A 5 years’ follow-up of root anatomy-based maturogenesis achieved in infected immature molars using regenerative techniques – A case series

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Abstract

Infected immature molars are commonly encountered but seldom are they treated using principles of regenerative endodontics. The case series describes a feasible technique for attempting maturogenesis based on molar tooth anatomy. A total of 9 infected immature molars in the patients between 6 and 18 years of age were treated as part of this case series. All the canals were disinfected using 3% sodium hypochlorite and 17% ethylenediaminetetraacetic acid following minimal instrumentation. After using triple antibiotic paste for 3 weeks, bleeding was induced in mesial or constricted canals and platelet-rich fibrin was placed in distal or open wide canals till the orifice level. Coronal seal was obtained using mineral trioxide aggregate. Outcome was evaluated clinically and radiographically at the periods of 3, 6, 12, 24, 36, and 60 months. All the teeth showed continued root development and maintained functionality but none responded to vitality testing. Anatomical aspects of individual roots within a tooth can be utilized as a guide to decide the appropriate approach for attempting maturogenesis in a molar. Root changes can be expected even if the pulp vitality is not restored.

Keywords: Blood clot; maturogenesis; molars; platelet-rich fibrin; root closure

INTRODUCTION

Regenerative endodontics is a well-known treatment modality in modern day practice. Multiple treatment protocols have been suggested to accomplish continued root development.[1] There exists a high variability in protocols and each technique has its own limitation. This has led to a decision-making dilemma for treating infected immature teeth.

It is an established fact that in young children with large apical diameter of infected immature tooth, regenerative endodontic procedures (REP) perform better than apexification.[2] However, literature available on PubMed database on molars is scarce.[1,3-6] To the best of our knowledge, not many case reports on successful management of immature molars using REP have been reported on PubMed database.

We followed a new approach toward maturogenesis to treat immature infected or necrotic mandibular molars. Mesial and distal roots were treated differently owing to different anatomy of both the roots. As mesial canals are commonly constricted in the middle third which does not allow insertion of pluggers, bleeding was induced inside the canal after disinfection followed by minimal instrumentation. In the distal canals, as the canal orifice is large and canal is straight and larger in dimension, platelet-rich fibrin (PRF) was used as a scaffold and matrix. The technique was designated as “Anatomic approach to maturogenesis.” A total of 9 cases were treated using this technique after University ethical clearance (MPDC_011/CONS-5/13-F) and

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obtaining informed consent from each patient/guardian of the patient. The root formation stage was categorized as per Nolla classification and outcome of the treatment was assessed as per types of response suggested by Chen et al.\[7,8\]

**CASE REPORTS**

**Case 1**
A 9-year-old male patient reported to the department with the chief complaint of continuous pain in the lower left back tooth region for 1 week. Clinical examination revealed large disto-occlusal caries on tooth 36. Vitality tests (sensibility) revealed an exaggerated response with lingering pain. Intraoral periapical (IOPA) radiograph revealed blunderbuss canals for both mesial and the distal roots [Figure 1a]. Both the roots depicted Nolla stage 9 where the roots are almost complete but with an open apex. A diagnosis of irreversible pulpitis was formulated. The new devised technique of maturogenesis was deemed appropriate for the tooth after reading the radiograph and determining the anatomical constrains and procedural difficulties.

**First appointment**
After the administration of local anesthesia (2% Lignox with adrenaline, Indoco Remedies Ltd, Mumbai, India) and rubber dam application, access cavity was made and three canals were located. Mesial canal orifices were slit shape and the distal orifice was large oval shaped. In the mesial canals, #30 k-file (Mani Inc., Tochigi, Japan) was snugly fit in the middle third [Figure 1b]. Working length for the distal canal was determined using the bent file technique and confirmed radiographically [Figure 1b]. In the distal canal, #80 k-file was loose and going beyond the apex without any resistance. Circumferential filing was done in the distal root and the mesial root was minimally prepared till #30 k-file. 5 mL of 3% sodium hypochlorite solution (Parcan, Septodont Healthcare India Pvt. Ltd, Maharashtra, India) was used as an irrigant for each canal along with normal saline. Triple antibiotic paste (TAP) (metronidazole, ciprofloxacin, and minocycline) was made as paste slurry\[9\] and placed inside the canal using lentulospiral (Mani Inc., Tochigi, Japan) and k files.

