-625.

44. **Hjortsjö C, Jonski G, Thrane PS, Saxegaard E, Young A**. Effect of stannous fluoride and dilute hydrofluoric acid on early enamel erosion over time in vivo. Caries Res 2009;43(6):449-454.
45. **Hjortsjö C, Jonski G, Young A, Saxegaard E.** Etching effect of acidic fluorides on human tooth enamel in vitro. Archives of Oral Biology 2014 ; 59 (12):1328–1333.
46. **Ismail AI**. What is the effective concentration of fluoride? Community Dent Oral Epidemiol 1995;23:246-251.
47. **Kayahan MB, Nekoofar MH, McCann A.** Effect of acid etching procedures on the compressive strength of 4 calcium silicate-based endodontic cements. J Endod 2013 ; 39(12): 1646–1648.
48. **Kim J, Song Y,Min K, Kim S et al**. Evaluation of reparative dentin formation of ProRoot MTA, Biodentine and BioAggregate using micro-CT and immunohistochemistry. Restor Dent Endod 2016;41(1):29-36.
49. **Koruyucu M, Topcuoglu N, Tuna EB, Ozel S et al**. An assessment of antibacterial activity of three pulp capping materials on Enterococcus faecalis by a direct contact test: An in vitro study. Eur J Dent 2015 ; 9(2):240-245
50. **Koubi G, Colon P, Franquin JC.** Clinical evaluation of the performance and safety of a new dentine substitute, Biodentine, in the restoration of posterior teeth—a prospective study. Clinical Oral Investigations 2013 ;17(1): 243–249.
51. **Laurent P, Camps J, De MEo M, Dejou J, About J.** Induction of specific cell responses to a Ca3SiO5-based posterior restorative material. Dent Mater 2008; 24(11):1486-1494.

52. **Laurent P, Camps J, About I.** Biodentine induces TGF-β1 release from human pulp cells and early dental pulp mineralization. Int Endod J 2012; 45(5): 439–448.
53. **Mathis RS & Ferracane JL .** Properties of a glass ionomer/ resin-composite hybrid material. Dent Mater 1989;5: 355 – 358.
54. **McLean J.W & Wilson A.D.** The clinical development of the glass ionomer cements. Aust Dent J 1977;22(2):120-127.
55. **Nicholson JW** Fluoride-Releasing Dental Restorative Materials: An Update. Review Paper Balk J Dent Med, 2014; 18:60-69.
56. **Nowicka A, Lipski M, Parafiniuk M, Sporniak-Tutak K.** Response of human dental pulp capped with biodentine and mineral trioxide aggregate. J Endod 2013 :39(6)743-747.
57. **Parirokh M & Torabinejad M.** Mineral Trioxide Aggregate: A Comprehensive Literature Review—Part III: Clinical Applications, Drawbacks, and Mechanism of Action. J Endod 2010; 36(3): 400-413.
58. P**eng W, Liu W, Zhai W, Jiang L et al.** Effect of Tricalcium Silicate on the Proliferation and Odontogenic Differentiation of Human Dental Pulp Cells .J Endod 2011;37:9-13
59. **Pioch T, Jakob H, García-Godoy F, Götz H, Dörfer CE, Staehle HJ.** Surface characteristics of dentin experimentally exposed to hydrofluoric acid. Eur J Oral Sci2003 ;111(4):359-364.
60. **Pradelle-Plasse N, Tran XV, Colon P, Laurent P, Aubut V, About I.** Emerging trends in (bio)material research. An Example of new material: preclinical multicentric studies on a newCa3SiO5-based dental material. In:

Goldberg M (ed) Biocompatibility or cytotoxic effects of dental composites, 1st edn. Oxford, UK: Coxmoor Publishing Company,pp.2009;1:184-203.

61. **Priyalakshmi S & Ranjan M** , Review on Biodentine-A Bioactive Dentin Substitute ,IOSR-JDMS 2014; 13(1):13-17.
62. **Rada RE.** New options for restoring a deep carious lesion. Dent Today 2013;32:4-5.
63. **Rakshith PK.** Studies on the estimation of fluoride and defluoridation of drinking water [Project Report]. [Bangalore] : Indian Institute of Sciences; 2004. 63 p.
64. **Raskin A, Eschrich G, Dejou J, About I.** *In vitro* microleakage of Biodentine as a dentin substitute compared to Fuji II LC in cervical lining restorations. J Adhes Dent 2012;14:535-542.
65. **Rawls HR &Zimmerman BF (1986):** Fluoride interpolymeric resin US Patent 4,572,920.
66. **Rawls HR.** Esthetic materials with active agent control release capabilities and their future role. In: Symposium on Esthetic Restorative Materials 1991, Chicago, Illinois, USA.
67. **Saku S, Kotake H, Scongall-Vilchis RJ, Ohashi S et al**. Antibacterial activity of composite resins with glassionomer filler particles. Dent Mater J 2010; 29:193-198.
68. **Sanghavi T, Nimisha S, RuchiRani S.** Comparative Analysis Of Sealing Ability Of Biodentine And Calcium Phosphate Cement Against Mineral Trioxide Aggregate(Mta) As A Furcal Perforation Repair Material (An In Vitro Study) Natl J Integr Res Med. 2013; 4(3): 56-60.

