10.5005/jp-journals-10024-2438

ORIGINAL ARTICLE

the Journal of CONTEMPORARY DENTAL PRACTICE at www.thejcdp.com

# Revascularization in Immature and Mature Teeth with Necrotic Pulp: A Clinical Study

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## ABSTRACT

**Aim:** The aim of this *in vivo* study was to evaluate the revascularization procedure both in immature and mature teeth with necrotic pulp and open apices, disinfected with triple antibiotic paste followed by inducing blood clot in the root canal.

**Materials and methods**: Fifteen patients were selected who presented with immature and mature permanent teeth with pulpal necrosis and open apices. In the first visit, the root canal was accessed with LA and rubber dam isolation; then the canal was disinfected using triple antibiotic paste containing ciprofloxacin, metronidazole, and clindamycin in the ratio of 1:1:1 and closed with IRM. In the second visit, after administering local anesthesia and isolating with a rubber dam, the triple antibiotic paste was washed out by saline irrigation, and apical papilla beyond the confines of the root canal was stimulated with sterile H file to produce a blood clot. Finally, the access was closed using a double seal with mineral trioxide aggregate (MTA) placed apical to cementoenamel junction and resin bonded cement over the MTA. Radiographic examination and pulp sensibility test was done during the follow-up period of 2,4,6,8 and 10 months.

**Result:** After 10 months follow-up, 10 out of 13 patients showed root development and apical closure. The eight patients out of 13 showed root development, apical closure and lateral thickening of radicular dentin and 2 out of 13 patients showed a positive response to electric sensibility test.

**Conclusion:** Within the limitation of this study, it can be concluded that there is evidence of root development, increase in lateral wall thickness, apical closure and positive response to pulp sensibility test in both mature and immature teeth with necrotic pulp.

**Clinical significance:** The conventional approach for management of teeth with necrotic pulp and the open apex is altered with the possibility of tissue regeneration within the pulp space and continued root development through revascularization procedures. It also re-establishes the vitality in a previously nonvital and necrosed tooth.

**Keywords:** Blood clot, Mineral trioxide aggregate (MTA), Revascularization, Triple antibiotic paste.

**How to cite this article:** Neelamurthy PS, Kumar RA, Balakrishnan V, Venkatesan SM, Narayan GS, Karthikeyan I. Revascularization in Immature and Mature Teeth with Necrotic Pulp: A Clinical Study. J Contemp Dent Pract 2018;19(11):1394-1400.

**Source of support:** Nil

**Conflict of interest:** None

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## INTRODUCTION

Management of permanent anterior teeth with necrotic pulp with/without apical closure poses a challenge to endodontists. This is because of incomplete formation of the natural apical constriction causing thin dentinal walls against which obturation material is placed. Open apex can occur as a result of pulpal necrosis due to caries or trauma, before the completion of root development. It can also form in a mature root as a result of extensive apical resorption due to orthodontic treatment, inflammatory root resorption, and periradicular surgery.[1]

The traditional management of the open apex was to obturate with customized gutta-percha cone method without or with apical surgery and retrograde filling.

The potential drawback of the surgical approach is, it can damage the very thin dentinal wall of the open apex tooth. Unfavorable crown root ratio is another reason for concern. Both these causes, lead to complication after post-endodontic restoration (*viz.* crown and root fracture).[2]

For the past five decades, apexification has been the treatment of choice in teeth with open apex which was introduced by Kaiser in 1964. This technique basically induces the formation of the apical barrier at the apex by placement of calcium hydroxide. But the main drawback of apexification is the duration of the treatment period (multiple visits). The increased susceptibility of the root to fracture after calcium hydroxide exposure and varying degree of apical closure it exhibits, are other potential disadvantages.[1]

The evolution of MTA as an artificial apical barrier material has drastically changed the conventional means of managing an open apex tooth. There is no denying the fact that MTA induces a barrier in open apex cases. It does not induce the thickening of the radicular dentin. In this scenario, the role of contemporary techniques in the management of open apex is highly questionable.[1]

