COMPARATIVE HISTOLOGICAL EVALUATION OF DIRECT PULP CAPPING WITH BIODENTINE AND MINERAL TRIOXIDE AGGREGATE ON HUMAN PULP TISSUE

- AN IN VIVO STUDY

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled "**COMPARATIVE HISTOLOGICAL EVALUATION OF DIRECT PULP CAPPING WITH BIODENTINE AND MINERAL TRIOXIDE AGGREGATE ON HUMAN PULP TISSUE- AN IN VIVO STUDY**" is a bonafide and genuine research work carried out by me under the guidance of **Dr.M.KAVITHA, Professor & HOD,** Department Of Conservative Dentistry and Endodontics, TamilNadu Government Dental College and Hospital, Chennai- 600 003.

DR.S.HEMA JANE CHRISTA

CERTIFICATE BY GUIDE

This is to certify that **Dr. HEMA JANE CHRISTA**, Post Graduate student (2012-2015) in the Department of Conservative Dentistry and Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai- 600003 has done this dissertation titled "**COMPARATIVE HISTOLOGICAL EVALUATION OF DIRECT PULP CAPPING WITH BIODENTINE AND MINERAL TRIOXIDE AGGREGATE ON HUMAN PULP TISSUE- AN IN VIVO STUDY**" under my direct guidance and supervision in partial fulfillment of the regulations laid down by the TamilNadu 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

ENDORSEMENT BY HEAD OF THE DEPARTMENT / HEAD OF THE INSTITUTION

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

Dr.S.PREMKUMAR, M.D.S.,
Principal i/c

TamilNadu Government Dental College and Hospital.

Chennai- 600003.

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DECLARATION

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NAME OF THE GUIDE	Dr. M. Kavitha
HEAD OF THE DEPARTMENT	Dr. M. Kavitha

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And

Mrs. Dr.S.HEMA JANE CHRISTA aged 33 years currently studying as **Post Graduate student** in Department of Conservtive Dentistry & Endodontics, TamilNadu Government Dental College and Hospital, Chennai 3 (herein after referred to as the PG student and coinvestigator').

Whereas the PG student as part of her curriculum undertakes to research on **"COMPARATIVE HISTOLOGICAL EVALUATION OF DIRECT PULP CAPPING WITH BIODENTINE AND MINERAL TRIOXIDE AGGREGATE ON HUMAN PULP TISSUE- AN IN VIVO 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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1.

2.

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ABBREVIATIONS

MTA	**Mineral Trioxide Aggregate**
BD	**Biodentine**
CH	**Calcium Hydroxide**
CSMs	**Calciumsilicate–Based Materials**
ALP	**Alkaline Phosphatase**
hDPCs	**Human Dental Pulp Cells**
OFMSCs	**Orofacial Bone Mesenchymal Stem Cells**
OCN	**Osteocalcin**
DSPP	**Dentin Sialophosprotein**
DMP1	**Dentin Matrix Protein 1**
BSP	**Bone Sialo-Protein**
BMP-2	**Bone Morphogenic Protein-2**
CEM	**Calcium Enriched Mixture**
MIP-2	**Macrophage Inflammatory Protein**
HO-1	**Heme Oxygenase-1**
LB_4.	**Leukotrine B_4**
IL-1 β	**Interleukin-1 Beta**
EMD	**Emdogain**
NEC	**Novel Endodontic Cement**

ABSTRACT:

AIM:

The aim of the study is to compare the pulpal response of human premolars to direct pulp capping with MTA and BIODENTINE by light microscopic histological evaluation.

MATERIALS AND METHODS:

Direct pulp capping procedure was carried out on human premolars scheduled for orthodontic extractions under local anaesthesia. Class I cavities were prepared and MTA or BIODENTINE was placed over the exposed pulp,followed by composite restoration.A total of 42 teeth were subjected to direct pulp capping. After the experimental periods of 7, 30, and 90 days the teeth were extracted and histological processing was carried out. The test materials were evaluated histologically for the degree of inflammation, dentin bridge formation and thickness of dentin bridge at all the three different observation periods.

RESULTS:

At 7 days observation all the samples in group I and II showed mild inflammatory response. At 30 days thin or partial dentin bridge was evident in all the samples in both groups. At 90 days all the samples in both groups showed evidence of complete dentin bridge formation. There was no statistically significant difference between the two groups in all the three criteria.

CONCLUSION:

Biodentine was effective in inducing a pulpal reaction with minimal inflammation and dentin bridge formation, comparable to MTA .

Key words: MTA, Biodentine, histologically.

Introduction

Introduction:

Dental pulp is a highly vascularized tissue of the tooth and has immense healing potential. It performs many functions throughout the life of a tooth, so every effort should be made to maintain its integrity and vitality. The aim of conservative pulp therapy is to maintain the coronal and radicular pulp tissue in a viable condition.

Direct pulp capping is the treatment of an exposed vital pulp by sealing the pulpal wound with a dental material placed directly on a mechanical or traumatic exposure to facilitate the formation of reparative dentin and maintenance of the vital pulp[26].

Direct pulp capping is indicated whenever the remaining pulp exhibits reversible pulpitis and can be selectively induced to produce a reparative barrier that protects the tissue from microbial challenges.

If successful, this procedure eliminates the need for more invasive, more extensive and more expensive treatment .The intention is to postpone more aggressive therapies that could eventually lower the long term prognosis for tooth retention and function. Since the vital pulp is capable of demonstrating competent immune defense mechanisms, it is desirable to preserve the vitality of an exposed pulp.

A number of factors have been shown to have an impact on the success of direct pulp capping. The ideal pulp capping agent should be the one that should stimulate reparative

dentin formation, not affect pulpal tissue vitality in the healing process, not unleash any adverse effects when used and have good sealing ability.[69]

A wide array of materials have been used for pulp capping. One such time tested material is calcium hydroxide. It acts as a stimulating agent for pulpal tissue repair because of its ability to induce hard tissue formation. Calcium hydroxide's ability to stimulate reparative dentine formation with high rates (more than 80% of the treated teeth) of pulpal survival after capping treatment have been reported in a long term follow up study[33].

But subsequent to pulp capping with this conventional alkaline agent,the adjacent pulp tissue to calcium hydroxide is usually completely deranged and distorted, forming a zone of obliteration. The non-adhesive nature of the cement and its dissolution over time may lead to micro leakage and entry of bacteria to the exposure site. Bacterial contamination may also occur through imperfections in the calcium hydroxide induced dentin bridges, the so-called 'tunnel defects', which may provide passage for bacteria from the exposure site to the pulp[15].

Impaction of particles of the capping agent, necrotizing action of Calcium hydroxide up to depths of 1.5 mm compromising vascularity of the pulp and interference by blood clot are among other factors held responsible for the failure of pulp capping with Calcium hydroxide. These may cause secondary inflammation of the pulp tissue and are thought to be responsible for failed maintenance of tooth vitality.[30,15]

Because of the above mentioned disadvantages of calcium hydroxide, a variety of materials have been developed, tried and tested as candidates for direct pulp capping, namely Resin-modified glass ionomers, Emdogain, Bioglass, Cyanoacrylate, Hydroxyapatite, Calcium phosphate ceramics, Antibiotic and Growth factor combinations, Dentin bonding agents and Mineral Trioxide Aggregate (MTA).

Use of calciumsilicate–based materials (CSMs) in dentistry became popularized with the advent of mineral trioxide aggregate (MTA) in 1993 as a root-end filling material. Mineral Trioxide Aggregate (MTA) has generated considerable interest as a direct pulp capping agent in recent years. Many of the advantages of MTA include its antibacterial properties, biocompatibility, high pH, radiopacity, lower solubility, improved mechanical strength, better marginal adaptation, better sealing ability and homogenous dentin bridge formation.[65]

Further MTA has been shown to induce reparative dentin formation by up regulation of various growth factors and signaling molecules[61,75].However, the major drawbacks of MTA are its handling properties, long setting time, and discoloration of the remaining tooth structure.

In recent years, bioactive tricalcium silicate cements have been introduced, that claim to overcome these limitations. One such material is Biodentine (BD; Septodont,Saint-Maur-des-Fosses, France). It is marketed as a bioactive dentin substitute with active biosilicate

technology. The manufacturer claims that Biodentine promotes pulp healing and remineralization by the production of reactionary dentin and dentin bridges.

Biodentine can be used as a dentin substitute on crowns and roots similar to MTA but with several added advantages like, cost effectiveness, better handling properties and reduced setting time. It has a positive effect on vital pulp cells and stimulates tertiary dentin formation. In direct contact with vital pulp tissue, it also promotes formation of reparative dentin.[16,75]

However there are limited in vivo studies using Biodentine. Hence in this study an attempt has been made to compare and evaluate the effects of Biodentine and MTA as direct pulp capping agents on human teeth .

Aim and Objectives

AIM AND OBJECTIVES:

AIM:

The aim of this in vivo study is to compare the outcome of direct pulp capping with Mineral Trioxide Aggregate [MTA-angelus] and Biodentine [septodont] cement through histological evaluation.

OBJECTIVES:

To evaluate the following criteria histologically after direct pulp capping with MTA/Biodentine; at one week ,one month and 3 month intervals

- Presence or absence of inflammation
- Formation of dentin bridge beneath the pulp capping material.
- Thickness of dentin bridge formed.

Review of Literature

REVIEW OF LITERATURE:

The first description of pulpal treatment in the literature was given by **Philip Pfaff** in 1756 **,** dentist to the King of Prussia in his famous German text on the teeth. He seems to have been the first to describe a procedure for applying a cap of lead foil to the exposed pulp hence the term ‘capping’.

Leonard Kocker in 1826 advocated cauterization of the exposed pulp with a red hot iron wire, after which the wound was covered with a piece of lead foil. Later during the 19th century , many others also reported incidences of pulp capping treatment like, **Rogers (1857)** in England, **Taft (1859)** in the United States and **Zur Nedden (1861)** in Germany. They replaced the lead cap by prefabricated sheets of tin or thin pieces of ivory. These were followed by filling materials given over the capping material and these included, Hills paste (chloropercha) introduced in 1848, zinc oxychloride patented by **Sorel** in **1856** and zinc oxysulphate (artificial dentine for exposed pulps) made by **Fletcher (1874)** in England.

Early attempts to pulpal healing utilized the placeament of metal foil against the exposed pulp to promote healing .Later a range of medicaments like asbestos, cork, beeswax, pulverized glass, a variety of calcium compounds, eugenol based compounds came into use.

Shroff stated that an exposed pulp has inherent capacity of healing, by the production of a calcified collagenous barrier, beneath which regeneration of normal dentin may occur and it is not material specific.

DIRECT PULP CAPPING:

Barthel et al [2000] [8]studied the significance of time of placement of the final restoration after pulp capping and reported that, there was a tendency for a higher failure rate in teeth with temporary restorations, compared with definitive restorations, such as amalgam, composite, or gold cast restorations during 5 year follow up . A significantly higher success rate was noticed in teeth with immediate placement of the final restoration (within 1 or 2 days versus a longer time period) at the 5 year follow up following direct pulp capping.

Murray et al [2002][49] conducted a study to collect quantitative information about the dentin bridge and secretory activity and number of odontoblast - like cells following pulp exposure. The cell density of subjacent reorganizing tissue was found to be strongly associated with that of odontoblast-like cells. Bacterial microleakage was found to impede dentin bridge secretion by odontoblast- like cells.

They concluded that pulp reparative activity occurs naturally beneath capping materials in the absence of bacterial microleakage. The outcome of pulp-capping treatments could be beneficially influenced by concentrating attention on limiting the width of pulp exposure, minimizing pulp injury by limiting the creation of operative debris and placing materials which prevent bacterial microleakage.

A recent systemic review by **Aguilar and Linsuwanont [2011],**[3] reported the outcome of vital pulp therapy, namely direct pulp capping, partial pulpotomy, and full pulpotomy, in vital permanent teeth with cariously exposed pulp .It revealed that the overall success rate was in the range of 72.9%– 99.4%. The fluctuation of the success rate of direct pulp capping was observed as >6 months–1 year, 87.5%; >1– 2years, 95.4%; >2–3 years, 87.7%; and >3 years, 72.9%.

Cenkhan Bal et al [2011][11] studied the effects of antiseptics on pulpal healing under calcium hydroxide pulp capping to evaluate the healing processes histopathologically and suggested that the antiseptic materials used, created an environment which may affect clinical and histological success in a positive way as there were significant differences in inflammatory response and tissue organization.

Mineral Trioxide Aggregate (MTA):

Use of calcium silicate–based materials (CSMs) in dentistry became popularized with the advent of mineral trioxide aggregate (MTA) in 1993 as a root-end filling material.

MTA has generated considerable interest as a direct pulp capping agent in recent years. Studies on MTA reveal that it not only exhibits good sealing ability, excellent long term prognosis, good biocompatibility but favors tissue regeneration as well.

Ford T R et al [1996][24] examined the dental pulp responses in monkeys to mineral trioxide aggregate (MTA) and calcium hydroxide preparation when used as pulp capping

materials. They observed a thick dentin bridge in all pulps capped with MTA. The bridge was continuous with the adjacent dentin, and dentinal tubules were observed in the bridge. Based on these results, it appears that MTA has the potential to be used as a pulp capping material during vital pulp therapy.

Torabinejad et al [1999][65] described the clinical procedures for application of MTA in capping of pulps with reversible pulpitis, apexification, repair of root perforations nonsurgically and surgically, as well as its use as a root-end filling material.

Faraco et al [2001][22] observed the response of dogs' dental pulp to mineral trioxide aggregate (MTA) and calcium hydroxide cement when used as pulp capping materials. The results showed a healing process with complete tubular dentin bridge formation and no inflammation in any of the pulps capped with MTA. Concluding that MTA exhibited better results than the calcium hydroxide cement for the capping of the pulp in dogs.

Aeinehchi et al [2003][2] presented a preliminary report comparing MTA and calcium hydroxide as pulp capping agents on human teeth through histologic evaluation after 2,3,4 and 6 months. Their study demonstrated that MTA produced less inflammation, hyperemia and necrosis. MTA was also seen to promote thick dentinal bridge formation and odontoblastic layer formation than calcium hydroxide.

Holland et al [2004][32] observed the response of dogs' dental pulp to white mineral trioxide aggregate (MTA) when used as pulp capping material. The pulp capped with white MTA showed a healing process with complete dentin bridge formation in all

samples. The pulp tissue showed vitality in all cases. This study also demonstrated the absence of bateria, which confirmed the sealing ability of MTA preventing microleakage.

Menezes et al [2004][45] investigated the pulpal response of dogs' teeth after pulpotomy and direct pulp protection with MTA Angelus, ProRoot MTA, Portland cement and white Portland cement. All the materials demonstrated similar results when used as pulp-capping materials. Pulp vitality was maintained in all specimens and the pulp had healed with a hard tissue bridge.

