TITLE- CONCENTRATED GROWTH FACTOR (CGF), PLATELET RICH FIBRIN (PRF), MINERAL TRIOXIDE AGGREGATE (MTA) AS DIRECT PULP CAPPING AGENTS - CASE REPORTS

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INTRODUCTION

The vitality of the pulp is essential for the longevity of the tooth as pulp is the only living tissue of the tooth which nourishes, aids in reparative tissue formation and also exhibits immune functions. Direct pulp capping is the treatment of an exposed vital pulp by sealing the pulpal wound with a dental material placed directly on an iatrogenic or traumatic exposure to initiate the formation of reparative dentin and maintenance of the vital pulp.[1]

The search for an ideal pulp capping material still continues to exist. Traditionally, Calcium hydroxide formulations were used as pulp capping agent and was once considered the gold standard for pulp-capping material. However, the material had several drawbacks like poor sealing ability with dentin, lack of ability to promote odontoblast differentiation in a consistent manner , was found to be cytotoxic in cell cultures and the reparative dentin formation induced by calcium hydroxide had tunnel defects[2]. MTA is a bioactive, Portland cement based, tricalcium silicate cement that has been shown to be a successful pulp-capping agent based on recent in vitro, animal and in vivo studies, The material is non-absorbable, sets in the presence of moisture, has a relatively high compressive strength and has a sustained high alkaline pH.[3] However, it poses drawbacks like its potency to cause tooth discoloration, presence of toxic elements in its composition, difficult handling properties, long setting time, cost ineffectiveness, the absence of a known solvent to retrieve the material and the difficulty faced during of its removal post hardening.

Recently, platelet concentrates like CGF and PRF are being used in various fields in dentistry due to their regenerative properties. They can be used as pulp capping agents, as the growth factors present in these platelet concentrates are known to play a crucial role in hard and soft tissue repair mechanisms,by exhibiting chemotactic and mitogenic properties that promote and modulate cellular functions .They can stimulate dental pulp cells into odontoblastic like cells, leading to reparative dentin formation.[4]

Following are three case reports on direct pulp capping using MTA, PRF and CGF.

CASE REPORT – 1

HISTORY

A 27- year old male patient reported to the department with a chief complaint of pain in right lower back teeth for past 1 month. He gave a history of sharp pain, which was stimulated by cold food, which lasted for a few seconds and subsided on the cessation of stimuli

CLINICAL EXAMINATION

Intraoral examination revealed Deep dentinal caries involving buccal and occlusal surfaces in 46. The tooth was not tender on percussion

INVESTIGATIONS

Vitality testing using EPT and cold test (Endo- Ice, COLTENE) showed a positive response in 46.

Radiographic findings revealed radiolucency in the occlusal aspect approximating pulp in relation to 46, with no evidence of thickening/ widening of periodontal ligament

PROVISIONAL DIAGNOSIS

Reversible pulpitis in 46

TREATMENT PLAN

Caries excavation → If carious exposure within 0.5-1mm → Direct pulp capping with CGF