The emergence of regenerative endodontics as a contemporary therapeutic modality has entirely altered the conventional approach for management of open apex. The primary role of regenerative endodontics is to give life to a bioengineered tooth. It can drastically alter the compromised structural integrity of diseased tooth in a positive way.[1]

Revascularization is a regenerative endodontic procedure which was introduced by Ostby in 1961. Since the plasma from the blood clot is a rich source of growth factor, it is always possible that it can lead to differentiation, growth, and maturation of fibroblast, odontoblast, and cementoblast. Rule and Winter showed that by revascularization, root development and apical barrier formation could occur in the tooth with necrotized pulp. Literature survey clearly shows this treatment modality of revascularization can result in the radiographically visible normal matured tooth which had previously immature open apex.[3]

It is a well-established fact that the development of root takes place from Hertwig's epithelial root sheath. As the root develops the HERS disintegrates and disappears but not completely. The remnants of hertwig's epithelial root sheath (HERS) are normally found in the open apex of the developed root which is highly resistant to polymicrobial infection and pulpal necrosis. As the spread of infection in the pulp is per tissue compartment, there is always a possibility that the apical pulpal tissue can remain vital. It is now being argued, this vital apical pulpal tissue with the remnants of HERS can contribute to continued root development and lengthening, even after the establishment of necrosis.

The other two mechanisms which are being put forth for continued root development are the role played by stem cell of apical papilla (SCAP) and multipotent pulp stem cells which are seen abundance in immature tooth and which have the capacity to lay down bone and cementum.[4]

The infected root canal system is a sanctuary for a plethora of microorganisms both aerobic and anaerobic. Elimination of these microorganisms is the prime goal for modern endodontic therapy. because of the varying degree of sensitivity exhibited by the organism towards the different antibiotics and due to the ability of a microorganism to rapidly exhibit resistant to antibiotics, a combined antibiotic regimen has been advised. The recommended regimen includes metronidazole, ciprofloxacin and minocycline.[5]

An alternate antibiotic regimen includes clindamycin instead of minocycline due to the potential discoloring ability of minocycline.[5] Literature is replete with documented case report of revascularization of necrotic root canal by inducing bleeding into the canal by over instrumentation. The two main advantages of revascularization by induced bleeding are, firstly, it is less technique sensitive. Secondly, the possibility of immune rejection and pathogen transmission are very minimal since regeneration occurs by patient's own biological tissue.

## MATERIALS AND METHODS

### Source of the Patient

The study was approved by the Ethical Committee of Ragas Dental College and hospital and due clearance was obtained for carrying out the investigation. A total number of 15 cases were selected from those patients attending the outpatient department for root canal therapy. An informed consent was signed by all the patients participating in the study and the duration of the study period was 10 months.

### Inclusion Criteria

Subjects willing to participate in the study were selected with following inclusion criteria.

- Both males and females aged between 7 to 35 years were included.
- Patient with an immature permanent maxillary or a mandibular single-rooted tooth with necrotic pulp.
- Patient with open apices in the matured permanent maxillary or a mandibular single-rooted tooth with a diagnosis of pulp necrosis.
- Only immunocompetent subjects were included.
- Teeth only with restorable crowns.

## Exclusion Criteria

- Patient with non-restorable teeth
- Immuno-compromised patients, including patients who self-reported to be a carrier of HIV, Hepatitis, undergoing steroid therapy, those who self-reported with genetic or systemic diseases that could result in a reduced immune response,
- Root fractures (checked with pre-operative radiographs using horizontal and vertical angulations)

According to the above inclusion and exclusion criteria, 15 patients were selected for the study.