**Second appointment**
After 3 weeks, the next phase was initiated in the absence of any signs and symptoms of infection. After administering local anesthesia (2% Lignox with adrenaline, Indoco Remedies Ltd, Mumbai, India), TAP was removed and irrigation was performed using 10 mL of 3% sodium hypochlorite (Dentwash, Prime Dental Products Pvt Ltd; Thane, Mumbai, India), followed by 10 mL of saline and a final flush of 10 mL of ethylenediaminetetraacetic acid (EDTA) (Dentwash, Prime, Bhiwandi, Maharashtra, India). At this time, 10 mL venous blood was drawn by venepuncture of the antecubital vein and centrifuged for 10 min at 3000 rpm as per guidelines to separate PRF from

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**Figure 1:** (a) Preoperative intraoral periapical; (b) Working length determination; (c) Bleeding induced in mesial canals; (d) Immediate postoperative; (e-i) Follow-up radiographs for 6-month, 1-year, 2-year, 3-year, and 5-year follow-up
acellular plasma and red blood cells. In the meantime, bleeding was induced in the mesial root canals using a #15 k file by taking it beyond the apex [Figure 1c]. No effort was made to control the blood below the cemento-enamel junction as mineral trioxide aggregate (MTA) (Prevest Dentpro, Jammu and Kashmir, India) had to be placed in the pulp chamber only. PRF was then separated from the blood corpuscles and squeezed between sterile gauze pieces to remove the trapped fluids. Following which, the PRF membrane was separated into small fragments using sterile scissors. A metal plugger (Manipal instruments, Parkala, India) with a rubber stopper set at 1 mm short of working length was used to pack the first increment inside the distal root. On reaching the desired length, a 0.5 mm diameter metal plugger was used in circumferential tapping manner to pack the PRF and a flat surface was achieved. Following which, the butt end of sterile No. 80 paper point (DiaDent, Chungcheongbuk-do, Korea) was used to compact the PRF further and to remove any remaining entrapped fluid. Next increment was placed in a similar way after shortening the length. Once the entire canal was filled till the orifice with the PRF, MTA was packed inside the pulp chamber to a thickness of 3 mm [Figure 1d]. Before temporizing, a moist cotton pellet was placed and the patient was recalled the next day for the placement of Glass Ionomer Cement (GC type 2, GC Corporation, Tokyo, Japan) seal and Composite restoration (3M Z350 XT, 3M ESPE Dental Products, MN, USA). Follow-up for the patient was taken at the periods of 6 months, 1 year, 2 years, 3 years, and 5 years [Figure 1e-i]. Follow-up radiograph at 2-year recall showed complete closure of the root end along with increased root length. The 5-year recall radiograph showed no signs of pathology and canals were patent and not obliterated. The patient was completely asymptomatic during all the follow-up visits. Pulp sensibility tests done using Endofrost ( Coltèn/ Whaledent Private Ltd, Raigad, Maharashtra) were negative for the tooth at all recall visits.

**Case 2**

A 16-year-old female patient complaining of pus discharge for 2 weeks was diagnosed with chronic periapical abscess for 47. Periapical Radiograph revealed nonblunderbuss cylindrical shaped open apex in the mesial root and blunderbuss apex in the distal root, curvature in the mesial root and periapical radiolucency around the roots [Figure 2a]. Both the roots represented Nolla stage 9. It was treated in a similar way as mentioned for the first case considering the anatomy of the tooth. Two years’ follow-up radiograph revealed complete closure of the root ends with increase in thickness of root dentin [Figure 2b and c]. The patient was completely asymptomatic with no signs of pathology at 3 years and 5 years recall periods [Figure 2d].

**Case 3**

A 14-year-old female patient complaining of pain since 2 weeks was diagnosed with chronic apical periodontitis for 47. IOPA revealed a nonblunderbuss cylindrical open apex with respect to the distal root [Figure 2e]. The Mesial root had a closed apex but both the roots were associated with periapical radiolucency. Similar treatment modality was opted. Follow-up radiographs revealed complete healing and closure of apex at 2-year follow-up [Figure 2f-h].

**Case 4**

A 7-year-old female patient complaining of pain for 1 week and diagnosed with chronic apical periodontitis for 36 was treated using the same technique. Clinical examination revealed disto-occlusal caries on tooth 36. IOPA revealed periapical rarefaction around both the mesial and the distal roots with cylindrical open apex [Figure 2i]. The roots represented Nolla stage 9. The distal root apex was wider as compared to the mesial root. It was treated in a similar manner. Follow-up radiographs revealed root end closure and increased distal root width [Figure 2j-l].