Caries excavation → If carious exposure > 1 mm → endodontic treatment

CLINICAL PROCEDURE

DIRECT PULP CAPPING PROCEDURE

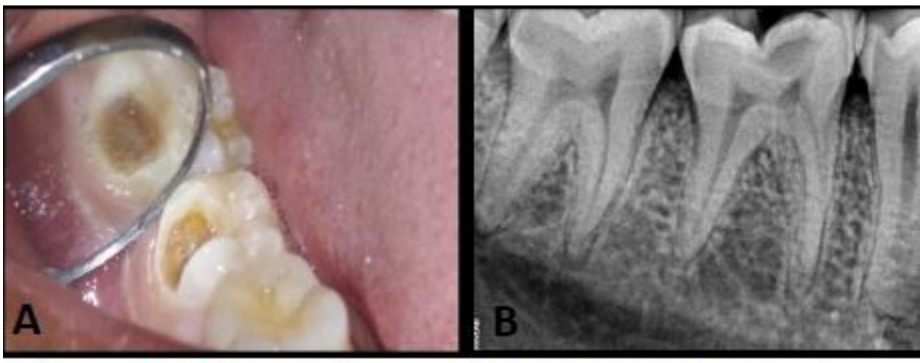
Oral prophylaxis was done prior to the commencement of the treatment. Patients were given a prophylactic oral rinse of 0.2% Chlorhexidine (Rexidine mouth rinse, ICPA products) just before the procedure. Under local anaesthesia, Lignocaine with 1:80,000 Adrenaline (Lignox 2%, Warren, Indoco Remedies Ltd, India) and rubber dam isolation , Caries detector dye (Prime Dental Products Pvt Ltd, India) was applied to the tooth for 30 seconds and rinsed to disclose the infected dentin. Caries excavation was done using sterile diamond points (Round diamond bur BR 41/ Straight fissure bur (SF 11 Mani Dia-Burs Inc.,) and high speed airotor hand piece (NSk,Tokyo, Japan). Complete caries removal was ensured with Caries Indicator. Once there was a carious pulp exposure of 0.5 – 1 mm, hemostatis was achieved by placing a sterile cotton soaked in 3% NaOCl. The cavities were then examined visually for arrest of bleeding.

CGF PREPARATION

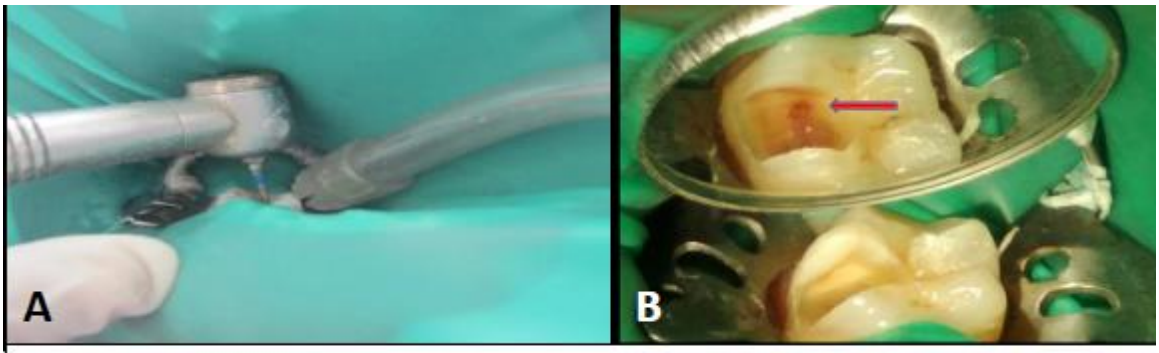
CGF was prepared just before its placement in the cavity as a pulp capping agent. 9 ml of venous blood was drawn from the patient's antecubital vein and transferred into a sterile Vacuette test tube (Greiner Bio-One, Kremsmunster, Austria) without an anticoagulant. These tubes were immediately centrifuged in the CGF centrifugation machine(Medifuge, Silfradent, Italy) using a variable rpm as follows: 30 seconds acceleration,2 min at 2,700 rpm, 4 minutes at 2,400 rpm, 4 minutes at 2,700 rpm, 3 minutes at 3,000 rpm and 36 seconds deceleration. At the end of the process, three blood fractions were identified: 1) upper layer, platelet poor plasma (PPP), which represents the liquid phase of plasma, 2) middle layer, representing the solid CGF, 3) lower layer, red blood cells. CGF was separated from red corpuscles base (preserving a small RBC layer) using sterile tweezers just after removal of PPP (platelet-poor plasma) and then transferred into a sterile dappen dish. The CGF clot was compressed gently between moist sterile cotton gauze to remove the serum and to procure the CGF membrane.The CGF membrane was then carried to the exposure and positioned with the tine of the sterile explorer.