69. **Simsek N, Alan H, Ahmetoglu F, Taslidere E, Bulut ET, Keles A.** Assessment of the biocompatibility of mineral trioxide aggregate, bioaggregate, and biodentine in the subcutaneous tissue of rats. Niger J Clin Pract 2015;18:739-743.

70. **Sonis AL & Snell W.** An evaluation of a fluoride releasing, visible light-activated bonding system for orthodontic bracket placement. Am J Orthod Dentofacial Orthop 1989Apr; 95(4): 306-311.

71. **Subramani K & Ahmed W.** Emerging nanotechnologies in dentistry: materials, processes, and applications. 1st ed. Waltham, USA: Elsevier; 2012. 412 p.

72. **Takagi S, Chow LC, Sieck BA.** Deposition of loosely bound and firmly bound fluorides on tooth enamel by an acidic gel containing fluorosilicate and monocalcium phosphate monohydrate. Caries Res 1992;26(5):321-327.

73. **Tantbirojn D, Rusin RP, Bui HT, Mitra SB.** Inhibition of dentin demineralization adjacent to a glass-ionomer/composite sandwich restoration. Quintessence Int 2009;40(4):287-294.

74. **Tarn LE, Chan GP, Yim D.** *In vitro* caries inhibition effects by conventional and resin modified glass-ionomer restorations. Oper Dent 1997;22(1):4-14.

75. **Ten Cate JM.** *In vitro* studies on the effects of fluoride on de- and remineralization. J Dent Res 1990;69 :614-619.

76. **Torabinejad M, Hong CU, McDonald F, Pitt Ford TR.** Physical and chemical properties of a new root-end filling material. J Endod 1995;21:349–353.

77.**Tran XV, Gorin C, Willig C.** Effect of a calcium-silicate based restorative cement on pulp repair. J Dent Res 2012; 91(12) :1166–1171.

78.**Tylenda CA , Jones D, Ingerman L, Sage G, Chappell L.** Toxicological Profile For Fluorides, Hydrogen Fluoride, And Fluorine U.S. Department Of Health And Human Services Public Health Service Agency For Toxic Substances And Disease Registry (US); 2003 Sept :243-253. (https://www.atsdr.cdc.gov/ToxProfiles/tp11.pdf)

79.**Vogel GL, Mao Y, Carey CM, Chow LC, Takagi S.** In vivo fluoride concentrations measured for two hours after a NaF or a novel two-solution rinse. J Dent Res 1992 ;71(3):448-452.

80.**Weir MD, Moreau JL, Levine ED, Strassler HD et al.** Nanocomposite containing CaF_2 nanoparticles: Thermal cycling, wear and long-term water-aging Dent Mater 2012 ; 28(6): 642–652.

81.**Wilson NHF & Burke FJT.** When should we restore lesions of secondary caries and with what materials? Quintessence Int 1998;29(9):598-600.

82.**Wilson AD & Kent BE.** A new translucent cement for dentistry. The glass ionomer Cement. Br Denr J 1972;132: 133-135.

83.**Yaman SD, Er O, Yetmez M, Karabay GA.** *In vitro* inhibition of caries-like lesions with fluoride releasing materials. J Oral Sci 2004;46(1):45-50.

84.**Zanini M, Sautier JM, Berdal A, Simon S.** Biodentine induces immortalized murine pulp cell differentiation into odontoblast-like cells and stimulates biomineralization. J Endod 2012 ;38(9):1220–1226.

85. **Zhou HM, Shen Y, Wang ZJ.** In vitro cytotoxicity evaluation of a novel root repair material. J Endod 2013; 39(4).478–483.

86. **Zolgharnein J, Shahrjerdi A, Azmi G, Ghasemi J.** Spectrophotometric determination of trace amounts of fluoride using an Al-xylenol orange complex as a colored reagent. Anal.Sci 2009; 25 :1249–1253.