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Comparative Evaluation of Response ofHuman Dental Pulp On Direct Pulp Capping With MTA, ERRM (Endosequence Root Repair Putty Material).

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Abstract: *Introduction: ERRM is a new bioactive cement that is similar to the widely used mineral trioxide aggregate (MTA). These 2 materials simulate dentin in various properties, which may be considered a preferable material for clinical indications of dentin-pulp complex regeneration such as direct pulp capping. The aim of the present study was to compare the response of the pulp-dentin complex in human teeth after direct capping with ERRM cement with that of MTA. Methods: Pulps in 15 caries-free maxillary and mandibular permanent intact human premolars arranged for extraction for orthodontic reasons were selected & mechanically exposed and assigned to 1 of 2 experimental groups, MTA or ERRM, and 1 control group. Each group has 5 samples. Assay of periapical response and clinical examination were performed. After 3 weeks, the teeth were extracted, stained with hematoxylineosin, and categorized by using a histologic scoring system. Results: The majority of specimens showed complete odontoblastic layer formation and an absence of inflammatory pulp response. Statistical analysis showed significant differences between MTA& ERRMwhen compared to these 2 ERRM showed inferior result outcome. Conclusions: Within the limitations of this study, ERRM may be considered an interestingalternative to MTA in pulp-capping treatment during vital pulp therapy. ERRM also has good results but it is inferior when compared to MTA.*

I. Introduction

The first pulp capping procedure was produced, in 1756 by the Phillip Pfaff, who stuffed a small piece of gold over an exposed vital pulp to promote healing. The success of the pulp capping procedure considerably depends upon the condition under which it is performed and the prediction depends upon the age, type, site and size of pulp exposure.[1]Direct pulp capping is a procedure in which an exposed dental pulp is covered with a protective dressing or cement that protects the pulp from additional injury and permits healing and repair.[2]

Importance of pulp capping agent, save the pulp against chemical irritation by operative procedures, and bacterial penetration due to microleakage.[3,4]$Ca(OH)_2$is commonly used for pulpcapping[5], but major disadvantage of $Ca(OH)_2$is that there is no chemicaladhesion to tooth and it dissolves over time, and dentin bridges adjacent to the material is porous in nature.[6]Studies have shown that newer materials like MTA may be used as an alternativeto $Ca(OH)_2$ in direct and indirect pulp capping procedures.[7]Torabinejad (1993) was first introduced MTA as surgical root repair materials. It has been developed by modification of Portland cement as calcium silicate based endodontic material. MTA stimulates formation of dentinbridges at faster rate than calcium hydroxide, and results in high success rates in clinical procedures.[8]MTA is a bioactive,biocompatible, antibacterial material with unique stability and high sealing ability.[9] However, MTA is has longersetting time, poor handling properties, high costs, and the discolorationpotential.[10] Many attempts have been made to improve theclinical manageability of MTA by adding a setting accelerator or a dual functionalmodifier.[11]

ERRM (Brasseler USA, Savannah, GA, USA), a new bioceramic material, is a hydrophilic, insoluble, radiopaque, and aluminum- free material. It is delivered as a premixed product in both low viscosity paste form dispensed from a syringe and a high viscosity putty form. Moisture is required for the materials to set and harden. The working time is more than 30 minutes, and the setting time is 4 hours under normal conditions. ERRM is of alkaline pH, biocompatible, antibacterial, and able to seal root-end cavities.[12]It consists of Calcium Silicates, Zirconium Oxide, Tantalum Oxide, Calcium Phosphate Monobasic, Filler Agents. The material has nanosphere particles with a maximum diameter of 1 x 10-3µm that allow for the material to enter dentinaltubules, be moistened by dentin liquid, and create a mechanical bond upon setting.This material has been manufactured to overcome some of the difficult handling characteristics of MTA.[13] This material is bioactive due to its ability to form a hydroxyapatite or apatite-like layer on its surface when it comes in contact with phosphate-containing fluids. Hansenet al.[14]compared the diffusion of hydroxyl ions for ERRM and WMTA

through root dentin. They found that although both materials showed diffusion of ions through dentin,the effect was less pronounced and of shorter duration for EndoSequence than WMTA.