They concluded that MTA, Portland cement, and white Portland cement represent biocompatible substrates to which formative cells can attach and produce new soft or hard tissue, preserving pulp vitality. The tissue reaction patterns and cell reactions were identical for ProRoot, MTA Angelus, Portland cement, and white Portland cement.

Queiroz et al [2005][56] evaluated histopathologically the response of the pulp tissue and the periapical region when applying MTA directly over the exposed pulp in dog's teeth, using calcium hydroxide plus distilled water as a negative control. They concluded that Mineral trioxide aggregate presented similar response to calcium hydroxide in the pulp tissue and periapical region when used for direct pulp capping.

Sarkar et al [2005][60] showed that MTA materials were a mixture of a refined portland cement and Bismuth oxide as radiopacifier and trace amounts of SiO_2, CaO, MgO, K_2SO_4, and Na_2SO_4. The major component of portland cement was a mixture of

dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum and tetracalcium aluminoferrite.

In a long term clinical study by **Farsi et al [2006][23]** to evaluate the effectiveness of Mineral trioxide aggregate as a direct pulp capping material in caries exposed young permanent teeth it was observed that none of the cases reported spontaneous pain at the six months follow up and the pulp showed signs of vitality and absence of periapical changes. At 2 years, the clinical and radiographic success rate was 93% with evidence of continued root growth.

Tuna et al [2008][67] in a long term clinical stuy to evaluate the effectiveness of mineral trioxide aggregate (MTA) in comparison with calcium hydroxide when used as a pulp capping material in primary teeth observed that none of the MTA and calcium hydroxide groups exhibited clinical or radiographic failure after a follow up period of 12 months. The author concluded that Mineral trioxide aggregate was found to be as successful as calcium hydroxide when used for direct pulp capping in primary teeth.

Bogen et al [2008][9] presented an observational study on direct pulp capping with MTA in carious teeth diagnosed with reversible pulpitis. Over an observation period of nine years, the authors followed 49 of 53 teeth and found that 97.96 percent had favorable outcomes on the basis of radiographic appearance, subjective symptoms and cold testing. They concluded that MTA can be a reliable pulp capping material on direct carious exposures in permanent teeth when a two visit treatment protocol is planned and vital

pulp therapy using MTA is a treatment option for teeth diagnosed with a condition no more severe than reversible pulpitis.

Accorinte et al [2008][1] evaluated the histomorphologic response of human dental pulps capped with mineral trioxide aggregate (MTA) and Calcium hydroxide cement (CH). All groups performed well in terms of hard tissue bridge formation and inflammatory response. However the pulp healing with calcium hydroxide was slower than that of MTA in the one month samples , but both materials were successful for pulp capping in human teeth. Despite the similarity in the responses of MTA and calcium hydroxide,a faster hard tissue bridge formation occurred when MTA was used.

Nair et al [2008][50] have reported a randomized control trial investigating the response of healthy human pulps to experimental capping with MTA through histological, ultrastructural and quantitative evaluations in comparison with calcium hydroxide. The results showed that iatrogenic pulpal wounds treated with MTA were mostly free from inflammation after one week and became covered with a compact, hard tissue barrier of steadily increasing length and thickness within 3 months of pulp capping. The control teeth capped with Dycal showed numerous tunnel defects and inflammation even at the end of the 3 month period. They concluded that MTA should be the choice for direct pulp capping procedures instead of calcium hydroxide.

Kuratate et al [2008][39] in their study investigated the reparative process of mechanically exposed pulps capped with mineral trioxide aggregate (MTA).Their findings suggest that

pulpal responses to MTA capping involve proliferation and migration of progenitors followed by their differentiation into odontoblast- like cells, a mechanism basically similar to calcium hydroxide.

Yasuda et al [2008][72] investigated the effect of mineral trioxide aggregate (MTA) on cell viability and mineralization ability of rat dental pulp cells. MTA exhibited no cytotoxicity, whereas almost all cells had died after 72 hours of culture with Dycal. Furthermore, MTA increased BMP-2 protein production by about 40%, whereas Dycal significantly reduced it. Their results suggest that BMP-2 may play an important role in mineralization stimulated by MTA.

Min et al [2008][48] conducted a study to evaluate the pulpal response to direct pulp capping with either mineral trioxide aggregate (MTA) or calcium hydroxide (CH), with a focus on dentin bridge formation and dentin sialoprotein (DSP) and heme oxygenase-1 (HO-1) expression. The mean thickness of the dentin bridges observed in the MTA group was statistically greater than that of CH group. In addition, DSP and HO-1 were expressed in the odontoblast-like cells and pulp fibroblasts beneath the dentin bridge. Collectively, their results indicate that MTA is superior to CH in terms of inducing the dentinogenic process in human pulp capping.

In a systematic review on direct pulp capping by **Hilton TJ [2009]**[30], it is suggested that on the basis of the literature to date, it would appear that MTA's success is due to the fact that it serves as a reservoir for calcium hydroxide and/or its capacity to provide a seal

at the site of the pulp exposure. The author has concluded in the light of the previous studies that MTA is a promising material.

Tabarsi et al [2010][64] examined the in vivo response of dental pulps in dogs to three pulp-capping agents: calcium hydroxide (CH), mineral trioxide aggregate (MTA) and a new endodontic calcium enriched mixture (CEM) cement. Mineral trioxide aggregate and CEM cement were associated with a similar favourable biological response to pulpotomy treatment and demonstrated a more effective induction of dentinal bridge formation compared to CH.

Gomes et al [2010][27] conducted a study to investigate the role of macrophages and mast cells in mineral trioxide aggregate (MTA) - induced release of neutrophil chemotactic factor. They found that MTA-induces the release of neutrophil chemotactic factor substances from macrophages and mast cells with participation of IL-1$^{\beta}$,MIP-2 and LB_4.

Zarrabi et al [2010][74] conducted a study to compare human pulp response to mineral trioxide aggregate (MTA) and a novel endodontic cement (NEC) when used as pulp capping materials after a time period of 2 and 8 weeks. They concluded that MTA and NEC showed similar biocompatibility and favorable response to pulp capping by inducing the formation of the dentinal bridge.

Paranjpe et al [2010][52] cultured human dental pulp stromal cells (DPSCs) on gray MTA, and the levels of gene expression, secretion of vascular endothelial growth factor,

and the surface morphology were analyzed. MTA promoted cell survival and proliferation, which was significantly different from the controls in human DPSCs. MTA up-regulated the expression of transcription factors like Runx2 and genes like osteocalcin, alkaline phosphatase, and dentin sialoprotein, which are important odonto-blastic genes, thereby showing the ability to promote differentiation of the pulpal cells into odontoblast-like cells, which, in turn, are responsible for dentin bridge formation. They concluded that MTA promoted cell survival and proliferation, which was significantly different from the controls.

Mente et al [2010][46] in a retrospective study investigated the outcome of 108 patients with 122 pulp exposures, of which 69 teeth were directly capped with MTA (ProRoot MTA) and 53 with a non-setting Ca(OH)2 paste (Hy- pocal SN). They concluded that MTA appeared to be more effective than calcium hydroxide for maintaining long-term pulp vitality after direct pulp capping.

Kim et al [2010][34] investigated changes in gene expressions related to mineralization when mineral trioxide aggregate (MTA) is applied in vitro to human dental pulp cells (HDPCs). They found that MTA plays a positive effect on a series of genetic changes in the pulp cells. It appears to influence mineralization greatly and induces slight inflammation and has a protective role against inflammation.

Parirokh et al [2011][54] compared a combination of MTA with/ without $CaCl_2$ as pulp-capping agents in dogs' teeth. Pulpal response to the two capping materials was evaluated in terms of necrosis, inflammation, and formation of a calcified bridge after two months. Histological results showed a higher percentage of inflammation and necrosis and a lower percentage of calcified bridge formation in MTA/$CaCl_2$ samples compared with MTA. According to this in vivo study, the addition of $CaCl_2$ to MTA as a pulp-capping biomaterial has a deteriorating effect on calcified bridge formation, inflammation, and necrosis.

Eskandarizadeh et al [2011][21] investigated the human pulpal response to white and grey mineral trioxide aggregate (WMTA, GMTA) and Dycal (CH) as pulp capping agents. The results showed that the calcified bridge in teeth that were capped with GMTA was significantly thicker than Dycal at 30 and 60 days; whereas WMTA showed significantly thicker calcified bridge than Dycal at 90 days. In addition, GMTA specimens showed significantly less inflammation compared to Dycal samples at 90 days interval. Both types of MTA can be suggested as the materials of choice for direct pulp capping procedure instead of Dycal.

Al-Hezaimi et al [2011][4] evaluated the formation of reparative hard tissues in baboon pulps after Emdogain (EMD) application in conjunction with 3 pulp-capping materials namely calcium hydroxide, ProRoot White MTA(white Portland cement) and the control group (no pulp-capping material). They found that MTA produced a better quality reparative hard tissue response along with Emdogain, when compared with the use of calcium hydroxide.

Paranjpe et al [2011][52] cultured human dental pulp cells on gray MTA, either in direct contact or away from the cells on a cell culture insert, and the levels of gene expression, secretion of vascular endothelial growth factor (VEGF), and the rates of cell proliferation were analyzed. MTA when placed in direct contact with the cells promoted up-regulated expression of important odontoblastic genes like osteocalcin and dentin sialoprotein, thereby showing that direct contact of the cells with the MTA is necessary to promote differentiation of the pulp cells into odontoblast-like cells, which in turn are responsible for dentin bridge formation.

Dreger et al [2012][18] studied the interaction of MTA and white Portland cement with dentin in vivo. Human dentin tubules filled with/without the experimental cements were implanted subcutaneously in rats. In the periods of 30 and 60 days, the mineral deposition in the material- dentin interface (interfacial layer) and in the interior of dentinal tubules was detected. MTA was found to be more effective in promoting the biomineralization process than Portland cements, mainly after 30 and 60 days.

Hirschman et al [2012][31] compared the cytotoxicity of white mineral trioxide aggregate cement (MTA-Angelus), Brasseler En-dosequence Root Repair Putty (ERRM), Dycal, and Ultra-blend Plus (UBP) by using human dermal fibroblasts and MTT assay. They found that after exposure to the 8-day elutes, the percentage of cell survivability was 91% (Brasseler), 88% (MTA-Angelus), 76% (Ultra-blend Plus), and 37% (Dycal) respectively.

Seo et al[2013][61] conducted a study to identify the early genetic changes related to odontogenic differentiation when mineral trioxide aggregate (MTA) is applied to dental pulp stem cells (DPSCs). In this study they found that MTA significantly up-regulated genes associated with cell migration not only in the uninduced DPSCs but also in the odontogenic differentiated cells. Their results suggest that MTA can stimulate the migration both of DPSCs and odontoblast-like cells at the injured site.

Even with such numerous advantages and utilities in sphere of application for MTA, there are many shortcomings of MTA such as difficult handling characteristics, long setting time, high cost and potential of discoloration. Leading to the development of new CSMs such as Biodentine {Septodont}.

BIODENTINE:

Biodentine is a new calcium silicate – based restorative cement with dentin-like mechanical properties. It can be used as a dentin substitute on crowns and roots similar to MTA . It has a positive effect on vital pulp cells and stimulates tertiary dentin formation. It also promotes formation of reparative dentin when in direct contact with vital pulp tissue.

Laurent et al [2008][40] assessed the ability of a recently developed tricalcium silicate-based cement (Biodentine) to induce reparative dentine synthesis and to investigate its capacity to modulate pulp cells' TGF-β1 secretion and found that Biodentine induced mineralized foci formation in the form of osteodentine and expressed markers of

odontoblasts. Biodentine significantly increased TGF-β1 secretion from pulp cells independently of the contact surface increase. Histologically, the bioactive tricalcium silicate demonstrated the ability to induce odontoblast differentiation. The resulting mineralized matrix had the molecular characteristics of dentin.

Till Dammaschke et al [2010][16] reported a case in which the pulp of tooth 14 (iatrogenic exposure during caries excavation) of a 43 year old male patient was directly capped with Biodentine. The cement was used as a base to replace the dentin layer and a composite restoration was placed to replace the enamel layer. At the follow-up visit after six months the tooth was clinically normal and tested positive for sensitivity and negative for percussion. The IOPAR showed the apical region without any pathological findings. Due to its improved material properties, Biodentine can be used as an alternative to conventional calcium hydroxide-based materials.

According to **Gandolfi et al [2011][25]**, calciumsilicate cements have the ability to release calcium and hydroxyl ions and to form hydroxyapatite crystals on the surface after contact with phosphate containing liquids like body fluid. They tested the hypothesis that material extracts of calcium-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 calciumsilicate cements sustained OFMSC survival and maintained steady state levels of vascular cell adhesion molecule-1, bone sialoprotein and alkaline phosphatase while upregulating gene transcripts. Thus

concluding that, ion-releasing calciumsilicate cements support a biomimetic microenvironment conducive to survival and differentiation of OFMSCs .

Peng et al [2011][55] 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. Ca_3SiO_5 enhanced the expression of odontogenic marker genes. Moreover, the extract of Ca_3SiO_5 stimulated mineralization and increased ALP and DSP production conspicuously. These results revealed that Ca_3SiO_5 can induce the proliferation and odontogenic differentiation of hDPCs in vitro and might be a potential candidate for preparation of a new type of Ca_3SiO_5 based cement as a pulp-capping agent.

Tran et al [2012][66] evaluated the capacity of a new calcium-silicate-based restorative cement to induce pulp healing in a rat pulp injury model. For this, cavities with mechanical pulp exposure were prepared on maxillary first molars of 27 six week-old male rats, and the damaged pulps were capped with either Biodentine, MTA, or calcium hydroxide. Cavities were then sealed with glass-ionomer cement, and the repair process was assessed. At day 7, the results showed that both the evaluated cement and MTA induced cell proliferation and formation of mineralization foci.These foci were strongly positive for osteopontin.

At longer time-points, they observed the formation of a homogeneous dentin bridge at the injury site. The dentin bridge was secreted by cells displaying an odontoblastic phenotype. However, the reparative tissue induced by $Ca(OH)_2$ showed porous

organization, suggesting a different reparative process from those induced by calcium silicate cements. Thus concluding that the evaluated cement can be used for direct pulp-capping.

Koubi et al [2012][37] performed a quantitative evaluation by glucose diffusion of microleakage in aged Calcium Silicate based open-sandwich restorations and found that the calcium silicate-based material performs as well as the resin-modified glass ionomer cement in open-sandwich restorations.

This was supported by, **Raskin A et al [2012]**[58] who, in an in vitro study evaluated the microleakage of Biodentine as a dentin substitute compared to Fuji II LC in cervical lining restorations. The study results concluded that, Biodentine in cervical lining restorations or as a restorative material in approximal cavities when the cervical extent is below the CEJ seemed to perform well without any conditioning treatment.

But, **Leiendecker AP et al [2012]**[41]examined whether prolonged contact of mineralized dentin with recently commercialized versions of these materials adversely affects dentin collagen matrix integrity and suggested that, extended contact of mineralized dentin with CSMs has an adverse effect on the integrity of the dentin collagen matrix.