## Preparation of Triple Antibiotic Paste

Triple antibiotic paste used in this study consisted of the following antibiotics

Metronidazole 400 mg, ciprofloxacin 500 mg, clindamycin 150 mg equal amount of these antibiotics were dispensed in the ratio of 1:1:1 on the mixing pad and was mixe\d with sterile distilled water to form a creamy paste.

## Procedure

In the initial visit, teeth were anesthetized by 2% lignocaine with 1:80,000 adrenaline. After rubber dam isolation, endodontic access was performed and working length was determined radiographically by placing #15 size K-file into the canals at the evaluated preoperative working length. After rubber dam isolation, endodontic access was performed. The working length was measured radiographically by placing #15 size K-file into the canals at the evaluated preoperative working length. The canals were cleaned by passive irrigation system with 20 mL of 3% NaOCl and 10 mL of saline for 10 minutes. The canals were then dried with a paper point. The triple anti-microbial paste used in this study comprised of newly blended antibiotic powders of metronidazole, ciprofloxacin, and clindamycin in the proportion 1:1:1. This powder was additionally blended with sterile double distilled water to shape glue like consistency. The prepared anti-microbial paste was then placed into the canals using a sterile Lentulo spiral file till the level of the cementoenamel junction. The entrance was then cleaned with sterile cotton pellets, and the coronal seal was set with IRM. The intracanal medicament of triple anti-biotic paste was left in the canals for 2 weeks.

In the second visit, evaluation of the response to initial treatment was done. If there are signs and symptoms of continuous infection, additional treatment time with triple antibiotic paste dressing were considered.

If the tooth was asymptomatic, patients were anesthetized using 2% lignocaine without vasoconstrictor. After rubber dam isolation, the temporary restoration was removed and using sterile saline irrigation; the antibiotic paste was washed out. The canal was dried with paper points. The apical tissues beyond the confines of the root canal were stimulated with a sterile H-file (35–50) to induce bleeding into the canal space. A blood clot was produced below the level of the cement-enamel junction to provide a scaffold, followed by a double seal of MTA in the cervical area and a bonded resin coronal restoration over it.

Patients were reviewed for a subsequent period following 2, 4, 6, 8, and 10 months. In the initial three visits radiographic examination were completed, on the follow up of 8, 10 months, along with radiographic examination pulp sensibility examination was done using an electric pulp tester.

## RESULT AND DISCUSSION

Regeneration in endodontics aims to heal infected, nonvital, immature and open apex teeth by regeneration of pulp tissues and apexogenesis. Revascularization is the technique to re-establish the vitality in a previously nonvital and necrosed tooth.

It was earlier thought the periapical tissue of a nonvital infected tooth will not regenerate. Therefore, treatment option followed for such teeth was to perform the surgical endodontic procedure to seal the wide-open apex, with retrograde seal. It is an invasive procedure which has its drawbacks causing discomfort to the patient and common complications associated with any surgery. The crown root ratio is compromised in the already weakened tooth due to immature root development.

Kaiser in 1964 documented calcium hydroxide apexification in non-vital incisor tooth which was later popularized by Frank.[6] It has several disadvantages (a) first, it requires a period of 6 to 24 months for barrier formation; (b) the barrier thus formed is often porous and non-continuous and therefore requires obturation of the canal after without splitting the tooth;[2] (c) apexificaiton can only induce a hard tissue barrier at the apex and further development of the root does not take place; (d) calcium hydroxide, a physical barrier will block the migration of multipotent undifferentiated mesenchymal cells into the canal and thereby preventing regeneration of tissues at the lateral dentinal walls; (e) by repeated intracanal calcium hydroxide dressing tooth becomes brittle because of the hygroscopic and proteolytic properties of Ca $(OH)_2$ which can lead to fracture.