RESTORATIVE PROCEDURE

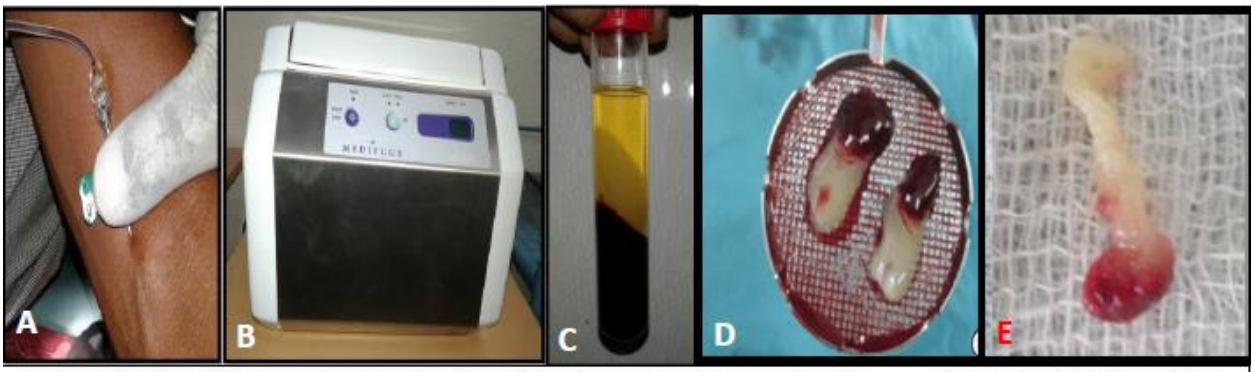
After placement of the CGF membrane, the floor of the prepared cavities were lined with type I GIC (Fuji I, GC Tokyo, Japan) . After ensuring that GIC was set, the cavity walls were etched with 37% Orthophosphoric acid (Tetric Etch, Ivoclar Vivadent) for 20 seconds, rinsed and dried followed by application of bonding agent (Tetric Bond , Ivoclar Vivadent) and cured for 20 seconds in a light curing unit . This was followed by restoration of the cavities with incremental addition and curing of Light Cure Composite (Tetric N Ceram, Ivoclar Vivadent) for 20 seconds and the final increment was cured for 40 seconds. The restorations were assessed for the presence of premature contacts and visible marginal defects. The restoration was finished and polished with composite finishing kit (Shofu, Kyoto, Japan).



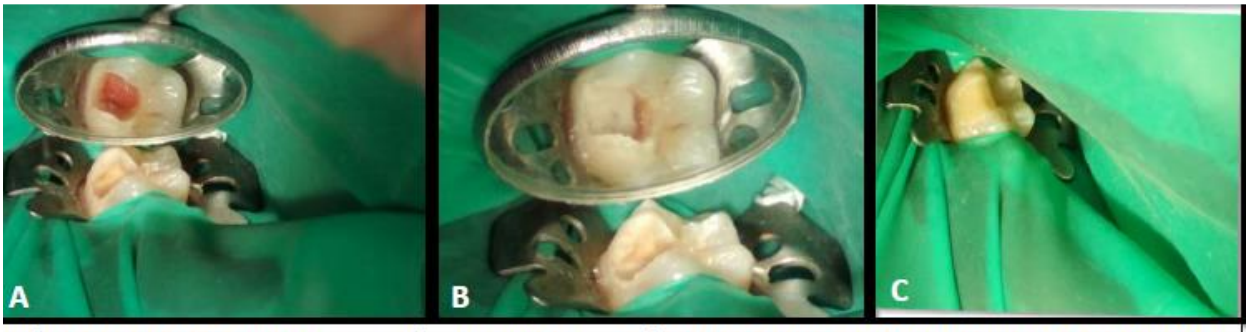
A) PREOPERATIVE IMAGE B)PREOPERATIVE RADIOGRAPH



A) CARIES EXCAVATION B) PINPOINT EXPOSURE



A-D) CGF PREPARATION IN MEDIFUGE (SILFRADENT, ITALY) E) CGF MEMBRANE



A) PLACEMENT OF CGF B) GIC LINER C) LIGHT CURE COMPOSITE RESTORATION

CASE REPORT – 2

HISTORY

A 22- year old male patient reported to the department with a chief complaint of pain in right lower back teeth for past 2 months. He gave a history of mild pain, which was stimulated by cold and sweet food, which lasted for a few seconds and subsided on the cessation of stimuli

CLINICAL EXAMINATION

Intraoral examination revealed Deep dentinal caries involving disto-occlusal surfaces in 46. The tooth was not tender on percussion

INVESTIGATIONS

Vitality testing using EPT and cold test (Endo- Ice, COLTENE) showed a positive response in 46**.**

Radiographic findings revealed radiolucency in the disto-occlusal aspect approximating pulp in relation to 46, with no evidence of periodontal or periapical involvement.