The purpose of the present study was to evaluate the clinical,radiographic, and histologic responses of the pulp-dentin complexafter direct capping with ERRM and MTA in human teeth. The hypothesis was that there wereno differences in the pulp-dentin complex response to 2 cappingmaterials, applied as a direct pulp cap inhuman teeth.

II. Materials And Methods

Fifteenintact human caries-free maxillary and mandibular premolars scheduled for extraction for orthodontic reasons were selected in 5 patients ranging in age group from 19–28 years. Teeth were free from any physical & chemical alterations.Informed consents were obtained. All experimental procedures were assessed and accepted by the Local Ethical Committee, DMIMS Sawangi (Meghe) Wardha, Maharashtra, India. (Approval number 2014-15/1112).Before the operative procedure, each tooth was radiolographically analysed to eliminate the presence of caries or periapical pathology. A standardized operative procedure was followed in both experimental groups. Thermal testing (Kalte spray; M&W Dental GmbH, Budingen, Germany) and electric pulp testing (Vitality Scanner pulp vitality tester; Sybron Endo, Orange, CA) were achieved to assess pulp vitality. Before cavity preparation, teeth were mechanically polished and sterilized with 0.2% chlorhexidine solution. After local anesthesia and rubber dam application, occlusal conventional Class I cavities were developed by using round sterile diamond burs no.6 at high speed under air-distilled water spray coolant. An exposure of approximately 1.2 mm in diameter was made, under air distilled water cooling. New burs were applied during each operation. Bleeding was restricted with saline irrigation, and a sterile cotton pellet was placed onto the pulp exposure sites.

The teeth were divided into 3 groups, MTA (n = 5), ERRM (n=5) and 1 control group (n = 5). In group I, pulps of teeth were restored directly using GIC (control). According to the manufacturer's recommendations. In group II, uncovered pulps and the surrounding dentin were covered with a 2-mm-thick layer of ProRoot White MTA (Dentsply, Tulsa Dental, Tulsa, OK) according to the manufacturer's recommendations. After placing the MTA, the operator placed a flat, water-moistened cotton pellet immediately over the material. Group III pulps were capped with ERRM. All the samples were provisionally restored the tooth with glass ionomer cement (Ketac Molar; 3M ESPE, Seefeld, Germany). Patients in all 3groups returned to the clinic for clinical examination & follow up on seventh post operative day. All procedures were performed by experienced operator in the Department of Conservative Dentistry, DattaMeghe Institute of Medical Sciences, Sawangi (M) Wardha, India.

Clinical Examination

Patients were checked for postoperative sensitivity or pain throughout the study period. Thermal testing and electric sensitivity testing were done to analyze the pulp health. Radiographs were taken before extraction to observe symptom of periapical pathology. The Duration of theclinical treatment was 3 weeks. The teeth were extracted as atraumatically as possible by a designated oral surgeon (DMIMS) in the Department of Oral Surgery.

Histologic Examination

After fixation for 2 days in 10% buffered formalin solution, thespecimens were demineralized in a decalcifying solution containing 10% formic acid for 20 days, continued for 3 days in10% nitric acid. After complete demineralization specimens were embedded in paraffin. Two- to 3-micron-thick serial sections in the buccolingual plane were prepared from the paraffin-embedded teeth which were later stained with hematoxylin-eosin. Coded samples were used throughout the study to ignore possible bias. By using an Stereomicroscope (Carl Zeiss Imager D1 Axio, Goettingen, Germany) connected to a high-resolution video camera (Axio Cam MRc5; Carl Zeiss Micro imaging, Thornwood, NY), samples were evaluated under normal and ultraviolet light by using 3 filters by an skilled oral pathologist in Dept of Oralpathology (DMIMS). The quantity of hard tissue formation at the contact of the capping material (continuity, morphology), pulp inflammation (type, intensity, and extension), and further histologic features of the pulp tissue including the odontoblast cell layer and bacterial penetration.