**Case 5**

An 8-year-old male patient complaining of pain for 3 days and diagnosed with chronic apical periodontitis for 36 was referred to the department. Clinical examination revealed large Class 2 Glass ionomer restoration on tooth 36. IOPA revealed large periapical radiolucency around the distal root and a small lesion around the mesial root. Both the roots presented with blunderbuss apex and represented Nolla stage 8 [Figure 2m]. Similar treatment modality was opted. Constriction in the middle third of the mesial root was evident. Follow-up radiographs revealed complete healing and closure of apex at 5-year follow-up [Figure 2n-p].

**Cases 6-9**

All the cases were treated in similar manner. Case number 6 [Figure 3a-d] and 7 [Figure 3e-h] could be followed up for 1 year and case number 8 [Figure 3i-l] and 9 [Figure 3m-p] were followed up for 5 years. Case number 8 was a retreatment case of maxillary molar and incomplete palatal root formation. Case number 9 involved inadvertent dislodgement of temporary restorative material into the periapical area of distal root. All the cases presented with satisfactory healing and fulfillment of treatment goals.

**DISCUSSION**

The primary goal of REP is elimination of clinical signs/symptoms and resolution of apical periodontitis. Increased thickening of the canal walls and/or continued root development are considered as secondary goals. Both the goals could be achieved for all the cases. As per Chen et al., the outcome of all the cases could be categorized as increased thickening of the walls and root maturation along (Type 3) with pulp canal obliteration (Type 4) in few. Lack of quantifying the root length and width changes could be considered as the limitation of the case series.
The term "maturogenesis" was proposed by Wigler et al. as continuation of root development in an immature tooth and hence it was used here. Inclusion criteria of immature teeth with apical periodontitis and age between 6 and 18 years were followed. The only deviation from the normal protocol was the inclusion of a retreatment case.

EDTA was used as it releases growth factors from human dentin, promotes the survival of stem cells of apical papilla (SCAP) and optimizes the environment of regeneration of tissue. Three percent sodium hypochlorite was used here as 6% sodium hypochlorite significantly reduced the survival of SCAP. TAP was used in thick paste form in the above cases as was advocated in some previous case reports, it is however recommended now to use diluted TAP in concentration of 1 mg/mL to avoid detrimental effect on stem cells.

All the above cases were treated with minimal instrumentation as failure of regenerative endodontic treatment could be caused by either lack of mechanical instrumentation or over-zealous instrumentation. PRF was used here as a scaffold material instead of platelet rich plasma (PRP) because it is easier to manipulate inside the canal, it is gel like consistency provides good resistance to back fill MTA, the process of making it is less redundant and it leads to slow release of growth factors which facilitates angiogenesis and cellular growth. A recent study also proved that PRF has huge potential to serve as a scaffold as compared to PRP and Blood clot. Synergistic activity of MTA and PRF in promoting the differentiation of Human Dentin Pulp Stem Cells into odontoblast like cells is also known. PRF served as a predictable matrix to simplify the procedure.

Blood clot was not utilized as a scaffold in the distal roots because inducing the bleeding and controlling its
level is difficult, its stability is questionable and packing MTA against it is a challenge. In the mesial canals on the other hand, since the curvature of canal and the anatomic constraints did not allow pluggers to be used till the working length, only bleeding was induced inside the canal using a #15 k file. There was no significant impact of the different techniques used for mesial and distal roots on the outcome as both the roots in all the cases showed successful healing and root closure except for one case.

SCAP and Hertwig’s Epithelial Root Sheath cells play a major role in regenerative endodontics and both can survive through chronic inflammation. Out of 9, 5 of the above cases had chronic lesions where healing was evident along with closure of root apex. It proves that chronic lesions are not an absolute contraindication for regenerative endodontics.

Even if the pulp vitality is not restored, root formation can occur. None of the cases treated above responded to vitality tests during the follow-up period but all showed clinical and radiographic success. A positive pulpal response only indicates a more organized vital pulp tissue. The completely restored pulp chambers can lead to negative response to vitality test. Obliteration/narrowing of the canals after regenerative endodontics is a common finding and so was observed in the above cases.