PROVISIONAL DIAGNOSIS

Reversible pulpitis in 46

TREATMENT PLAN

Caries excavation → If carious exposure within 0.5-1mm ⟹ Direct pulp capping with PRF

Caries excavation → If carious exposure > 1 mm ⟹ endodontic treatment

CLINICAL PROCEDURE

DIRECT PULP CAPPING PROCEDURE

The caries excavation procedure was carried out as described in CASE-1..

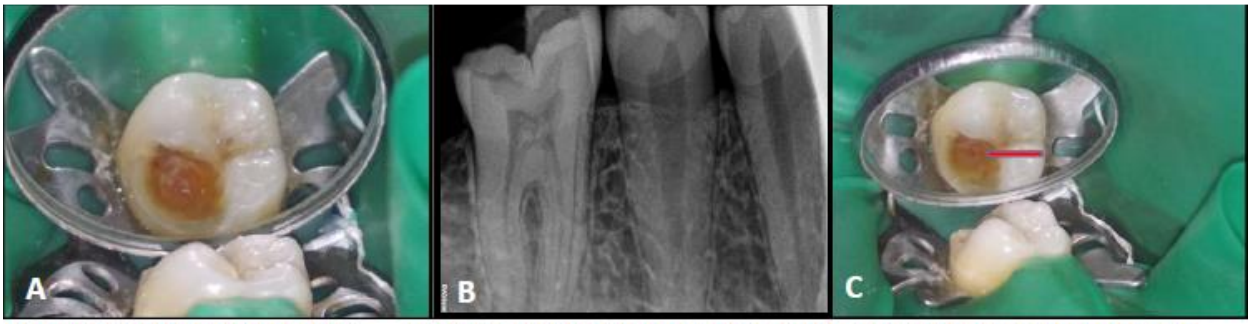
PRF PREPARATION

PRF was prepared after the operative procedure just before its placement in the cavity as a pulp capping agent in accordance with the protocol developed by **Choukroun et al**. 10 ml of venous blood was drawn from the patient's antecubital vein and transferred into a sterile test tube without an anticoagulant. These tubes were immediately centrifuged in the Remi centrifugation machine at 2700 rpm for 12 min . At the end of the process, a structured fibrin clot formed in the middle of the tube, just between acellular plasma (platelet-poor plasma) at the top and the red corpuscles at the bottom . PRF was separated from red corpuscles base using sterile tweezers just after removal of PPP (platelet-poor plasma) and then transferred into a sterile dappen dish. The

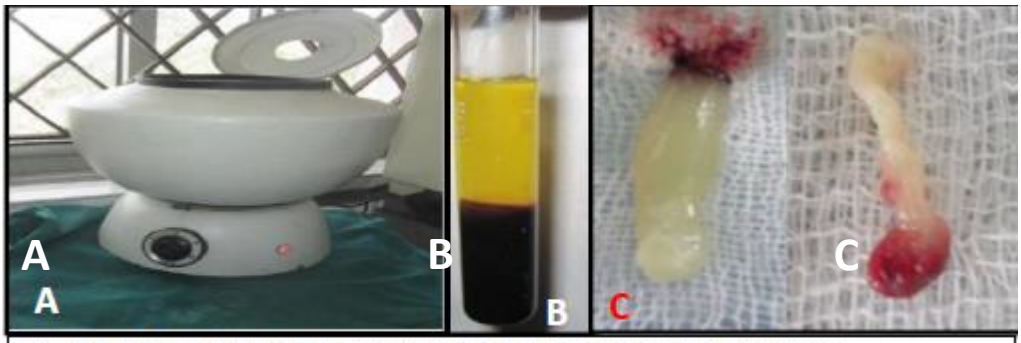
PRF clot was compressed gently between moist sterile cotton gauze to remove the serum and to procure the PRF membrane.The PRF membrane was then carried to the exposure and positioned with the tine of the sterile explorer.