These study results were further supported by, **Zanini M et al in 2012**[73] who conducted a study to evaluate the biological effect of Biodentine on immortalized murine pulp cells (OD-21). OD-21 cells were cultured with or without Biodentine. Cell proliferation was determined by MTS colorimetric assay after 2, 3, and 5 days of stimulation respectively.

The expression of several biomolecular markers were also analyzed. The results suggest that Biodentine is bioactive because it increased OD-21 cell proliferation and biomineralization in comparison with controls. Because of its bioactivity, Biodentine can be considered as a suitable material for clinical indications of dentin-pulp complex regeneration, e.g. direct pulp capping.

Du R et al [2013][19] conducted an invitro study to investigate the role of the extracellular signal-regulated kinase ½ (ERK1/2) pathway in regulating tricalcium silicate(C_3S) – driven proliferation and biomineralization of human dental pulp cells (hDPCs). He concluded that, C_3S stimulated the proliferation and biomineralization of hDPCs in vitro. ERK1/2 pathway was also found to play a key role in the regulation of these effects.

Zhou et al [2013][77] in his study examined the effect of a new bioactive dentin substitute material (Biodentine) on the viability of human gingival fibroblasts . Cells exposed to extracts from Biodentine and MTA showed the highest viabilities at all extract concentrations, whereas cells exposed to glass ionomer cement extracts displayed the lowest viabilities. Biodentine caused gingival fibroblast reaction similar to that by MTA. Both materials were less cytotoxic than glass ionomer cement. Hence they concluded that Biodentine maintains human gingival fibroblast viabililty on cell culture similar to that by MTA.

Han L et al [2013][29] compared white ProRoot MTA (WMTA), EndoSequence BC sealer (BC sealer) and Biodentine, for their ability to produce apatites and cause Ca- and Si-incorporation in adjacent human root canal dentine after immersion in phosphate-

buffered saline (PBS). All materials produced surface precipitates of acicular or lath-like morphology with Ca/P ratio similar to that of tooth .All three materials formed tag-like structures that were frequently composed of Ca- and P-rich and Si-poor materials within dentinal tubules, suggesting intratubular precipitation. 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][51] conducted a study to compare the response of the pulp-dentin complex in human teeth after direct capping with this new tricalcium silicate–based cement with that of MTA. Here, pulps in 28 caries-free maxillary and mandibular permanent intact human premolars scheduled for extraction for orthodontic reasons were mechanically exposed and assigned to each group. After 6 weeks, the teeth were extracted, stained and categorized by using a histologic scoring system.The majority of specimens showed complete dentinal bridge formation and an absence of inflammatory pulp response.

Layers of well-arranged odontoblast and odontoblast-like cells were found to form tubular dentin under the osteodentin. During the observation period, statistical analysis showed no significant differences between the Biodentine and MTA experimental groups. Thus concluding that, 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.

De Rossi et al [2014][16] evaluated the pulpal and peri-apical responses of dogs' teeth after pulpotomy and pulp capping with tricalcium silicate–based Biodentine when compared with mineral trioxide aggregate (MTA) by radiographic, histomicrobiological, and histopathologic analyses.They concluded that Biodentine presented tissue compatibility and allowed for mineralized tissue bridge formation after pulpotomy in all specimens with similar morphology and integrity to those formed with use of MTA.

Zhirong Luo et al [2014][76] investigated the response of human dental pulp stem cells (hDPSCs) to biodentine. Their results showed that Biodentine significantly increased alkaline phosphatase activity and formation of mineralized nodule and the expression of osteocalcin (OCN), dentin sialophosprotein (DSPP), dentin matrix protein 1 (DMP1), and bone sialo-protein (BSP).

Corral Nuñez et al [2014][14] Conducted a study to assess whether Biodentine caused any changes in cell viability and cytokine expression in fibroblast cell culture [mouse embryonic fibroblast cells] compared with MTA. BD and MTA were placed in direct contact with fibroblast-rich tissues such as dental pulp and periodontal ligament. Cell viability was assessed with the nontoxic, nonradioactive, water-soluble compound AlamarBlue. This assay revealed that cells exposed to BD and MTA behaved, in terms of viability, similarly to each other. They concluded that BD and MTA showed similar effects in mouse embryonic fibroblast cells at both the cytotoxicity and cytokine expression levels.

Chang et al [2014][12] reported that biocompatibility, inflammatory response, and odontoblastic differentiation of Biodentine were similar to that of Ortho-MTA (OMTA; BioMTA, Seoul, Korea) and Angelus-MTA (AMTA; Angelus, Londrina, Brazil) in HDPCs. The results of this study showed that Biodentine, OMTA, and AMTA exhibited equally favorable cell viabilities that were superior to IRM. They concluded that Biodentine could be good alternative pulp capping agent to MTA.

Materials and Methods

MATERIALS AND METHODS:

Materials used:[Fig:2]:

- 2.2% Lignocaine HCl with 1:80,000 Adrenaline (D.J Laboratories Pvt ltd, India)
- MTA- Angelus[Angelus,Londrina,PR,Brazil]
- Biodentine[Septodont,France]
- Chlorhexidine 2%[Asep-RC,Anabond-Stedman,Chennai,India]
- Materials for COMPOSITE RESTORATION:
    - Tetric N- ceram composite [Ivoclar Vivadent,Schann,Liechtenstein]
    - Tetric N –Flow [Flowable comoposite]
    - Etchant [35% phosphoric acid]
    - Tetric N- bond [bonding agent]
    - Visible light curing unit [Heraeus Kulzer-Hulix Model-200]
    - Super Snap Mini Kit for Polishing [Shofu Inc.,Japan]

ARMAMENTARIUM USED FOR THE CLINICAL PROCEDURE:

INSTRUMENTS:[Fig: 1]:

- Mouth mirror,Dental explorer,Tweezer
- Sterile cotton and gauze
- Suction tip
- Dappen dish,Mixing spatula,Plastic instrument
- Electric pulp tester [Dental Pulp Tester,Mident Industries,Henan]
- Rubber dam Kit

- Airotor handpiece (NSK, Japan)
- Straight diamond abrasive [SF-11,Mani,Japan]
- No. 2 [1 mm] round tungsten carbide bur (FG-2, SS White, USA)
- Suction tips

HISTOLOGICAL PROCESSING:

- Diamond disc[#7020,KG,Sorensen Ind,Barueri-SP,Brazil]
- 10% formalin
- Aqueous ethanol
- 5% Nitric acid
- Paraffin wax
- Hematoxylin and eosin stain

MATERIALS AND METHODS:

This study was conducted on human subjects, with approval from The Institutional Ethical Committee- TamilNadu Government Dental College, Chennai.

SUBJECTS AND SPECIMENS:

Young adult patients [age group: 18 to 24 years] from the Department of Orthodontics, who were scheduled for extraction of premolars for orthodontic reasons, were selected for the study.

The subjects signed consent forms after they had received a thorough explanation regarding the experimental rationale, clinical procedure and possible risks. All the patients were recruited by a single operator. As this study was not based on a population basis, no randomization was used for patient selection.

Forty five first or second human premolar teeth from fourteen patients were selected. Seven patients contributed all four premolars each, four other patients contributed three teeth each,two of them gave consent for two teeth and one patient contributed one tooth.

All teeth were examined clinically and radiographically to ensure absence of caries and periapical pathosis.

EXCLUSION CRITERIA:

The teeth with the following criteria were excluded from the study:

- Decayed or fractured teeth
- Previous restorative history
- Symptomatic teeth
- Teeth with any other pathology
- Teeth not conducive for rubber dam isolation

EXPERIMENTAL GROUPS: [Table-1]

- **GROUP I** –MTA—[n=21]
- **GROUP II** –BIODENTINE-- [n=21]
- **GROUP III** --CONTROL-NORMAL TEETH—[n=3]

EXPERIMENTAL PROTOCOL:

- **PHASE I** – CAVITY PREPARATION AND PLACEMENT OF MATERIAL
- **PHASE II** – ATRAUMATIC EXTRACTION OF THE TEETH AFTER EXPERIMENTAL PERIODS
- **PHASE III** – HISTOLOGICAL EVALUATION

TABLE 1:

GROUP	DPC MATERIAL	PERIOD OF EXTRACTION	CODE
1	MTA	1 WEEK	SM
2	BD	1 WEEK	SB
3	MTA	1 MONTH	OM
4	BD	1 MONTH	OB
5	MTA	3 MONTHS	TM
6	BD	3 MONTHS	TB
7	NONE	---	C

n=7 for Group 1-6 , n=3 for Group 7

DPC : Direct Pulp Capping Material Used

C : CONTROL

CLINICAL PROCEDURES:

Electrical pulp testing was performed on all teeth before preforming the experimental procedure. Calculus and debris were removed from the tooth surfaces. After administering local anaesthesia , with 2% lignocaine and rubberdam isolation, the teeth were cleaned with a rubber cup and prophylactic paste at low speed. The site was then washed with 2% chlorhexidine.

CAVITY PREPARATION:

Occlusal cavities of dimensions approximately 2mm long , 2 mm wide and of variable depth till pulp exposure, about 3.5mm deep were prepared using a sterile straight fissure diamond [SF-11,Mani,Japan] at high speed under water spray coolant [fig- 4].The cavity walls were irrigated with normal saline.Then a pulp exposure was made using a no-2, round tungsten carbide bur [1mm dia],at high speed without penetrating the pulp space[fig- 5]. New sterilized burs were used for each procedure.

DIRECT PULP CAPPING:

Hemorrhage was controlled by a sterile cotton pellet soaked in saline. Then, the area of pulp exposure was capped with MTA/ Biodentine [fig- 6,7]. The materials were mixed according to the manufacturers' instructions.

A layer of non-bonded flowable composite resin was then placed over the capping material and light cured for 40 seconds [fig- 8,9] .The walls of the cavity were then etched,rinsed and bonded with light cured nanofilled composite resin.The immediate final restoration was finished and polished with polishing discs[fig- 10].

SPECIMEN PREPARATION AND HISTOLOGIC EVALUATION:

All the experimental teeth were carefully followed up and assessed before extraction.The assessment included history taking and a clinical examination for recording any signs or symptoms and response to electric pulp testing.The extractions were scheduled after a period of 1 week, 1 month and 3 months.The teeth were grouped according to the timing of extraction and the pulp capping material used.

Immediately after the atraumatic extraction procedure,the roots were sectioned midway between the cemento-enamel junction and the root apex, to facilitate diffusion of the fixing solution. After fixation with 10% formalin for 96 hours, the specimens were decalcified in 5% nitric acid, dehydrated in increasing concentrations of aqueous ethanol and embedded in paraffin.

Then serial bucco-lingual sections of four micron thickness were cut longitudinally through the center of the exposure site, with Leica Microtome [fig-11].The sections mounted on glass slides were then stained with hematoxylin and eosin in the Leica semi-automatic stainer [fig-12].

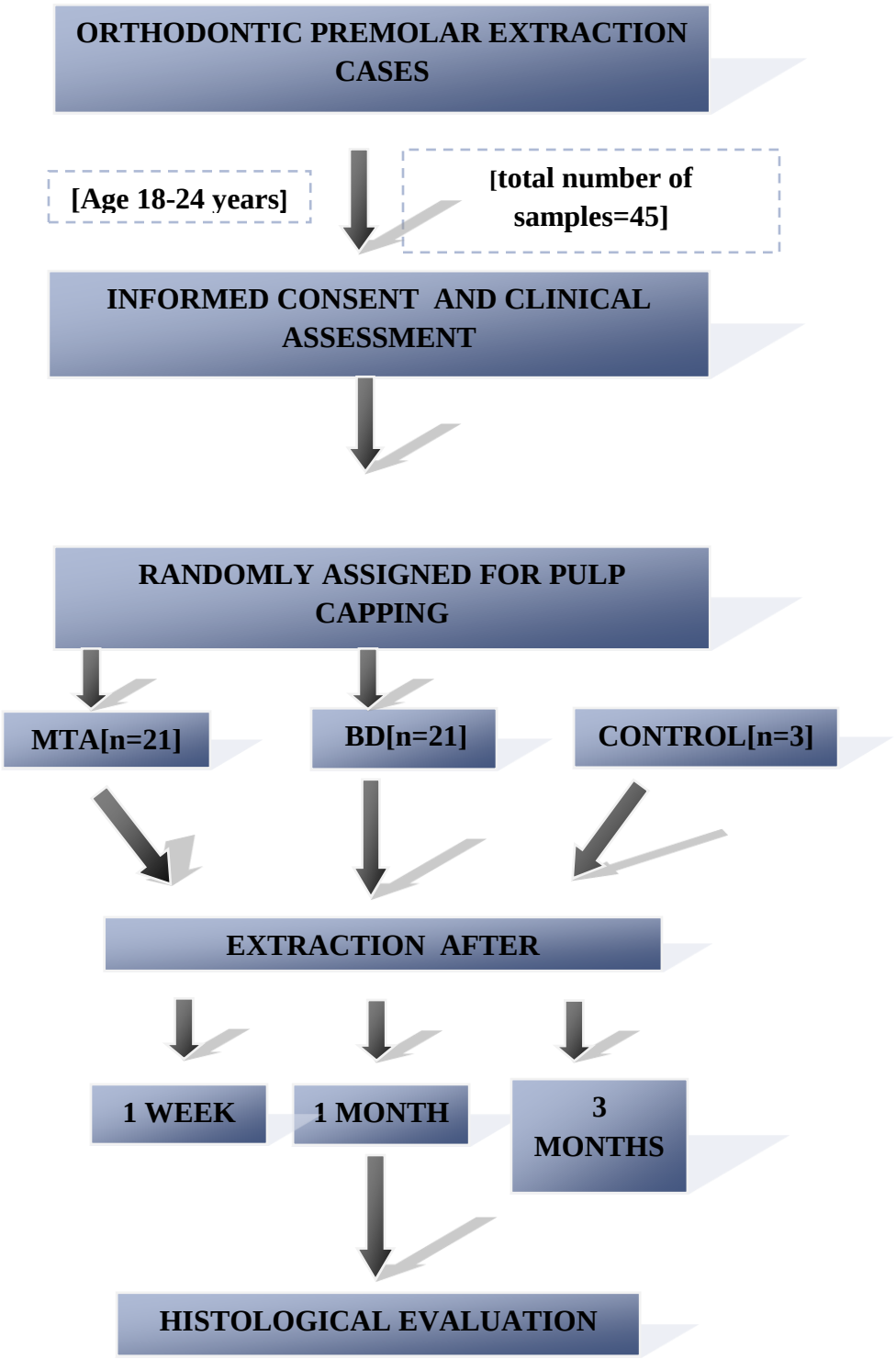
All histological processing procedures were carried out in the Department of Veterinary Pathology, Madras Veterinary College, Chennai. The sections were then blindly evaluated by an experienced pathologist and calibrated according to the following criteria [Table 2].