The emergence of MTA as an artificial apical barrier material has drastically altered the conventional means of management of open apex because of the several advantages it poses over calcium hydroxide-induced apexification. MTA is a biocompatible material, has osteoinductive properties, and can even set in the presence of moisture, and the entire treatment protocol can be completed in a single visit.[3]

The advantage for the success of treatment by MTA is less dependent on patient co-operation. $Ca(OH)_2$ apexification requires a motivated patient who has to return for multiple follow-up appointments,(the usual recall period for $Ca(OH)_2$ apexification is 3 months until the barrier form) whereas the MTA root-end barrier procedure can be completed in one or two appointments. Allowing an immediate restoration is another advantage of MTA. Ca $(OH)_2$ treatment requires that a patient be left in a provisional restoration for a considerable period which is the time required for $Ca(OH)_2$ paste to fulfill its prime function. This intermediate time period makes the tooth vulnerable to crown/root fracture. Andreasen et al. showed that Ca $(OH)_2$ intracanal dressing for more than 1 month significantly reduced the fracture resistance of the tooth. In spite of MTA having numerous advantages over $Ca(OH)_2$, it does not have the potential to induce thickening of root dentin, but it does help in increasing the root length which in turn doesn't improve the crown-root ratio.

In concern with the above aspects, revitalization procedure promises to overcome the negative aspect of Ca $(OH)_2$ and MTA method of apexification. The important step in revitalization procedure is to disinfect the canal using the right irrigation protocol along with local application of antibiotics. In the current study, a mixture of triple antibiotic paste was developed which consists of metronidazole, Ciprofloxacin, and clindamycin. Metronidazole is a nitro imidazole compound exhibiting a broad spectrum of activity against protozoa and anaerobic bacteria. Ciprofloxacin, a synthetic fluoroquinolone, is bactericidal in nature. Clindamycin is a lincosamide antibiotic. It inhibits bacterial protein synthesis.

The dental pulp is a soft tissue, developed from the dental papilla and composed of water, ground substance, connective tissue, blood vessels, nerves, lymphatics, fibroblasts, immune cells, and odontoblast. Odontoblasts secrete dentin which helps in root formation and this developed into apical papilla. The component of the apical region of immature teeth consists of dental pulp, apical papilla, and periodontal tissues. During the bell stage of tooth formation, dental papilla is surrounded by enamel organ and result in the formation of dental follicle.

The dental follicle is made up of progenitor cells for the developing periodontium, cementum, alveolar bone, and PDL. At the same time, HERS is formed by the fusion of inner and outer enamel epithelium. HERS plays an important role in root development and shape of the tooth.

In the current study, to evaluate the revascularization procedure both in immature and mature permanent teeth with necrotic pulp and open apices, disinfected with triple antibiotic paste followed by inducing blood clot in the root canal.

After the initial month follows-up, i n the second month of follow-up 15 cases showed no signs and symptoms with a reduction in the periapical radioluciences. In the fourth month of follow up, 2 cases failed to report. The remaining 10 out of 15 cases, were asymptomatic with a complete resolution of periapical radioluciences. 3 out of 15 cases were asymptomatic, and only minimal reduction in the periapical radiolucent was observed radiographically.

In the 6-month follow-up, complete resolution of periapical radioluciences were seen in all the 13 cases reported, and there was evidence of root lengthening and apical closure in 8 cases. The remaining 5 cases showed no evidence of root lengthening or apical closure (Tables 1 to 3).

In the 8-month follow up all the 13 cases reported, continued to show complete resolution of periapical radiolucency. Among the 13, 8 cases showed root lengthening and apical closure with evidence of lateral wall thickness in root dentin. Two cases showed satisfied levels of root lengthening and apical closure with no evidence of any lateral thickness. The remaining 3 cases showed no improvements compared with the previous follow-ups. Pulp sensibility test was done in this follow up using electric pulp tester. In this follow-up, there was no response to electric pulp testing.

In the 10-month follow-up, 10 cases showed shreds of evidence of root lengthening and apical closure. Out of which there were shreds of evidence of lateral wall thickness in radicular dentin only in 8 cases. Out of these 8 cases that showed evidence of lateral wall thickness, two cases responded to electric pulp testing showing pulp sensibility. The remaining 3 out of 13 cases that were reported continued to show no improvements.