RESTORATIVE PROCEDURE

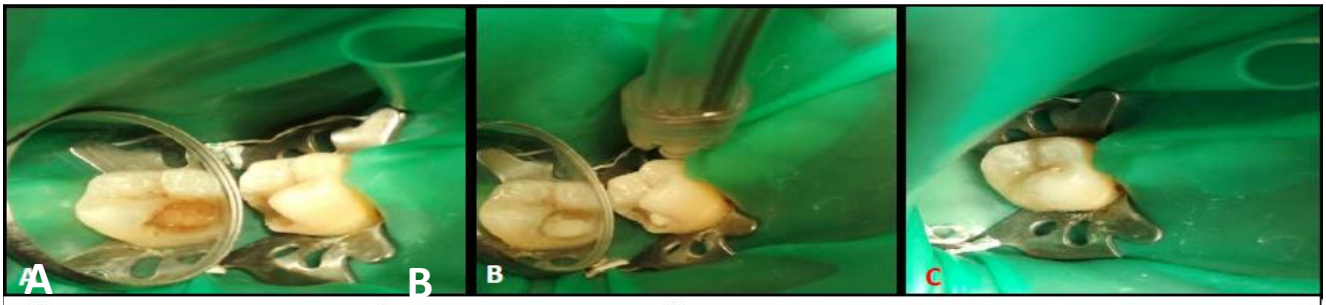
The restorative procedure was carried out as described in CASE-1



A) PREOPERATIVE IMAGE B)PREOPERATIVE RADIOGRAPH C) PINPOINT EXPOSURE



A, B) PRF PREPARATION IN REMI CENRIFUGATION MACHINE C) PRF GEL AND MEMBRANE



A) PRF PLACEMENT B) GIC LINER C) LIGHT CURE COMPOSITE RESTORATION

CASE REPORT – 3

HISTORY

A 32- year old female patient reported to the department with a chief complaint of pain in right lower back teeth for past 1 month. She gave a history of mild pain, which was stimulated by cold and sweet food, which lasted for a few seconds and subsided on the cessation of stimuli

CLINICAL EXAMINATION

Intraoral examination revealed Deep dentinal caries involving disto-occlusal surface in 46. The tooth was not tender on percussion

INVESTIGATIONS

Vitality testing using EPT and cold test (Endo- Ice, COLTENE) showed a positive response in 46

Radiographic findings revealed radiolucency in the disto-occlusal aspect approximating pulp in relation to 46, with no evidence of thickening/ widening of periodontal ligament

PROVISIONAL DIAGNOSIS

Reversible pulpitis in 46

TREATMENT PLAN

Caries excavation → If carious exposure within 0.5-1mm ⟹ Direct pulp capping with MTA

Caries excavation → If carious exposure > 1 mm ⟹ endodontic treatment

CLINICAL PROCEDURE

DIRECT PULP CAPPING PROCEDURE

The caries excavation procedure was carried out as described in CASE-1..