The light microscope used for this purpose was an Olympus DX 4 SF[fig-13].

TABLE 2:Scoring criteria for histopathological evaluation: [1, 74, 42,57]

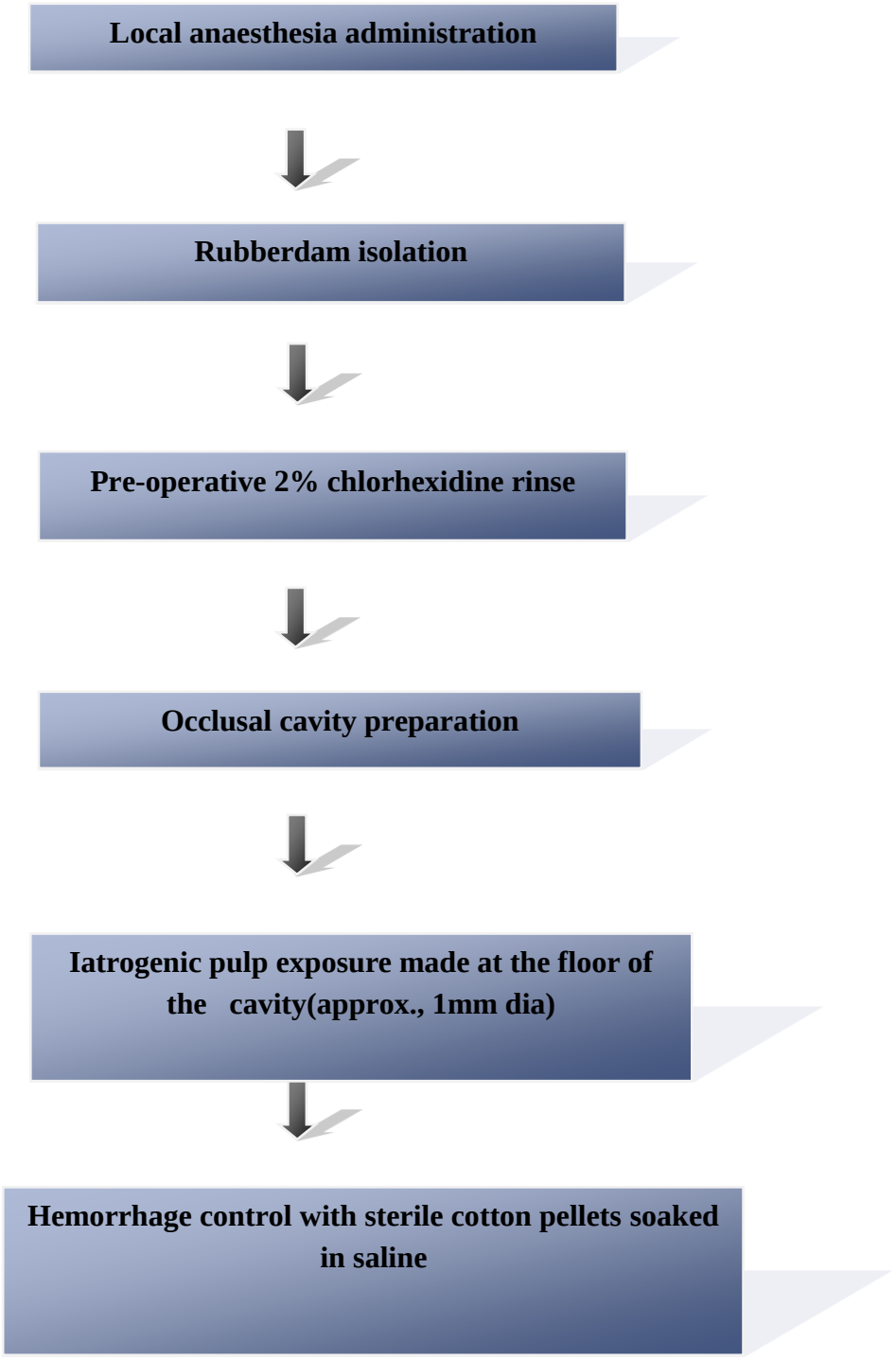
GRADING	CHARACTERIZATION
Score	**Inflammatory cell response**
1	None or few scattered inflammatory cells present in the site of pulp exposure, characteristic of normal tissue
2	Mild inflammatory cell infiltration with PMN or mononuclear leukocytes[< 10 cells per high power field]
3	Moderate inflammatory cell infiltration involving coronal pulp[10-25 cells]
4	Severe inflammatory cell infiltration[>25 cells] involving coronal pulp or abcess present
Score	**Hard tissue formation**
1	Absent
2	Lateral deposition of hard tissue on the walls of the cavity of pulp exposure
3	Partial hard tissue bridge-little communication of the capping material with pulp
4	Complete hard tissue bridge-closure of exposure area
Score	**Thickness of dentinal bridge**
1	0mm
2	<0.1 mm
3	0.1–0.25 mm
4	>0.25 mm

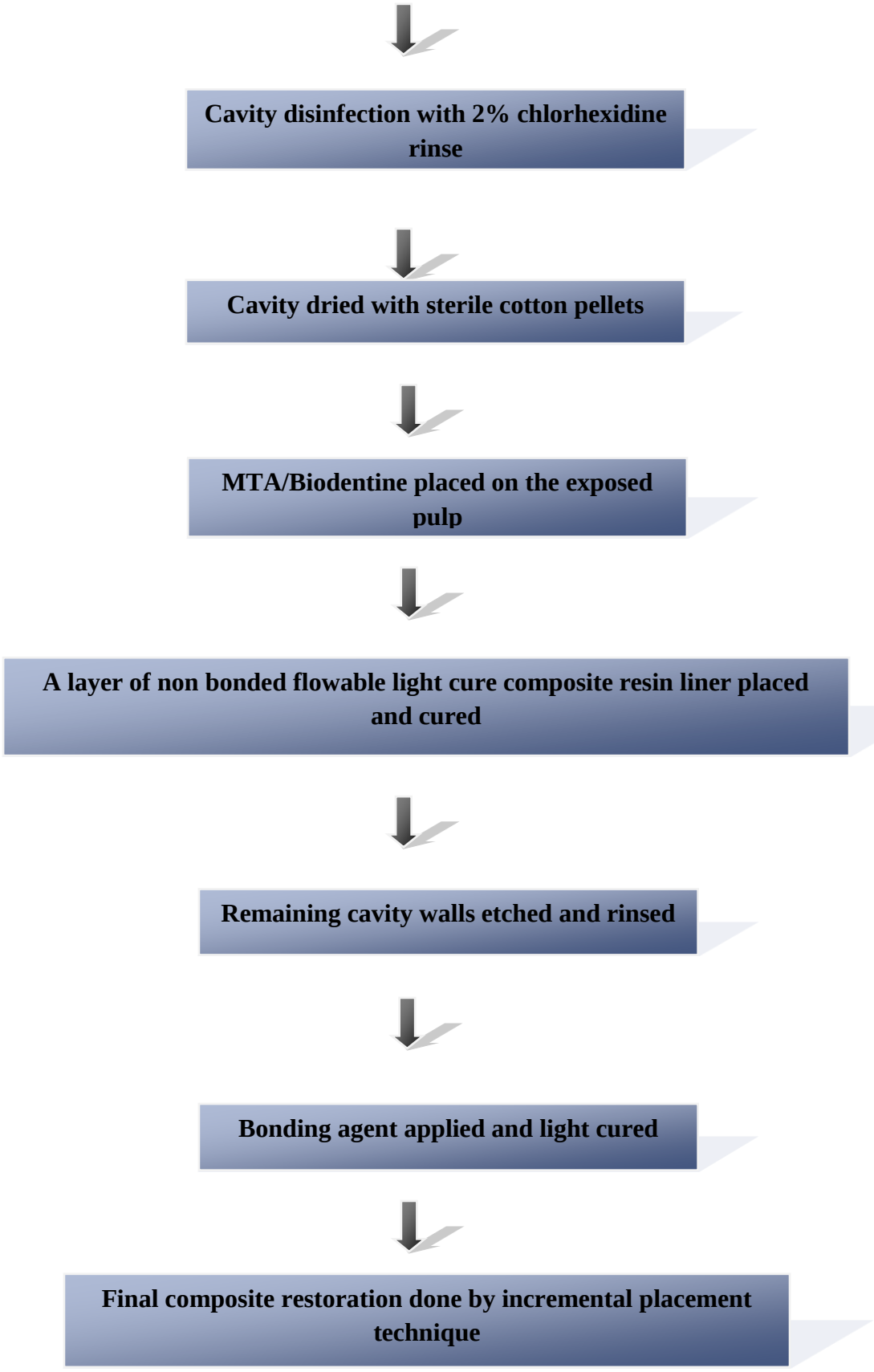
EXPERIMENTAL DESIGN:



EXPERIMENTAL PROTOCOL

PROCEDURAL-FLOWCHART FOR DIRECT PULP CAPPING:




Cavity disinfection with 2% chlorhexidine rinse
Cavity dried with sterile cotton pellets
MTA/Biodentine placed on the exposed pulp
A layer of non bonded flowable light cure composite resin liner placed and cured
Remaining cavity walls etched and rinsed
Bonding agent applied and light cured
Final composite restoration done by incremental placement technique

ARMAMENTARIUM FOR THE CLINICAL PROCEDURE:

FIG:1

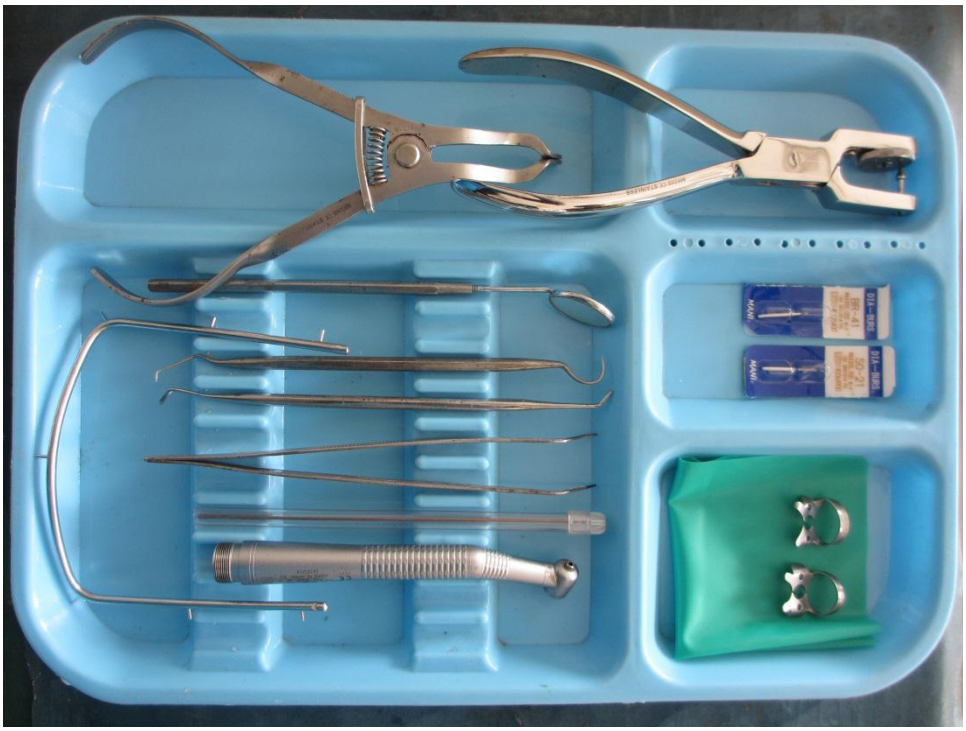


FIG:2

FIG:3 AMALGAMATOR FOR MIXING BIODENTINE:

FIG 4: INITIAL CAVITY PREPARATION BEFORE PULP EXPOSURE

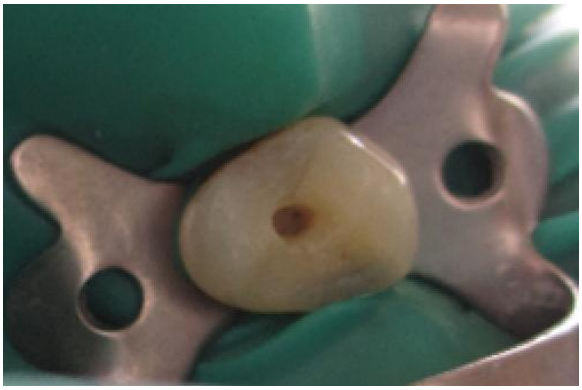


FIG 5: EXPOSURE OF PULP

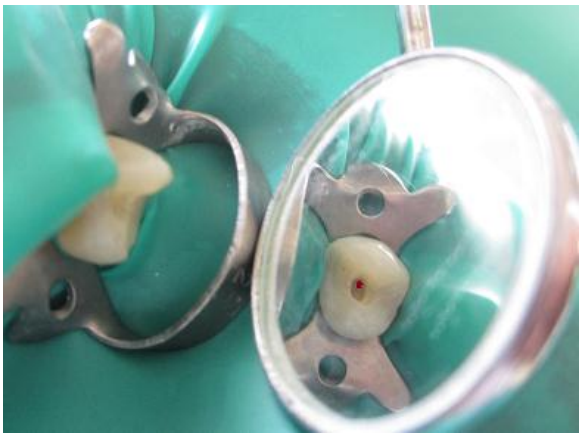


FIG 6: PLACEMENT OF THE PULP CAPPING MATERIAL AFTER CONTROLLING BLEEDING

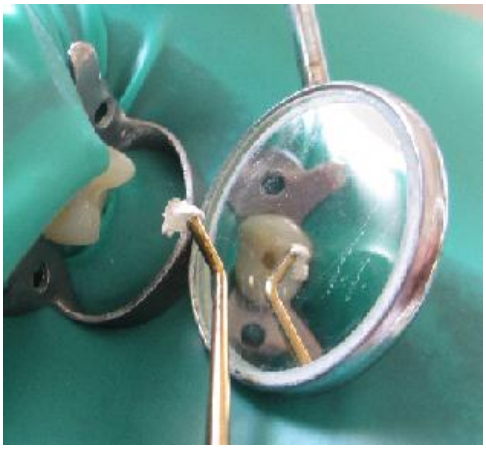


FIG 7 :TOOTH AFTER THE PLACEMENT OF THE PULP CAPPING MATERIAL

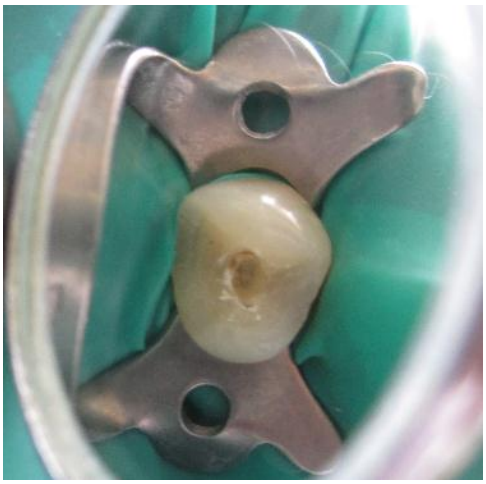


FIG 8: PLACEMENT OF FLOWABLE COMPOSITE

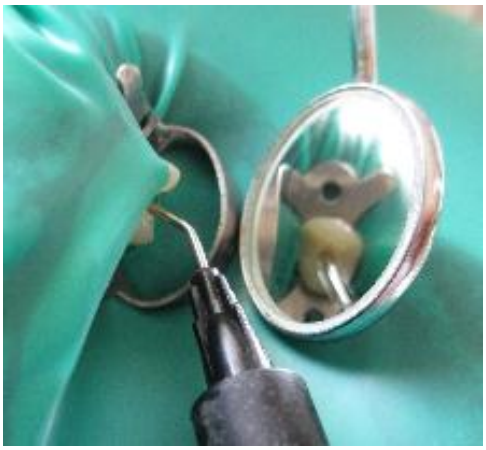


FIG 9: CURING WITH VISIBLE LIGHT

FIG 10:FINAL RESTORATION WITH COMPOSITE MATERIAL

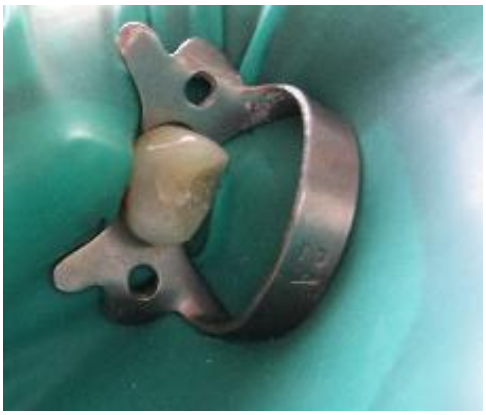


FIG 11:LEICA MICROTOME

FIG 12:LEICA SEMI-AUTOMATIC STAINER

FIG 13: OLYMPUS LIGHT MICROSCOPE

Results

Results:

No apparent adverse events were observed during the experimental periods. All the individual scores for the two experimental groups in all the observation periods were tabulated [TABLE 3,4 ,5].