The results of the present study indicated that it is possible to treat both immature permanent tooth and matured permanent open apex tooth which could render the patient asymptomatic without any evidence of sinus tract and resolving apical periodontitis. There is also radiographic evidence of continuing thickness of dentinal walls, apical closure, or further development of root length (Figs 1 to 3).

In the present study, 2 out of 13 demonstrated a return of pulp sensibility after complete revascularization treatment, which is per the study by Petersson et al. showing that teeth with necrotic pulps might give a positive response for the electrical test.

**Table 1:** Evaluation of pulp revascularization

| Case no. | Gender | Age | Follow-up months | Signs and symptoms | Radiographic examination: Resolution of periapical radiolucencies | Radiographic examination: Apical closure and root lengthening | Radiographic examination: Evidence of lateral dentin wall thickness | Electric pulp tester |
|---|---|---|---|---|---|---|---|---|
| 1 | Male | 15 | 10 | Absent | + | + | + | - |
| 2 | Male | 12 | 10 | Absent | + | + | + | - |
| 3 | Male | 12 | 10 | Absent | + | + | + | + |
| 4 | Female | 18 | 10 | Absent | + | + | + | - |
| 5 | Male | 35 | Not reported | Absent | NA | NA | NA | NA |
| 6 | Male | 23 | 10 | Absent | + | - | - | - |
| 7 | Female | 20 | 10 | Absent | + | + | + | + |
| 8 | Female | 19 | Not reported | Absent | NA | NA | NA | NA |
| 9 | Male | 16 | 10 | Absent | + | + | - | - |
| 10 | Male | 32 | 10 | Absent | + | - | - | - |
| 11 | Male | 23 | 10 | Absent | + | + | + | - |
| 12 | Male | 26 | 10 | Absent | + | + | + | - |
| 13 | Male | 29 | 10 | Absent | + | - | - | - |
| 14 | Female | 24 | 10 | Absent | + | + | - | - |
| 15 | Female | 24 | 10 | Absent | + | + | + | - |

**Table 2:** Inference till 8 months follow-up

| Features | No. of cases showing positive result: 2 months follow-up | 4 months follow-up | 6 months follow-up | 8 months follow-up |
|---|---|---|---|---|
| Resolution of periapical radiolucencies | 15 | 10 | 13 | 13 |
| Increase of root length | – | – | 8 | 8 |
| Apical closure | – | – | 8 | 8 |
| increased in width of root dentin | – | – | – | 8 |
| Root lengthening and apical closure | – | – | 8 | 8 |
| Response to pulp sensibility test using electric pulp tester | – | – | – | – |

**Table 3:** Inference after 10 months follow-up

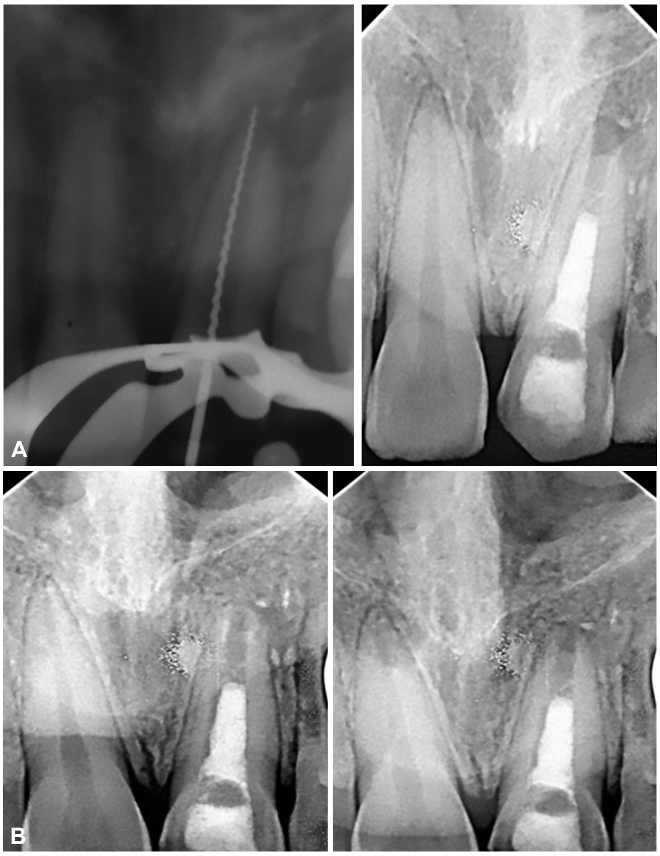
| Features | No of cases showing positive result (10 months follow-up) |
|---|---|
| Resolution of periapical radiolucencies | 13 |
| Increase of Root length | 10 |
| Apical closure | 10 |
| Increased in width of root dentin | 8 |
| Increase of Root length and apical closure | 10 |
| Response to pulp sensibility test using electric pulp tester | 2 |