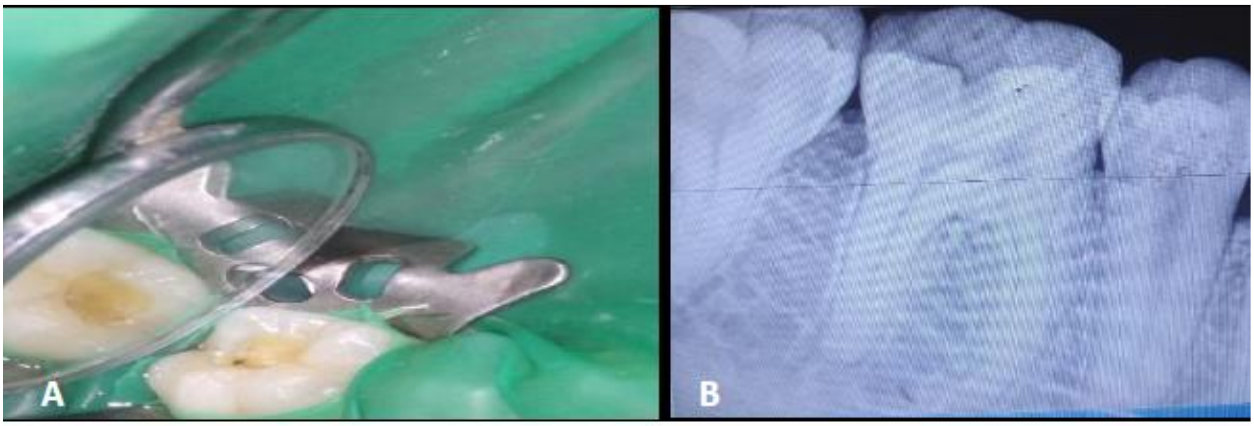
MTA PREPARATION

MTA(MTA ANGELUS) was mixed according to the manufacturer's instructions on a sterile glass slab using a stainless steel spatula . A 1mm thickness of the manipulated MTA was placed

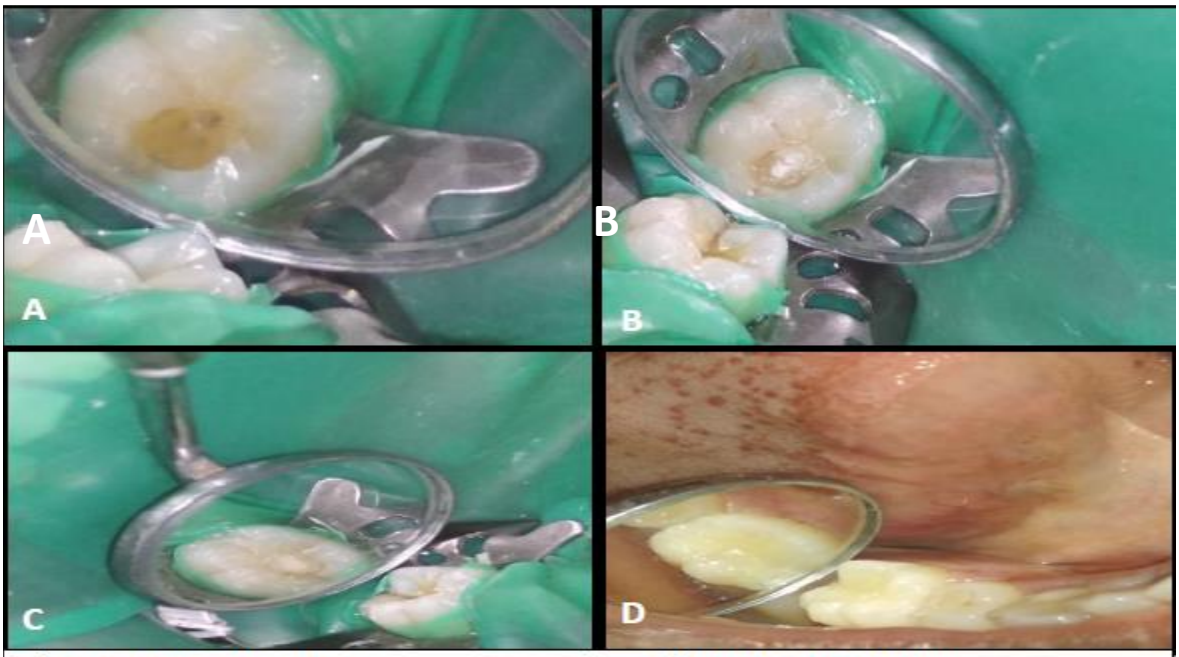
over the exposure site .After placement, the cavity floor was dabbed with moist sterile cotton pellet