TABLE 3:Histopathological scores for seven days observation

Si.No	Group Code	Material	Sub-Group Code	Age/ Sex		scores	
					Inflam-mation	Dentin Bridge	Thickness of Dentin Bridge
1	G1	MTA	G1 SM-01	21/F	1	1	1
2	G1	MTA	G1 SM-02	21/F	1	1	1
3	G1	MTA	G1 SM-03	21/F	1	1	1
4	G1	MTA	G1 SM-04	21/F	2	1	1
5	G1	MTA	G1 SM-05	19/M	2	1	1
6	G1	MTA	G1 SM-06	19/M	1	1	1
7	G1	MTA	G1 SM-07	19/M	2	1	1
8	G2	BD	G1 SB-01	18/M	1	1	1
9	G2	BD	G1 SB-02	18/M	2	1	1
10	G2	BD	G1 SB-03	18/M	2	1	1
11	G2	BD	G1 SB-04	18/M	1	1	1
12	G2	BD	G1 SB-05	24/M	2	1	1
13	G2	BD	G1 SB-06	24/M	2	1	1
14	G2	BD	G1 SB-07	24/M	1	1	1

TABLE 4: Histopathological scores for one month observation:

Si. No	Group Code	Material	Sub-Group Code	Age/ Sex		Scores	
					Inflamm-ation	Dentin Bridge	Thickness of Dentin Bridge
1	G1	MTA	G1OM-01	24/M	1	2	2
2	G1	MTA	G1OM-02	24/M	1	3	2
3	G1	MTA	G1OM-03	24/M	1	3	2
4	G1	MTA	G1OM-04	24/M	1	3	3
5	G1	MTA	G1OM-05	18/M	1	2	3
6	G1	MTA	G1OM-06	18/M	1	2	2
7	G1	MTA	G1OM-07	18/M	1	2	2
8	G2	BD	G2OB-01	18/F	1	3	2
9	G2	BD	G2OB-02	22/F	1	2	2
10	G2	BD	G2OB-03	22/F	1	2	2
11	G2	BD	G2OB-04	22/F	1	3	2
12	G2	BD	G2OB-05	22/F	1	2	3
13	G2	BD	G2OB-06	19/F	1	2	3
14	G2	BD	G2OB-07	19/F	1	2	3

TABLE 5: Histopathological scores for three months observation:

Si. No	Group Code	Material	Sub-Group Code	Age/ Sex		Scores	
					Inflamm-ation	Dentin Bridge	Thickness of Dentin Bridge
1	G1	MTA	G1TM-01	19/M	1	4	3
2	G1	MTA	G1TM-02	19/M	1	3	3
3	G1	MTA	G1TM-03	19/M	1	4	4
4	G1	MTA	G1TM-04	19/M	1	4	4
5	G1	MTA	G1TM-05	21/F	1	4	4
6	G1	MTA	G1TM-06	21/F	1	4	4
7	G1	MTA	G1TM-07	21/F	1	4	4
8	G2	BD	G2TB-01	21/F	1	4	4
9	G2	BD	G2TB-02	24/M	1	3	3
10	G2	BD	G2TB-03	24/M	1	4	4
11	G2	BD	G2TB-04	24/M	1	4	3
12	G2	BD	G2TB-05	24/M	1	4	3
13	G2	BD	G2TB-06	17/F	1	4	4
14	G2	BD	G2TB-07	17/F	1	3	4

Statistical Analysis:

The scores attributed to each group were recorded [Table 3,4 and 5]. The results of the histopathologic evaluation were statistically analyzed by using the Chi-Square Test using SPSS 16 (statistical package for social sciences version 16) software. A p value <.05 was considered statistically significant.

INFLAMMATION:

Chi-square test for inflammation for all the 3 intervals:

Table:6:

INTERVAL	Histopathological scores	Group I MTA	Group II BD	Chi-value	p-value
7 Days	1	4	3	0.424	0.515
	2	3	4		
	3	0	0		
	4	0	0		
One Month	1	0	0	--	--
	2	0	0		
	3	0	0		
	4	0	0		
3 Months	1	0	0	--	--
	2	0	0		
	3	0	0		
	4	0	0		

DENTIN BRIDGE:

Chi-square test for dentin bridge for all the 3 intervals:

Table:7

Interval	Histopathological scores	Group I MTA	Group II BD	Chi-value	p-value
7 Days	1	7	7	--	--
	2	0	0		
	3	0	0		
	4	0	0		
One Month	1	0	0	0.311	0.577
	2	4	5		
	3	3	2		
	4	0	0		
3 Months	1	0	0	0.00	1.0
	2	0	0		
	3	2	2		
	4	5	5		

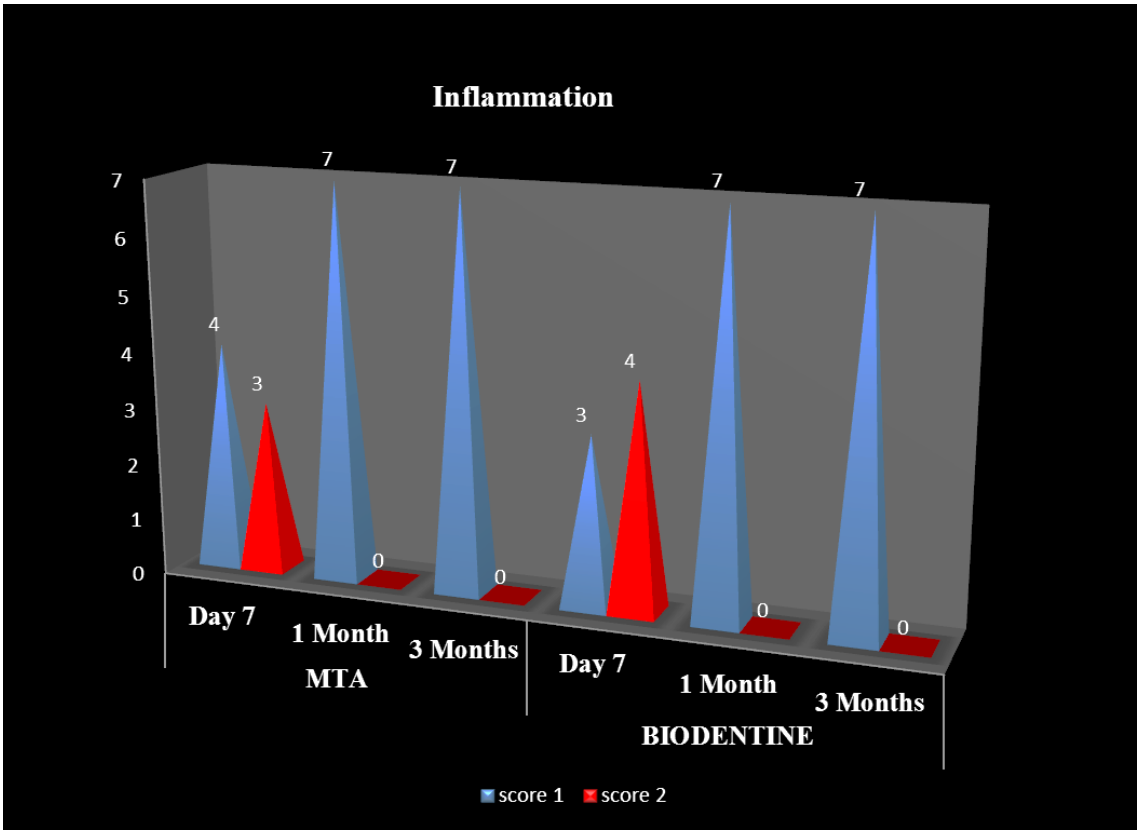
THICKNESS OF DENTIN BRIDGE:

Chi-square test for thickness of dentin bridge for all the 3 intervals:

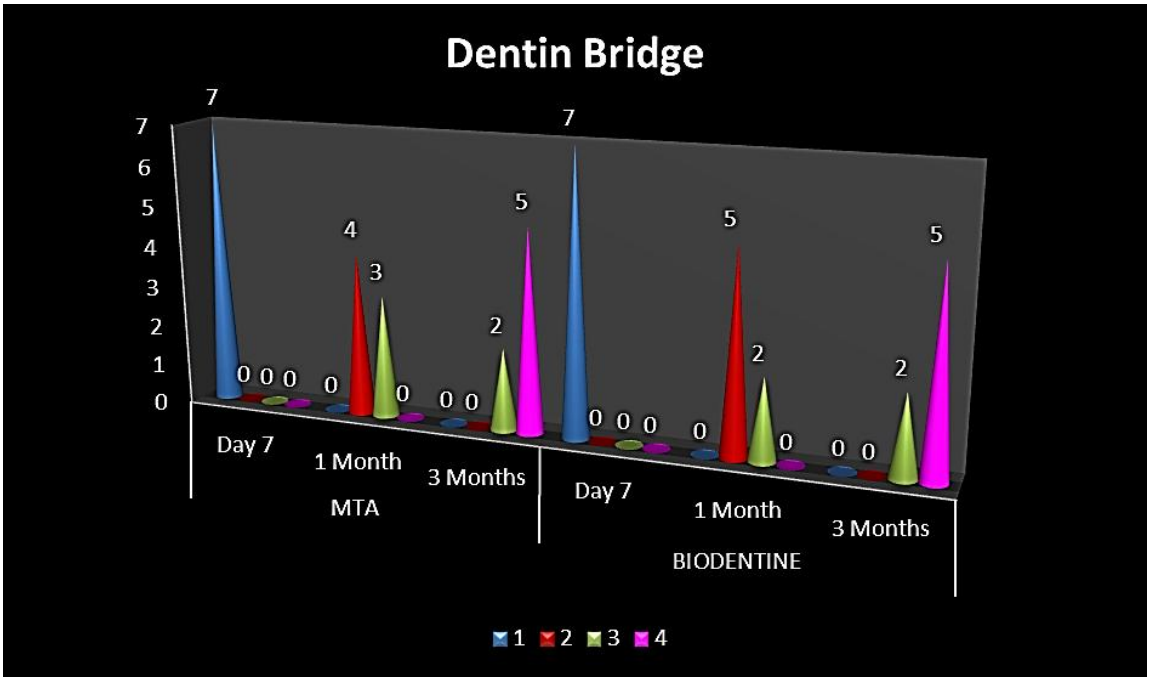
Table:8

Interval	Histopathological scores	Group I MTA	Group II BD	Chi-value	p-value
7 days	1	7	7	--	--
	2	0	0		
	3	0	0		
	4	0	0		
One month	1	0	0	0.311	0.577
	2	4	5		
	3	3	2		
	4	0	0		
3 months	1	0	0	0.311	0.577
	2	0	0		
	3	2	3		
	4	5	4		

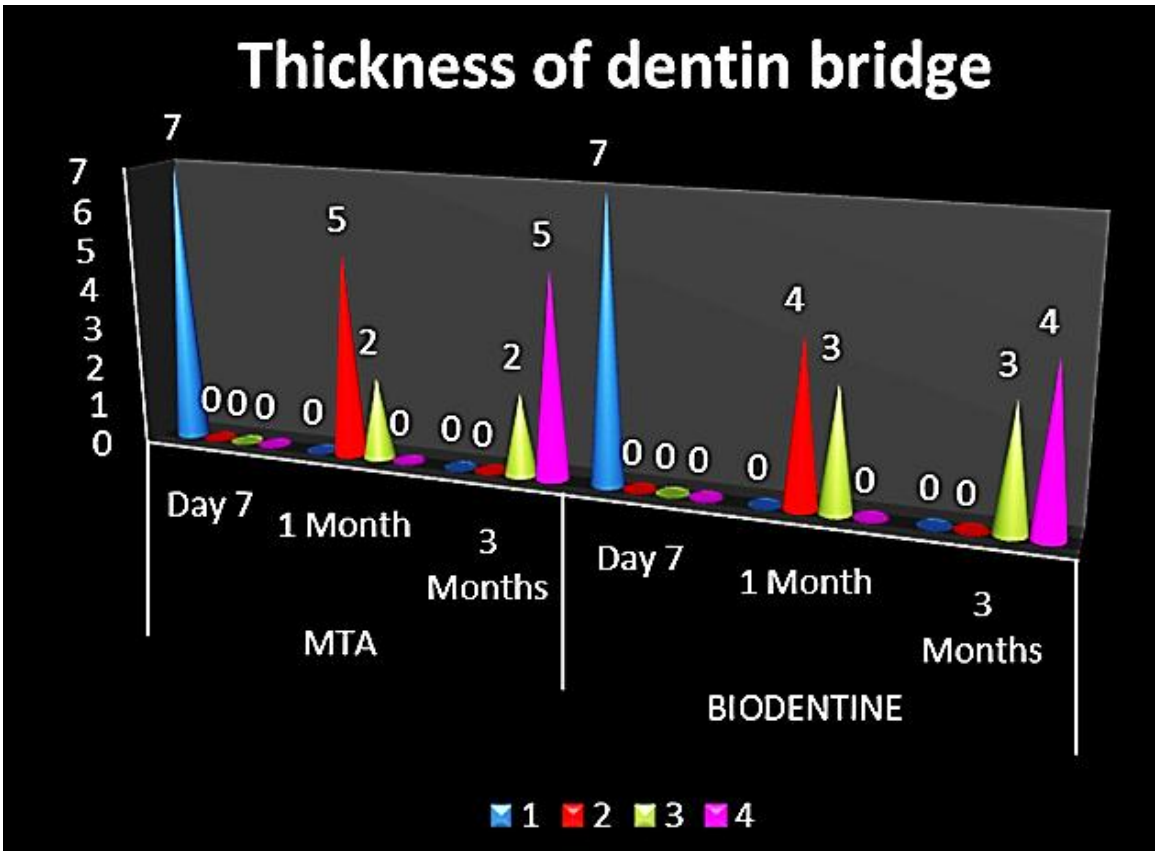
Graph-1: Comparison of Inflammation in MTA Vs BD Groups at Time Intervals of 7 Days, One Month, 3 Months