Scoring Criteria-

	Score -	1	2	3	4
A.	For continuity of the dentinal bridge	Complete dentin bridge formation	Partial/incomplete dentin bridge formation extending to more than one-half of the exposure site but not completely closing the exposure site	Initial dentin bridge formation extending to not more than one-half of the exposure site.	No dentin bridge formation
B.	For morphology of dentinal bridge	Dentin or dentin associated with irregular hard tissue	Only irregular hard tissue deposition	Only a thin layer of hard tissue deposition	No hard tissue deposition.
C.	For type of pulp inflammation	No inflammation	Chronic inflammation	Acute and chronic inflammation	Acute inflammation.
D.	For intensity of pulp inflammation	Absent or very few inflammatory cells	Mild, defined as an average of <10 inflammatory cells	Moderate, defined as an average of 10–25 inflammatory cells	Severe, defined as an average >25 inflammatory cells.
E.	For extensity of pulp inflammation	Absent	Mild, defined as inflammatory cells only next to dentin bridge or area of pulp exposure	Moderate, defined as inflammatory cells observed in part of coronal pulp (in one-third or more of the coronal pulp or in the midpulp)	Severe, defined as all coronal pulp is infiltrated or necrotic.
F.	For odontoblastic layer	Palisade pattern of cells	Presence of odontoblast cells and odontoblast-like cells	Presence of only odontoblast-like cells	Absent

Histopathological results were determined according to the modified criteria by Faraco et al[15] and Medina et al.[16] Each histomorphologic section was scored from 1–4, with 1 representing the most desired result and 4 representing the least desired result.

Statistical Analysis

The results of the histopathologic evaluation were statistically analyzed by using the Mann-Whitney U test. A P value <0.05 was evaluated statistically significant.

III. Result

On 21st day patients were checked for spontaneous minor pain, on electric pulp vitality test. None of them reported of pain on electric pulp testing. Patients reported no particular symptoms during the experimental time period. Before extraction, all teeth were cold-sensitive and electro-sensitive and involved vital pulp. In addition, no periapical pathologies were revealed by radiography before extraction.

Histologic evaluation of teeth showed that 2 materials were welltolerated by the pulp tissue. Results of all the specimens in MTA& ERRM groups are provided in **Table 1**

Group	N	Mean	Std. Deviation	Std. Error
MTA	5	2.80	1.30	0.58
ERRM	5	2.60	1.14	0.50
Control	5	1.00	0.00	0.00

Table 1: Comparison of continuity of dentinal bridge in three groups

In the MTA experimental group, the pulp responses were not similar to those observed in theERRM group where the pulp response was comparativelyless. The dentin bridge was formed directly underneath the capping materials at the injury site with all 3 materials. Complete dentin bridge formation was observed in 3 teeth in the MTA group, 3 teeth in the ERRM group. In 4specimens of MTAgroup, dentin was associated with an irregular hard tissue; similar to that of tooth morphology were observed, whereas it is seen in3 specimens ofERRM group.

There was no evidence of inflammation, abscess, or necrosis below the dentinal bridge. An absence of or few inflammatory cells and, rarely, dilated blood vessels were observed in a majority of pulp specimens. Chronic mild inflammation (<10 inflammatory cells) was seen in 2 specimens of ERRM. Chronic inflammation was observed next to dentin bridge or area of pulp exposure.In most specimens in all groups, odontoblast and odontoblastlike cells were discovered adjacent to the dentinal bridge with well distinguishable dentin tubules and with irregular pattern of tubules. The layers of well-arranged odontoblast and odontoblast-like cells were

observed to form tubular dentin under the osteodentin. Specimens in the control group exhibited normal pulp tissue with palisade columnar odontoblast cells, a zone of Weil, a cell-rich zone, and central pulp with normal characteristics.Wellformedodontoblastic layer with palisade pattern of cells was observed in all 3 in MTA & 3in ERRM. Presence of odontoblast&odontoblastic cells were seen in 2 specimens of MTA & 1 in ERRM.(Table 2)

Regarding the histologic evaluation criteria, our investigation showed that there was no statistically significant difference between the responses of teeth to MTA compared with ERRM as a pulp capping agent (P >0.05). ERRM also showed pronounced positive results; but when compared with MTA it is inferior in outcome.