RESTORATIVE PROCEDURE

The restorative procedure was carried out as described in CASE-1



A) PREOPERATIVE IMAGE B)PREOPERATIVE RADIOGRAPH



A) PINPOINT EXPOSURE B) MTA PLACEMENT
C) GIC LINER D) COMPOSITE RESTORATION

POST-OPERATIVE FOLLOW UP

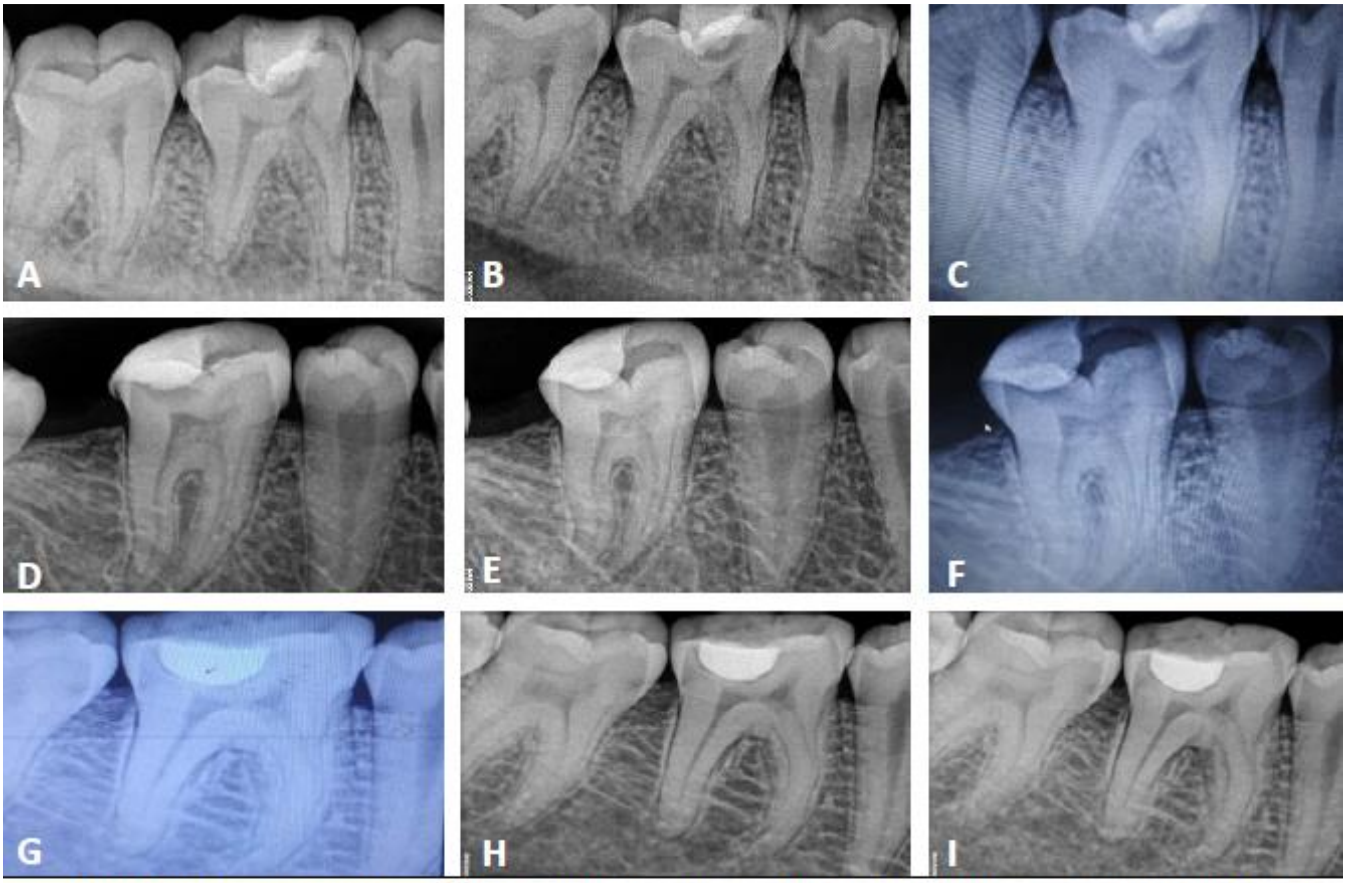
CLINICAL FOLLOW - UP

Patients were reviewed after 3 and 6 months. The pulp vitality using cold and electric pulp testing showed a positive response , periodontal probing depth was normal,there was no mobility, no pain and tenderness to percussion with respect to all the three cases.