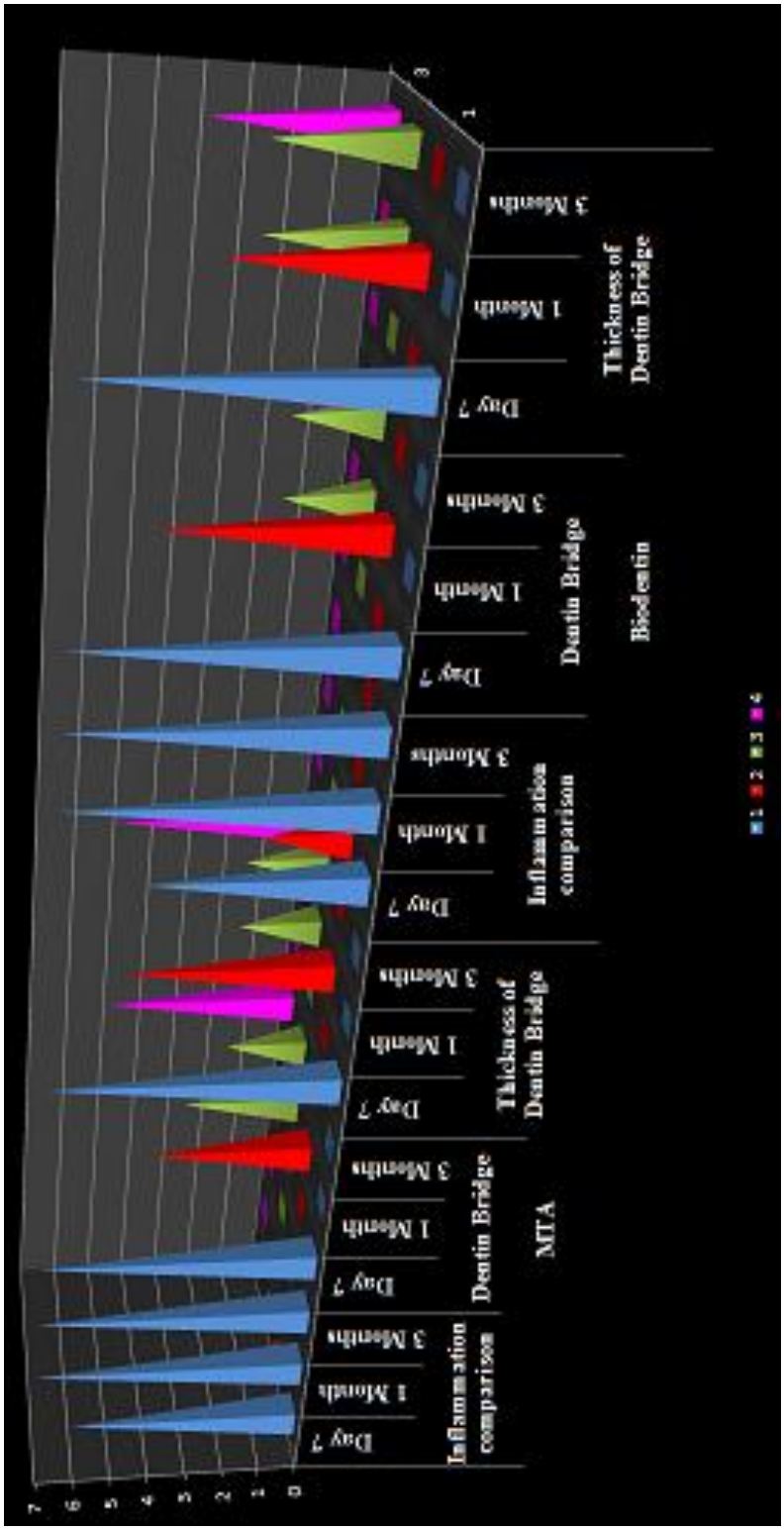
Graph-2: Comparison of Dentin Bridge Formation in MTA Vs BD Groups at Time intervals of 7 Days, One Month, 3 Months



Graph-3: Comparison of Thickness of Dentin Bridge in MTA Vs BD Groups at Time Intervals of 7 Days, One Month, 3 Months



Graph-4 : Comparison of all the 3 Parameters at Time Intervals of 7 Days, One Month, 3 Months



Interpretation of results:

Seven days observation:

In the 7 days samples groups 1 and 2 showed mild or no inflammatory response.The corresponding histopathological scores were 1 and 2 respectively, giving a Chi –value of 0.424. While the one month and three month samples showed only score 1. [TABLE:6,GRAPH-1]

One month observation:

The one month samples showed scores of 2 and 3 for formation of dentin bridge and thickness of the dentin bridge. The Chi – value was 0.311 for both the parameters in the one month samples.The score corresponding to inflammation was 1 in both groups as no inflammation was seen. [TABLE:7,GRAPH-2]

Three months observation:

The three month samples showed scores of 3 and 4 for formation of dentin bridge and thickness of dentin bridge. The Chi – value was 0.0 for formation of dentin bridge, while it was 0.311 for thickness of dentin bridge. The score corresponding to inflammation was 1 as in the one month samples. .[TABLE:8,GRAPH-3]

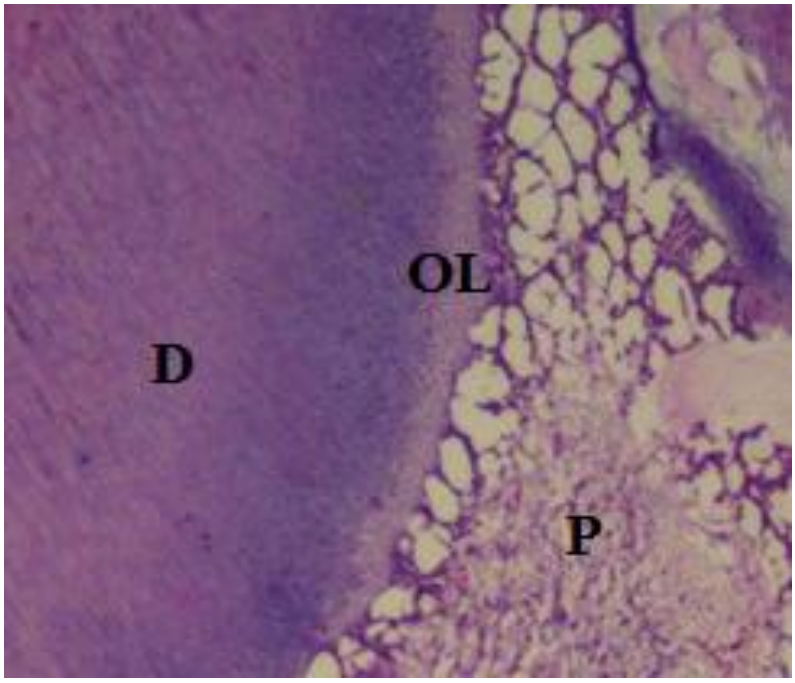
The results showed that there is no statistically significant difference between the groups in terms of inflammation, dentin bridge formation and the thickness of the dentin bridge formed as the p- value calculated for each of the groups was above 0.05.

HISTOLOGICAL PICTURES:

Normal Tooth Stained with Eosin And Hematoxylin.

P-Pulp, D-Dentin, OL - Odontoblastic Layer

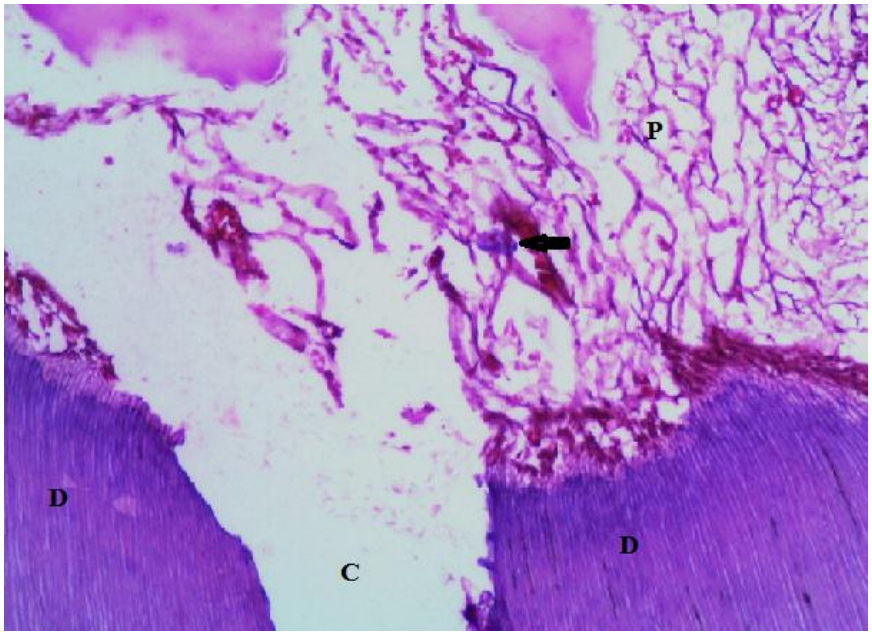
At 10x Magnification -- [Fig:14]



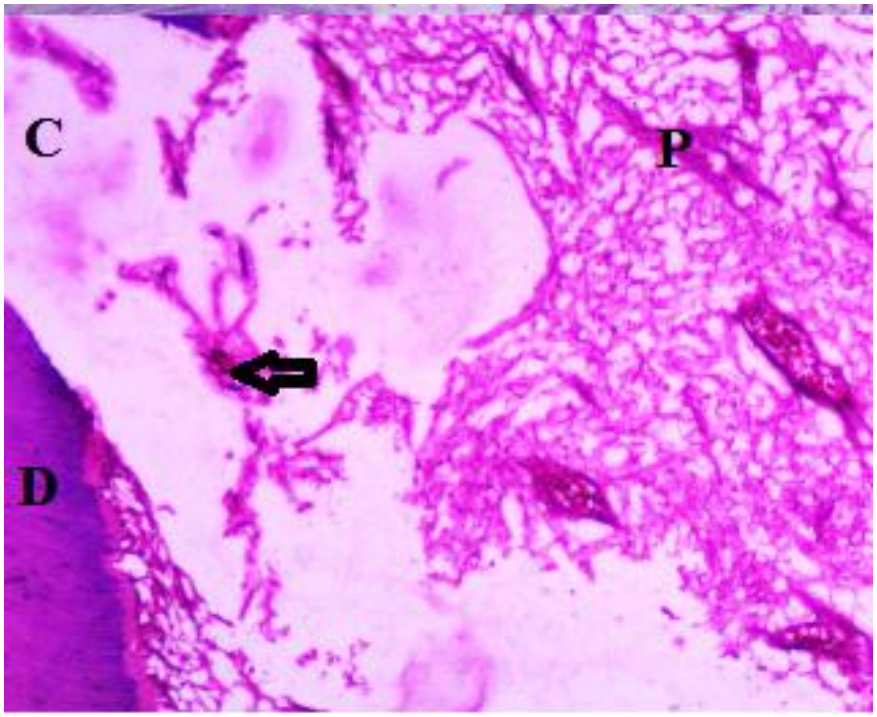
TEETH AFTER SEVEN DAYS OF MATERIAL PLACEMENT:

D-DENTIN, P- PULP, ARROW- INFLAMMATORY CELL, C- CAVITY

MTA - Magnification-10x--[Fig:15]



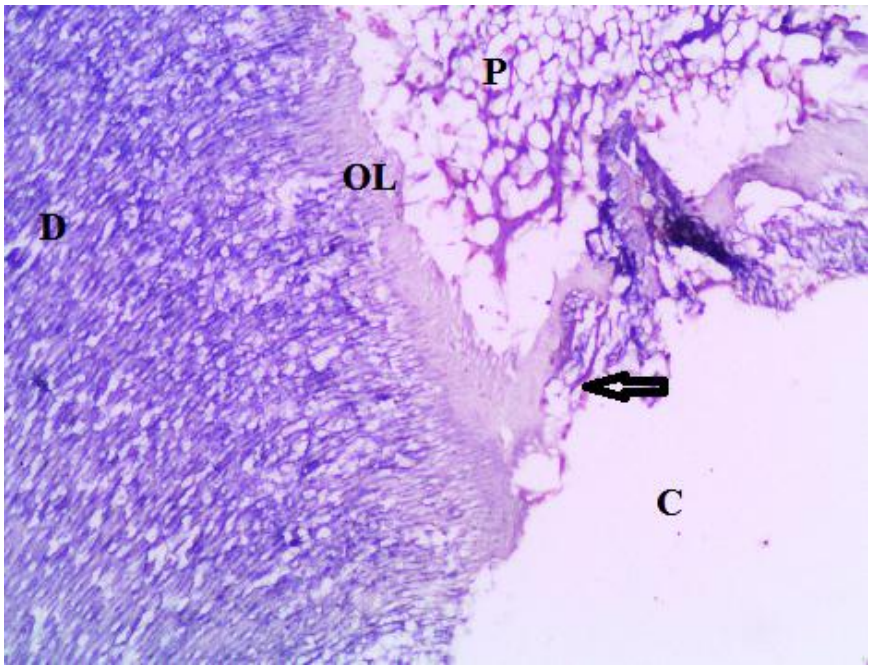
BIODENTINE - Magnification-10x -- [Fig:16]



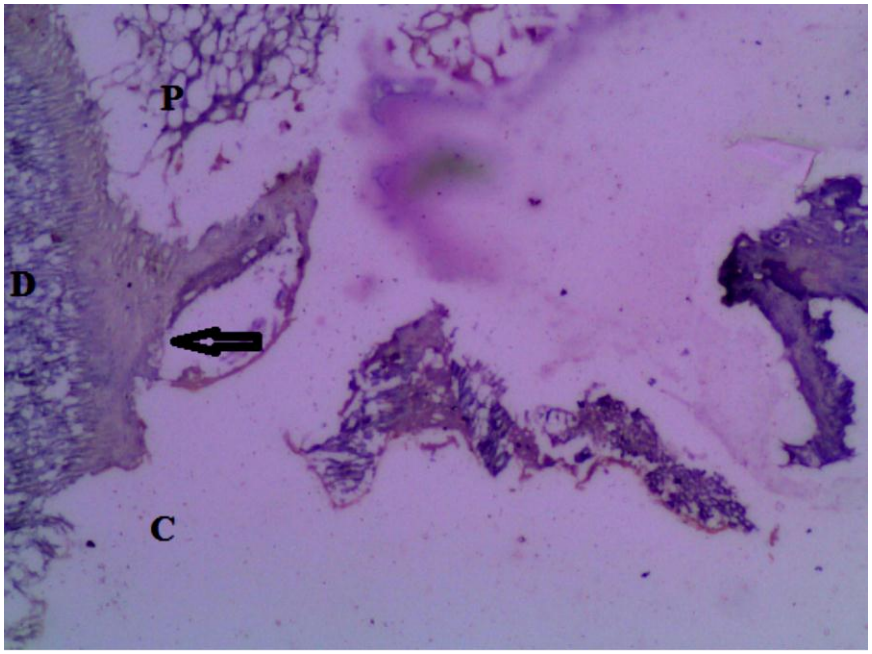
TEETH AFTER ONE MONTH OF MATERIAL PLACEMENT:

D-Dentin, P- Pulp, DB-Dentin Bridge, C-Cavity, Arrow- Dentin Deposition on the Lateral Walls ,OL-Odontoblastic Layer.