Group	N	Mean	Std. Deviation	Std. Error
MTA	5	0.33	0.54	0.24
ERRM	5	0.37	0.44	0.20
Control	5	2.80	0.83	0.37

Table 2: Comparison of intensity of pulp inflammation in 3 groups

IV. Discussion

This study presents a light microscopic analysis distinguish between MTA & ERRM in the pulpal response to direct pulp capping in healthy human premolars. The findings of this study indicate that iatrogenic pulp defects treated with both calcium silicate cements are principally free from inflammation and become covered with compact, dentin-like hard tissue bridges.

There are no differences in the pulp-dentin complex response to the 2 capping techniques (MTA,ERRM) as a direct pulp capping in human teeth can be accepted. In the present study, dentinal bridge formation & inflammation were interpreted as a positive reaction and as sign of healing.These three materials (**MTA & ERRM**) induced the formation of a dentinal bridge at its contact with the pulp tissue columnar cells, with polarized nuclei projecting into invaginations of the bridge observed in some specimens, which is clearly indicative of the formation of odontoblast cells and initiation of tubular dentin, palisade pattern of cells were also evident in few specimens.ERRM showed slightly less results when compared to MTA.

The major advantages of ERRM are improved handling characteristics over traditional MTA and the distribution of a consistent product with single application.In present study, ERRM has good mechanical properties and this material received good rates forhandling, whereas in MTA placement was more time consumingand technically difficult. Dentinal bridge formation at the junction of pulpand direct pulp-capping material is a speculative issue because it maybea due to healing of pulp or due to irritation. Though inflammation is required for healing.Fig 1,2

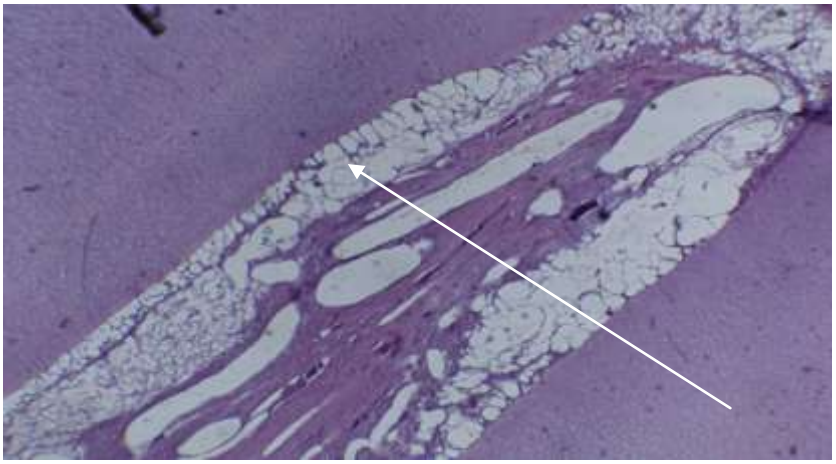

Fig 1- Group B- MTA Human pulp capped with MTA.Complete dentin bridge formation with MTA. Hard bridge tissue, new odontoblast cell layer, and dentinal tubules,palisade pattern of odontoblastic cells. (Box -hematoxylin-eosin; scanner view 4x)