RADIOGRAPHIC FOLLOW – UP

The pulp capped teeth were radiographically evaluated using Digital radiography (CS Image Software) with XCP film positioning device and standard exposure modes of 100 msec, 4 mA and 60 kVP**.**

There was no signs of periodontal space widening or periapical pathology in any of the three cases, at the 3 and 6 months follow up



A-C) DIRECT PULP CAPPING WITH CGF; A)IMMEDIATE POST-OP, B) 3 MONTHS FOLLOW UP, C) 6 MONTHS FOLLOW UP
D-F) DIRECT PULP CAPPING WITH PRF; D) IMMEDIATE POST-OP,E) 3 MONTHS FOLLOW UP, F) 6 MONTHS FOLLOW UP
G-1) DIRECT PULP CAPPING WITH MTA; G) IMMEDIATE POST-OP, H) 3 MONTHS FOLLOW UP, I)6 MONTHS FOLLOW UP

DISCUSSION

All the three materials used in these cases are based on the biological approach of vital pulp therapy, which explores the molecular and cellular mechanisms behind pulp tissue regeneration and identifies a biological strategy for the treatment of clinical exposures .[5] In the category of newer synthetic materials, MTA has shown promising clinical outcomes. A study showed that a high pH of 12.5 created in the area adjacent to the MTA remains high for at least 8 weeks and this high pH of MTA during setting has been found to affect cell growth and exert a cytotoxic effect on both macrophages and fibroblasts.[6]

Therefore, it is important to develop biocompatible treatments directed at maintaining pulp vitality and increasing tooth longevity, like the platelet concentrates . Though MTA is proved to induce dentin bridge formation by inducing biochemical pathways, platelet concentrates provide the necessary bioactive substances in ready made form and they circumvent the natural biological process of induction.[7]

PRF is a Second generation platelet concentrate, developed by Choukroun et al in 2001. Huang et al (2010) investigated effect of PRF on cultured primary dental pulp cells and they concluded that PRF causes proliferation of human dental pulp cells and increase the protein expression of osteoprotegerin activity leading to the odontoblastic differentiation and mineralization process.[8]

CGF is an Advanced second generation platelet concentrate, developed by Sacco in 2006. CGF appears to contain more abundant cytokines and was substantially studied in bone regeneration , but there has been little research performed to substantiate its role as a pulp capping agent

Platelet concentrates can increase DPC proliferation and differentiation, suggesting potential applications of these as a biological molecule to promote the regeneration of lost or injured dental pulp tissues and stimulate reparative dentinogenesis

CONCLUSION

Platelet concentrates like PRF and CGF were found to have clinical efficacy that was comparable to MTA . Thus, they could be considered as a promising alternative to MTA as direct pulp capping agents

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8) Huang FM, Yang SF, Zhao JH, Chang YC. Platelet- rich fibrin increases proliferation and differentiation of human dental pulp cells. J Endod 2010 Oct; 36(10):1628-32

Contributors' Form

I / We certify that I/we have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the writing of the manuscript, to take public responsibility for it and have agreed to have my/our name listed as a contributor. I/we certify that all the data collected during the study is presented in this manuscript and no data from the case report has been or will be published by the editors, I/we will provide the data/information or will cooperate fully in obtaining and providing the data/information on which the manuscript is based, their assignees.

We give the rights to the corresponding author to make necessary changes as per the request of the panel, do the rest of the correspondence on guarantor for the manuscript on our behalf.

All persons who have made substantial contributions to the work reported in the manuscript, but who are not authors, are named in the Acknowledgment permission to be named. If I/we do not include an Acknowledgment that means I/we have not received substantial contributions from non-authors and Name Signature Date signed

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(up to four authors for case report)