The 3 cases showing an unfavorable outcome in the present study might be related to a failure to induce any bleeding into the canal. It was evident in an animal study that the absence of a blood clot formation has a negative impact on successful revascularization of the pulp and one possible reason for absence of blood clot might be due to the resolution of inflammatory reaction after antibiotic paste intracanal dressing, making it more difficult to induce bleeding.

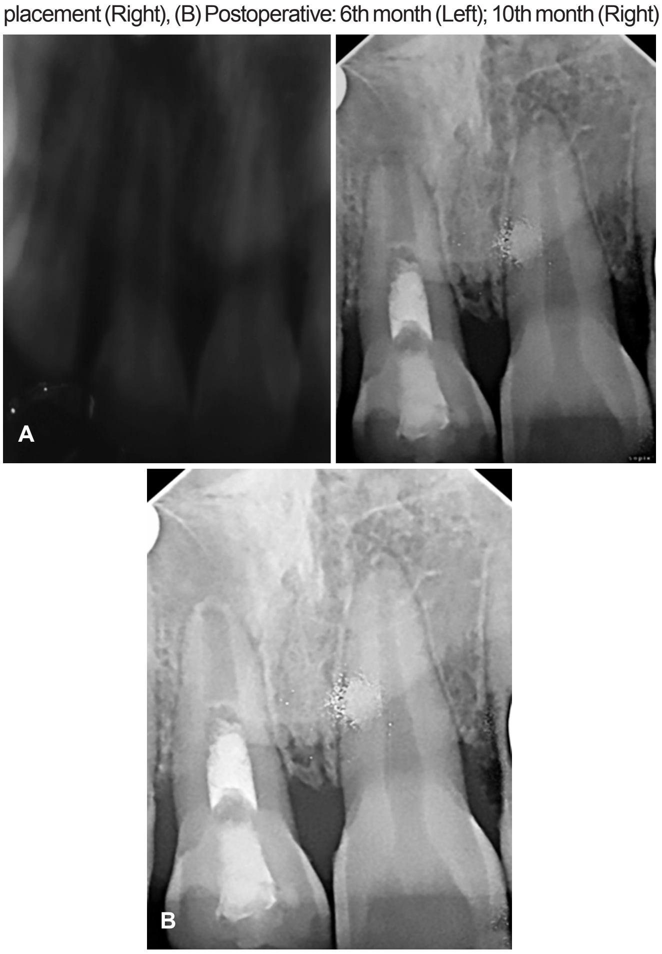
The possible explanation for the favorable treatment outcome of the present study may be beca use a few vital pulp cells would have remained at the apical end of the root canal. These cells must have proliferated into the newly formed matrix and differentiate into odontoblasts under the organizational influence of cells of HERS, which are highly resistant to disintegration, even in the presence of inflammation. The newly formed odontoblasts can lay down a tubular dentin at the apical end, causing apexogenesis.[7]