1.MTA - Magnification 10 X -- [Fig:17]



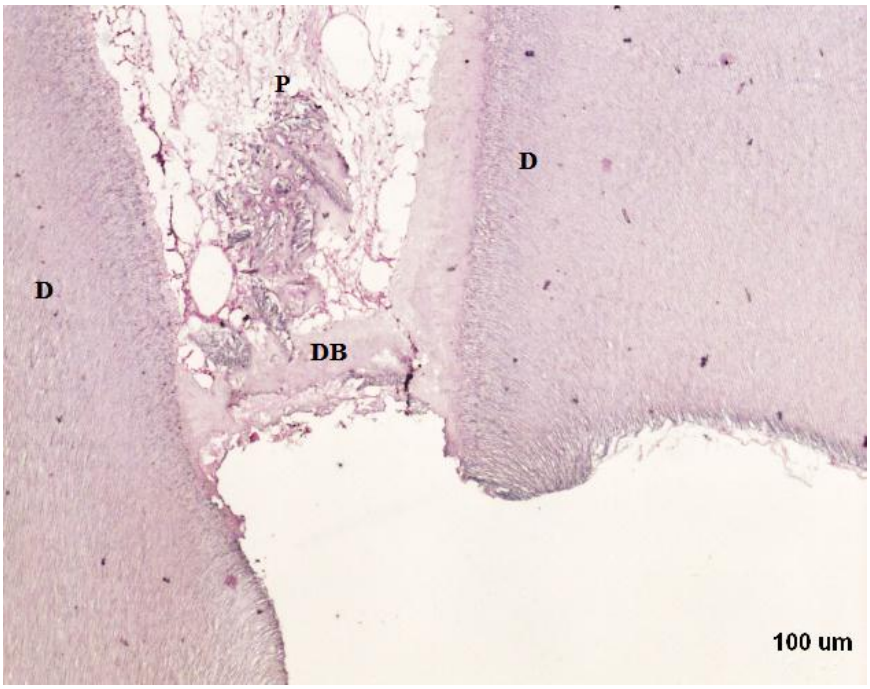
2.BIODENTINE - Magnification 10 X -- [Fig:18]



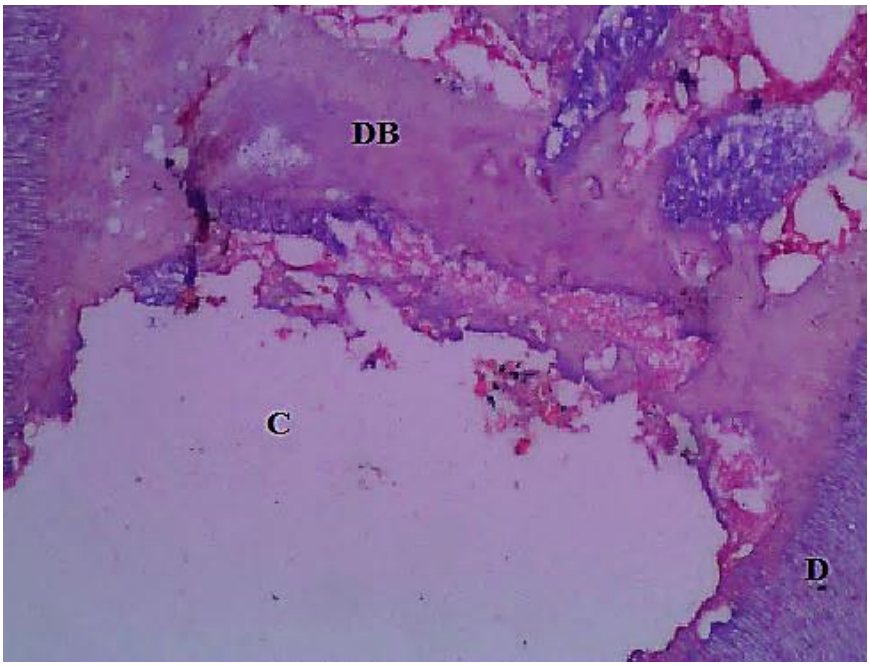
TEETH AFTER THREE MONTHS OF MATERIAL PLACEMENT:

D-Dentin, P- Pulp, DB-Dentin Bridge, C-Cavity.

MTA—Magnification—2X -- [Fig:19]



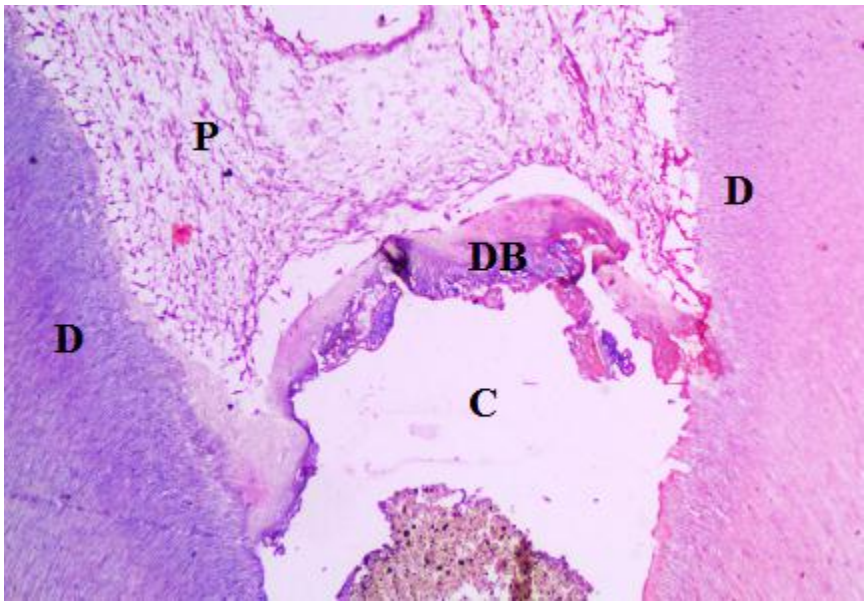
Magnification—10X -- [Fig:20]



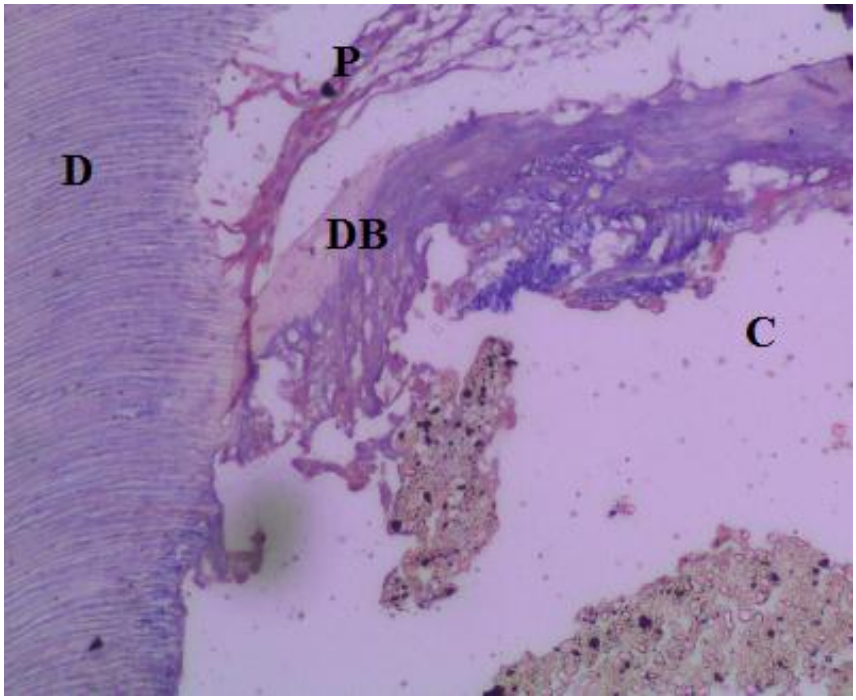
BIODENTINE

D-Dentin, P- Pulp, DB-Dentin Bridge, C-Cavity.

1.BD—MAGNIFICATION—2X -- [Fig:21]



MAGNIFICATION—10X -- [Fig:22]



Discussion

DISCUSSION:

Direct pulp capping is defined as the placement of a medicament on a pulp that has been exposed in the course of excavating the last portions of deep dental caries. The rationale behind this treatment is the encouragement of young healthy pulps to initiate a dentin bridge and wall off the exposure site[13].

Direct pulp capping is a vital pulp therapy technique which aims at maintaining pulpal tissue viability by protecting the pulpal system from bacterial ingress and hence enhancing its reparative capacity. This involves the placement of a biocompatible agent on pulp tissue that has been inadvertently exposed from traumatic injury or by iatrogenic means.

The success of vital pulp therapy requires a bacterial tight seal, minimal or no inflammation and stable hemodynamics within the pulp. The repair response of the tooth is tertiary dentin deposition. Tertiary dentin deposition could be either reactionary or reparative in nature.

Historically, the placement of a medicament or material against a direct pulpal exposure during caries excavation has been considered controversial, and instead, conventional endodontic therapy has been recommended. Currently modern dentistry is in an era of minimal intervention for maximum preservation of tooth structure and function.

Caries management has shifted its focus more towards prevention and control of the disease because of a better understanding of the basic disease process and advances in dental material science.

Retaining, reviving and **regenerating** the existing pulpal tissue are the current modalities of interest in endodontics. Preservation of pulp vitality by restorative intervention depends on the capacity and the extent to which the pulpal cells can detect, survive, react to injury and initiate repair.

Two main strategies to achieve a successful vital pulp therapy are:

1. To reduce further damage to remaining odontoblasts
2. To potentiate the differentiation of new odontoblasts.

Enhanced understanding of pulp physiology, caries progression, inflammatory mediators, and pulpal defense mechanisms has changed the clinical approach to caries removal and protocols for vital pulp therapy. Thus leading to a better understanding of the conditions, that are necessary for the pulp healing.

Ever since the introduction of calcium hydroxide [CH] to dentistry, by **Hermann (1920),** this medicament has been indicated to promote healing in many clinical situations. Traditionally, CH was the most popular material for vital pulp therapy and for direct pulp capping.

According to previous studies cited[1,47,46], the spectrum of success rates after direct pulp capping with calcium hydroxide ranges from 13% - 96%, although this material was regarded as the gold standard.

The difference in these rates is attributed to different potential prognostic factors that can influence the outcome of direct pulp capping such as duration of follow-up, type of pulp exposure (carious or mechanical), presence of an extra-pulpal blood clot between the pulp and the capping material , the area of pulp to which the capping material was applied (coronally or cervically), time elapsed to placement of a definitive restoration of the pulp-capped tooth, type of CH used, presence or absence of infection (as a result of bacteria still present or exposure to new bacteria from leakage) as well as the age of the patients. In addition, different definitions of success and failure must be considered when comparing and evaluating data in clinical studies.

Use of calcium silicate – based materials (CSMs) in dentistry became popularized with the advent of mineral trioxide aggregate (MTA) in 1993 as a root-end filling material. Studies on MTA reveal that it not only exhibits good sealing ability, excellent long term prognosis, good biocompatibility but also, favors tissue regeneration by providing biologically active substrate for cell attachment as well.

Mente et al [2010][46] conducted a long term clinical study to find out the ability of calcium hydroxide in the maintenance of long term pulp vitality after direct pulp capping in carious teeth, it was concluded that the longer the follow up period ,the more evident the trend became to a decline in the success rate of the teeth in the calcium hydroxide

group compared to MTA group. However there was no time-dependent decline in the success rate was observed in the same study when MTA was used as capping material. The decrease in success rate with increased follow-up time when CH was used for capping has been observed in many clinical studies. This can be attributed to the various drawbacks of calcium hydroxide like, poor bonding to dentin, material resorption and mechanical instability which are a few among them.

Aeinehchi et al [2003][2] compared the use of MTA and calcium hydroxide in direct pulp capping cases, using 11 pairs of third molars (patients 20–25 years old) with pulps mechanically exposed and capped with either MTA or calcium hydroxide, covered with zinc oxide–eugenol, and restored with amalgam. Teeth were extracted and then histologically evaluated at 1 week and 2, 3, 4, and 6 months. Odontoblastic layers appeared earlier; less hyperemia, inflammation, and necrosis were noted; and dentinal bridges were more pronounced in the MTA-treated teeth.

In a different randomized clinical trial, **Nair et al [2008]**[50] investigated the pulpal response to direct pulp capping in healthy human teeth with MTA versus calcium hydroxide cement (Dycal) as control. MTA was clinically easier to use as a direct pulp capping agent and resulted in less pulpal inflammation and more predictable hard tissue barrier formation than Dycal.

When considering the pulp capping materials, current evidence in literature has consistently demonstrated a better outcome when using MTA. Therefore, considering the favourable properties of MTA and various literature reviews[43,71,3] stating its success as a

pulp capping material, MTA is currently considered as a gold standard for pulp capping procedures.

However, the major drawbacks of MTA are its handling properties, long setting time, and discoloration of the remaining tooth structure. In recent years, bioactive tricalcium silicate cements have been introduced that claim to overcome these limitations. One such material is **Biodentine (BD; Septodont,Saint-Maur-des-Fosses, France).** It is marketed as a bioactive dentin substitute with active biosilicate technology. It is a tricalcium silicate cement that the manufacturer claims to promote pulp healing and remineralization by the production of reactionary dentin and dentin bridges.