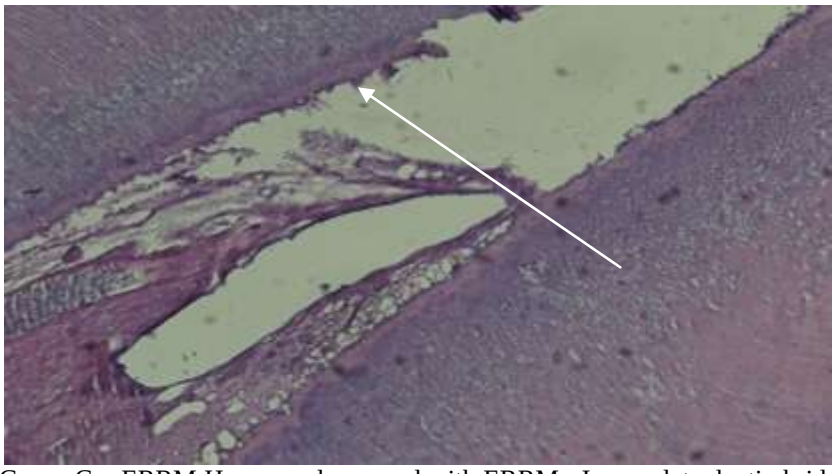

Fig 2 – Group C – ERRM Human pulp capped with ERRM. Incomplete dentin bridge formation with ERRM. Hard bridge tissue, new odontoblast cell layer, and dentinal tubules, presence of few inflammtory cells, mild inflammatory reaction. (arrow -hematoxylin-eosin; scanner view 4x)

MTA is considered as a bioactive material with possible osteoinductive properties.[17] The potential property of MTA to promote differentiation of dentinoblasts from clonogenic cells of the dental pulp has beendemonstrated by Zhao et al.[18]Calcium silicate-based material has been recently developed to overcome some ofshortcomings of MTA. ERRM is stated so as to bondadjacent dentin, to have no shrinkage, and to be highly biocompatible, hydrophilic,radiopaque, and antibacterial lead to a high pH during setting.The diffusion of hydroxyl ions for ERRM and through root dentin, although both materials showed diffusion of ions through dentin. ERRM is bioactive due to its ability to form a hydroxyapatite or apatite-like layer on its surface when it comes in contact with phosphate-containing fluids.[17]Clinical evaluationis inappropriate for the long-term treatment outcomeas the results& prognosis of pulp-capping materials requires histological diagnosis.[19]

Numerous investigationshave reported the successful application of MTA in pulp capping.In the present study, dentin bridgeformation was observed in almost all pulps capped with MTA&ERRM (>80). Parirokhet al[19]reportedthat adding $CaCl_2$ to MTA pulp-capping agent did not improvethe properties of this biomaterial. In contrast to present study, histologicresults showed higher percentages of inflammation anda lower percentage of calcified bridge formation in MTA + $CaCl_2$ samplescompared with MTA.The new tricalcium silicate–based cement was tested in vivo inanimals by Tran et al.[20] This study evaluated the capacity of MTA, and $Ca(OH)_2$ to induce pulp healing in a rodent pulps.

Similar to present study, the researchers have observed dentin bridge formation at the mechanical exposure site after 30 days that was secreted by cellsdisplaying a resemblance to odontoblastic cells. These odontoblastic activity & formation of dental hard tissue which were induced by calcium silicate cementswere homogenous and in continuation with primary dentin. Studies reported that pulp response after direct capping is linked tobacterial microleakage.[21] Micro-organisms interfere with thepulpal reaction to capping materials.[9] It was observed thatbacteria stimulate pulpal inflammatory activity but reduce the area ofdentin bridge formation irrespective of the material used for pulpcapping; as the area fordentin bridge is decreased cause it depend upon sharing the space with microbes.[22] Many authors have reported that pulpal survival afteran oral contamination is not so much a function of potential bioactivitybut its capacity to protect the pulp from bacterial exposures. Prevention of bacterial leakage by following strict isolation protocol isan important objective & contributes to thelongevity of cavity restorations.[22]In the present study, an absenceof bacteria in the stains may indicate that all three materials haveexcellent sealing properties and prevent microleakageby providing a barrier under theseal. All the cavities in present study were sealedwith GIC after the application of pulp capping material to prevent microbial leakage through saliva contamination.

In present study the teeth with ERRM showed mild inflammatory cells. Direct pulpcapping is used for iatrogenic mechanical exposure of healthy pulps as well as for pulps with partial/without caries involvement of or oral exposure after a mechanicaltrauma.

Limitations –

This is an ex vivo study conducted on small number of samples, and for shorter duration. For more accurate results larger sample size is required with longer duration of evaluation & assessment.

V. Conclusion

Within the limitations of this study, MTA had a better efficacy in the clinical setting and may be considered interesting alternatives to other pulp capping materials in pulp-capping treatment during vital pulp therapy. ERRM is newer calcium silicate based material which can be used for direct pulp capping but MTA produce superior results.

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