The second possible explanation of continued root development could be due to multipotent dental pulp stem cells, which can differentiate into odontoblasts and deposit tertiary or atubular dentin leading to elongation of root.[8]

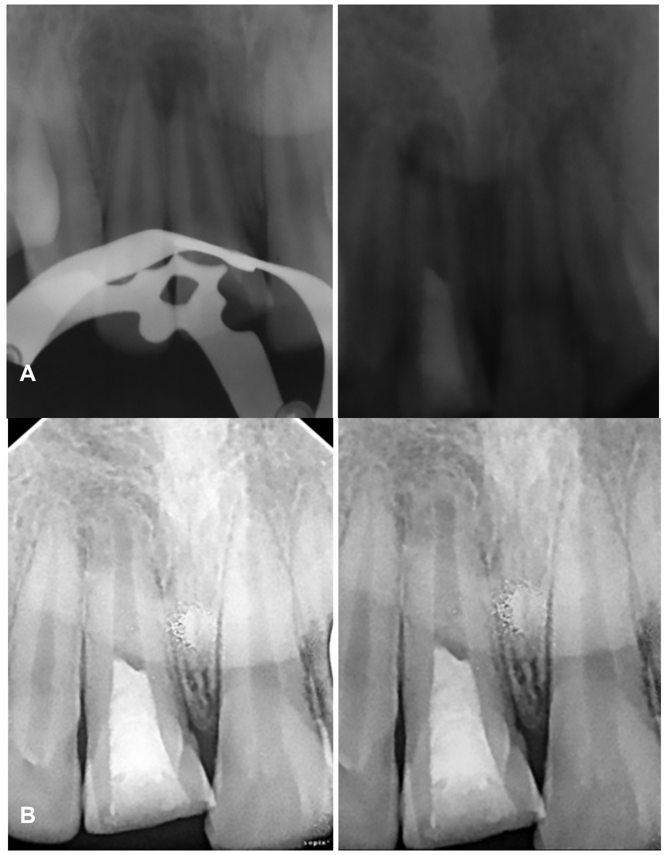
The third possible explanation could be attributed to the presence of stem cells in the periodontal ligament, which can deposit hard tissue at the apex and on the lateral root walls. The presence of cementum and




**Figs 1A and B:** (A) Preoperative working length (Left); after MTA placement (Right), (B) Postoperative: 6th month (Left); 10th month (Right)




**Figs 3A and B:** (A) Preoperative (Left); after MTA placement (Right); (B) Postoperative (10th month)




**Figs 2A and B:** (A) Operative (Left); After MTA Placement (Right), (B) Post Operative: 8th Month(Left), 10th Month (Right)

Sharpey's fibers in the newly formed tissues is being presented as a proof for this explanation.[9]

The fourth possible explanation is attributed to transplantation of stem cells from the apical papilla or the bone marrow to the canal lumen during instrumentation beyond the apex to induce bleeding.[10] The fifth possible explanation could be the blood clot itself, which is rich in growth factors. These growth factors include platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), platelet-derived epithelial growth factor (PDEF), and tissue growth factor (TGF) that have the capacity to stimulate differentiation, growth, and maturation of fibroblasts, odontoblasts, cementoblasts.[9]

However, this study needs further follow-up one year to demonstrate progress in pulp revascularization and to check the responses of the teeth to electric pulp sensibility testing.

## CONCLUSION

According to the result of the study, All the patients who reported till 10 months follow up showed complete resolution of periapical radiolucencies. Ten patients out of 13 patients, who reported till a period of 10 months follow up showed root development and apical closure.[8] Out of 13 patients who reported till a period 10 months follow up showed root development, apical closure and lateral thickening of radicular dentin. Two out of 13 patients at the end of 10-month follow-up showed positive response to electric sensibility test. All the patients are to be followed up further for 1 year to verify the pulp sensibility.

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