Biodentine is a new calcium silicate – based restorative cement with dentin-like mechanical properties, which can be used as a dentin substitute on crowns and roots similar to how MTA is used. It has a positive effect on vital pulp cells and stimulates tertiary dentin formation. In direct contact with vital pulp tissue, it also promotes formation of reparative dentine.

The principal component of Biodentine is tricalcium and dicalcium silicate ($3CaO\ SiO_2$ and $2CaO\ SiO_2$). The liquid consists of calcium chloride ($CaCl_2.\ 2H_2O$), which is used as a setting accelerator and water-reducing agent in aqueous solution with an admixture of polycarboxylate (a super plasticizing agent). The powder consists of fine hydrophilic particles that set when mixed with the liquid. Hydration of the powder results in a colloidal gel that solidifies to a hard structure in about 9-12 minutes.

The setting of Biodentine is illustrated by a sharp increase in the compressive strength, reaching more than 100 MPa in the first hour. The mechanical strength continues to improve to reach more than 200 MPa at 24hrs; it continues to improve with time, reaching 300 MPa after one month. Its hardness is 69 VHN after 1 month.

Biodentine shares both the indications and mode of action as MTA, but does not have its drawbacks. The chief features of biodentine include reactionary dentin genesis for pulp vitality preservation, complete remineralisation with calcific barrier formation, anti microbial property due to high pH (12.5) , natural micro mechanical anchorage for better sealing properties without surface preparation, similar mechanical properties and mechanical behaviour as human dentin, and 3.5mm aluminium radiopacity facilitating easy short & long term follow-up with the advantage of its use in a single visit as a base / dentine substitute under permanent restorations like composite for vital pulp therapy.

Laurent et al [2008][40] assessed the ability of Biodentine to induce reparative dentine synthesis and to investigate its capacity to modulate pulp cells TGF-ß1 secretion and found that Biodentine induced mineralized foci formation early after its application. The mineralization appeared under the form of osteodentine and expressed markers of odontoblasts.

He also found that, Biodentine significantly increased TGF-ß1 secretion from pulp cells independently of the contact surface increase. Histologically, the bioactive tricalcium silicate demonstrated the ability to induce odontoblast differentiation from pulp progenitor cells. The resulting mineralized matrix had the molecular characteristics of dentin. Thus supporting its potential use as a pulp capping agent.

Zanini et al (2012)[73] evaluated the biological effect of Biodentine on immortalized murine pulp cells (OD-21). The expression patterns of several genes confirmed the differentiation of OD-21 cells into odontoblasts during the period of cell culture. Results suggested that Biodentine is bioactive because it increased OD-21 cell proliferation and biomineralization.

Biodentine with its comparable efficacy to MTA, which is currently the most preferred material for reparative and regenerative procedures, has many advantages over it.

Compared to Mineral Trioxide Aggregate, Biodentine has better handling properties with a homogenous consistency similar to phosphate cement, while MTA has a sandy consistency making it difficult to condense. Biodentine has a controlled setting time of 9-12 minutes. Unlike other Portland cement-based products, BD is sufficiently stable to be used for both pulp protection and temporary fillings. Also, it is cost effective when compared to MTA.

In vivo studies are required to understand the pulp response when BD is used for direct pulp capping. Studies on animal teeth[17,54] and human teeth[53,55] have demonstrated the various effects of applying MTA as a pulp-capping agent; to our knowledge, only a few clinical investigations have compared Biodentine and MTA in humans.

Hence in this study Biodentine was compared to MTA, the current gold standard for direct pulp capping procedures.This study presents a light microscopic analysis of pulpal

response to direct pulp capping, with Biodentine and MTA in healthy human premolars, which were scheduled for extraction due to orthodontic reasons.

The most effective method of evaluating the pulp-dentin complex after vital pulp therapy is by **histological criteria** as it reflects the true status. To appreciate the success of the pulp capping procedure, it should be well supported both clinically and histologically. Though a symptom-free tooth with normal function, sensitivity and radiographic appearance suggests a clinical triumph, only the histological evidence can shed light on the inflammation during the course of healing and so the long term prognosis cannot be evaluated clinically.

We have used a fast setting version of MTA namely MTA [Angelus], which has a setting time of 15 minutes. This was done to achieve standardization of both the pulp capping materials.The pulp capping material MTA/BD was stabilized with a thin layer of flowable composite and immediately sealed with a light cure composite restoration after etching and bonding.This procedure was done to prevent microleakage. Direct composite restorations provides the best seal of restorations against coronal leakage after direct pulp capping.

Studies report that pulp response after direct capping is linked to bacterial microleakage[37,58] . Microbes interfere with the pulpal response to capping materials [50] .It was noted that bacteria stimulate pulpal inflammatory activity and reduce the area of dentin bridge formation irrespective of the material used for pulp capping [49].

Many authors have suggested that pulpal survival after an oral exposure is not so much a function of an agent's potential bioactivity but its capacity to protect the pulp from bacterial exposures[16]. Prevention of bacterial leakage into cavity preparations is an important objective in treatment planning and contributes to the longevity of cavity restorations[49] . In the present study, an absence of bacteria in the stains may indicate that Biodentine and MTA have excellent sealing properties and prevent microleakage and pulpal inflammation by providing a predictable secondary barrier under the surface seal.

Although the mechanisms of tertiary dentin formation in humans are not yet completely known,it is believed that the dental pulp healing process begins with new blood vessel formation followed by the proliferation, migration, and subsequent differentiation of stem/progenitor cells into osteo-/odontoblast-like cells.

The purpose of a regenerative treatment in direct pulp capping is to induce odontoblast-like cells to differentiate and form tertiary dentin at the pulp exposure area and to stimulate the biosynthetic activity of surrounding primary odontoblasts. Depending on the severity of the injury, dentin defects are repaired by up-regulation of biosynthetic activity of primary odontoblasts (reactionary dentin formation) and/or by the formation of osteodentin (reparative dentin formation).

Stanley et al [1966][62] conducted a histologic study of 108 human teeth with Class V cavities to asses the formation of reparative dentin. Their findings state that the rate of formation of reparative dentin is highest initially in the 27- to 48-day interval (3.5 μm per day); it decreases markedly after the forty-eighth day of the experimental period to

0.74 μm per day; and it decreases further to 0.23 μm in the 72- to 132-day period. The average rate for the total length of the study was 1.49 μm per active day.

In our study a period of one week, one month and three months was used to evaluate pulp response to capping materials based on the rate of reparative dentin formation. After the specified time period for each group, the teeth were atraumatically extracted.

Immediately after extraction,the teeth were sectioned midway between the root apex and cemento enamel junction to allow better penetration of the fixative solution. Immediate placement in10% formalin followed by decalcification with 5% Nitric acid solution . Formalin is the widely used fixative in pathology labs worldwide owing to its convenience in handling, high degree of accuracy and extreme adaptability.

A mild inflammatory response is essential as a part of normal pulp healing response. This is most commonly due to operative trauma. All the samples during the 7 days observation period showed a zone of tissue destruction , which consisted of dispersed dentin particles along with the test material and a zone of pulp tissue damage. This tissue destruction was mainly due to the mechanical trauma during the pulp capping procedure. In **7 days observation period** all the samples in group 1 and 2 showed **mild or no inflammatory response** with very few inflammatory cells infiltrating the exposed area.

In **30 days observation period** a reduction in the inflammatory response and re-organization of the pulp tissue was evident in group 1 and 2. Also formation of a **thin or**

partial dentin bridge was evident in all the samples in both groups. This represents a positive healing outcome.

After **90 days** all the samples in group 1 and 2 showed evidence of **complete dentin bridge formation** closing the exposed area. **There was no significant difference between the groups in all the three criteria.**

Both materials induced the formation of a dentinal bridge at its interface with the pulp tissue. Both these calcium silicate cements induced an early form of reparative dentin synthesis, probably because of modulation of pulp cell transforming growth factor–beta 1 secretion .[61.]

A recent study[40] showed that particles of Biodentine were entrapped in the newly formed foci, and mineralization appeared as osteodentin, suggesting that material physicochemical properties might promote the mineralization process as shown with MTA-based cements. Stimulation of cell proliferation and differentiation might be related to the tricalcium silicate itself, which is one of the main components of Biodentine, and the presence of both calcium and silicon ions [72,66,55].

Numerous investigations have reported the successful application of MTA in pulp capping. In the present study, dentin bridge formation was observed in all three month samples of pulps capped with MTA[74] and Biodentine [51] as previously observed.

Tran et al [2012][66] evaluated the capacity of a new calcium-silicate-based restorative cement (biodentine) as compared to calcium hydroxide and MTA to induce pulp healing in a rat pulp injury model. At day 7, results showed that both the evaluated cement and MTA induced cell proliferation and formation of mineralization foci, which were strongly positive for osteopontin. At longer time-points, they observed the formation of a homogeneous dentin bridge at the injury site, secreted by cells displaying an odontoblastic phenotype. In contrast, the reparative tissue induced by $Ca(OH)_2$ showed porous organization, suggesting a reparative process different from those induced by calcium silicate cements.

A multicentric randomized, 3-year prospective study[38] was conducted to determine for how long Biodentine, can remain as a posterior restoration. It was concluded that resistance to marginal discoloration was superior with Biodentine compared to composite [Z 100]. When Biodentine was retained as a dentine substitute after pulp vitality control, it was covered systematically with composite. This procedure yielded restorations that were clinically sound and symptom free. Biodentine may be successfully used as a posterior restoration material for up to 6 months after direct pulp capping[38] . After validation of pulp health, it may be partially removed to place a permanent composite material [58].

Compared with Biodentine, MTA placement was more time consuming and technically difficult, although it was necessary to use a dental triturator for preparation of Biodentine. On the other hand, MTA does not require any additional equipment, except a messing gun.

Formation of the dentinal bridge at the interface between the pulp and pulp-capping material is a controversial issue because it can be a sign of healing or a reaction to irritation [6,74,4] . In the present study, formation of the dentinal bridge was interpreted as a positive reaction to stimulation and a sign of healing.

The results of this study should be carefully evaluated because the capping procedure was accomplished in sound teeth. In most clinical scenarios, the pulp exposure frequently occurs by a carious process in which the level of inflammation is much higher. However, although the use of vital healthy teeth for this kind of study has limitations, it still has the benefit of standardization and can be regarded as acceptable in respect to material selection and handling.

Direct pulp capping is used not only for accidental exposures of healthy pulps but also for pulps challenged by caries or oral exposure after a traumatic injury. Therefore, the relevance of these studies conducted in healthy human teeth may be clinically limited, and further long-term assessment is required to evaluate the pulp response to Biodentine in inflamed pulp.

Also, additional studies are needed with immunohistochemistry and transmission electron microscopy to assess the mode of action of Biodentine on the pulp.

Summary

SUMMARY:

The purpose of this study was to compare the outcome of direct pulp capping by MTA-[Angelus] and Biodentine through histological evaluation. The evaluation was done at pre-determined time periods of one week, one month and three months. Informed consent was obtained from the subjects, prior to the experimental procedures.

Forty – five intact premolar teeth to be extracted for orthodontic purposes were used for the study. The teeth were randomly allocated and equally assigned to pulp capping procedure by either MTA or Biodentine. The samples were grouped according to the material used for pulp capping and the time period of extraction.

GROUPING:

- **GROUP-1[GI]—MTA, [n=21]**
- **GROUP-2[G2]—BIODENTINE, [n=21]**
- **GROUP3[G3]—CONTROL,[n=3]**

SUB-GROUPING:

- **G1 SM**—SEVEN DAYS MTA , [n=7]
- **G2 SB**—SEVEN DAYS BD, [n=7]
- **G1 OM**-- ONE WEEK MTA, [n=7]
- **G2 OB**— ONE WEEK BD , [n=7]
- **G1 TM**-- THREE MONTHS MTA , [n=7]
- **G2 TB**—THREE MONTHS BD . , [n=7]

The pulp capping procedure was performed under rubber dam isolation. The teeth were extracted atraumatically after their respective observation periods. The roots were sectioned midway to allow for fixation and the teeth were then decalcified and subjected to normal histologic procedures for H & E staining.

The sections were then evaluated for the following criteria:

1.Inflammatory response,

2. Dentin bridge formation and

3.Thickness of Dentin Bridge.

The samples were assigned scores based on the histopathological findings.The scores were tabulated and the results were analyzed statistically using Chi-Square Test. The results showed that there was no statistically significant difference between MTA and Biodentine in the pulp healing response for all the three observation periods.

Thus it can be concluded that Biodentine shows comparable pulp healing potential to that of MTA.

Conclusion

CONCLUSION:

Within the limitations of this study, the following conclusions can be arrived at:

1. The pulp healing potential of Biodentine is comparable to that of MTA.
2. There is no statistically significant difference in the inflammatory process and formation of Dentin Bridge between the MTA and Biodentine groups.
3. Long term clinical trials are required to further evaluate the effect of Biodentine as a pulp capping material.

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Annexure

ANNEXURE I

DEPARTMENT OF CONSERVATIVE DENTISTRY &ENDODONTICS

TAMILNADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL

CHENNAI – 600003

Comparative histological evaluation of direct pulp capping with biodentine and mineral trioxide aggregate on human pulp tissue.

PROFORMA

Date: O.P. No: Group no:

Name: Age / Sex: Case no :

Address: Tel. no Mobile no:

Occupation: Income:

Details of the procedure:

Type of case: premolar orthodontic extraction case

Procedure: iatrogenic exposure of the pulp under LA

and capping with_____.

Any other information:

Name of the investigator:

Signature of the investigator:

ANNEXURE II

INFORMATION SHEET

- We are conducting a study on "**Comparative Histological Evaluation Of Direct Pulp Capping With Biodentine And Mineral Trioxide Aggregate On Human Pulp Tissue"**" among patients attending TNGDCH, Chennai and for this study, we are selecting patients .

- The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

- Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

Name of the patient Signature / Thumb impression

Name of the investigator Signature Date

ANNEXURE III

INFORMED CONSENT FORM

STUDY TITLE:

Comparative Histological Evaluation Of Direct Pulp Capping With Biodentine And Mineral Trioxide Aggregate On Human Pulp Tissue.

Name: O.P.No:

Address: S. No:

Age / Sex:

Tel. no:

I, _____ age _____ years exercising my free power of choice, hereby give my consent to be included as a participant in the study **"Comparative Histological Evaluation Of Direct Pulp Capping With Biodentine And Mineral Trioxide Aggregate On Human Pulp Tissue"**

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures.
- I understand that the study involves a restorative procedure in the teeth to be extracted for orthodontic treatment.
- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I agree that my extracted teeth may be used for the research purpose.
- I agree to report to the doctor for regular follow up, as and when required for the research.
- I have informed the doctor about all medications that I am currently taking and other systemic illnesses that I have.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.

Name of the patient Name of the investigator

Signature **/** Thumb impression Signature

Date