**CONCLUSION**

1. PRF can serve as a predictable matrix to pack MTA in wide canals
2. MTA does not slip to uncontrolled depth inside mesial canals because of anatomical constraints
3. Negative vitality tests do not warrant failure of maturogenesis for mandibular molars
4. Maturogenesis can be successfully carried out in chronic inflammation cases of immature infected molars.

5. Choice of treatment modality could be considered based on individual root anatomy while attempting regenerative endodontics.

Declarations of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Kontakisitis EG, Filipatos CG, Tzanetakis GN, Agrafioti A. Regenerative endodontic therapy: A data analysis of clinical protocols. J Endod 2015;41:146-54.

2. Estefan BS, El Batouty KM, Nagy MM, Diogeneas A. Influence of age and apical diameter on the success of endodontic regeneration procedures. J Endod 2016;42:1620-25.

3. Martin G, Ricucci D, Gibbs JL, Lin LM. Histological findings of revascularized/revitalized immature permanent molar with apical periodontitis using platelet-rich plasma. J Endod 2013;39:1384-44.

4. Ulusoy AT, Cehreli ZC. Regenerative endodontic treatment of necrotic primary molars with missing premolars: A case series. Pediatr Dent 2017;39:131-4.

5. Saoud TM, Martin G, Chen YH, Chen KL, Chen CA, Songtrakul K, et al. Treatment of mature permanent teeth with necrotic pulps and apical periodontitis using regenerative endodontic procedures: A case series. J Endod 2016;42:37-45.

6. Topcuoglu G, Topcuoglu HS. Regenerative endodontic therapy in a single visit using platelet-rich plasma and biodentine in necrotic and asymptomatic immature molar teeth: A report of 3 cases. J Endod 2016;42:1344-6.

7. Nolla CM. The development of the permanent teeth journal of dentistry for children. J Dent Child 1960;27:254-66.

8. Chen MY, Chen KL, Chen CA, Tayebaty F, Rosenberg PA, Lin LM. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. Int Endod J 2012;45:294-305.

9. Takushige T, Cruz EV, Asgar Moral A, Hoshino E. Endodontic treatment of primary teeth using a combination of antibacterial drugs. Int Endod J 2004;37:132-8.

10. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mounjyi J, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:136-44.

11. American Association of Endodontics, AAE Clinical Considerations for a Regenerative Procedure. Revised 4-1-2018. Available at: https://www.aae.org/specialty/wpcontent/uploads/sites/2/2018/06/ConsiderationsForRegEndo_AAEApril2018.pdf.

12. Wigler R, Kaufman AY, Lin S, Steinbock N, Hazan-Molina H, Torneck CD. Revascularization: A treatment for permanent teeth with necrotic pulp and incomplete root development. J Endod 2013;39:319-26.

13. Law AS. Considerations for regeneration procedures. J Endod 2013;39:544-56.

14. Diogeneas A, Ruparel NB. Regenerative endodontic procedures: Clinical outcomes. Dent Clin North Am 2017;61:111-25.

15. Trevino EG, Patwardhan AN, Henry MA, Perry G, Dybdal-Hargreaves N, Hargreaves KM, et al. Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. J Endod 2011;37:1109-15.

16. Chueh LH, Ho YC, Kuo TC, Lai WH, Chen YH, Chiang CP. Regenerative endodontic treatment for necrotic immature permanent teeth. J Endod 2009;35:160-4.

17. JadHAV GR, Shah N, Logani A. Comparative outcome of revascularization in bilateral, non-vital, immature maxillary anterior teeth supplemented with or without platelet-rich plasma: A case series. J Conserv Dent 2013;16:568-72.

18. Lin LM, Ricucci D, Huang GT. Regeneration of the dentine-pulp complex with revitalization/revascularization therapy: Challenges and hopes. Int Endod J 2014;47:713-24.

19. Ray HL Jr., Marcelino J, Braga R, Horwat R, Lisien M, Khalig S. Long-term follow up of revascularization using platelet-rich fibrin. Dent Traumatol 2016;32:80-4.

20. Adhikari HD, Gupta A. Report of a case of platelet-rich fibrin-mediated revascularization of immature 12 with histopathological evaluation. J Conserv Dent 2016;19:689-95.

21. Narang I, Mittal N, Mishra N. A comparative evaluation of the blood clot, platelet-rich plasma, and platelet-rich fibrin in regeneration of necrotic immature permanent teeth: A clinical study. Contemp Clin Dent 2015;6:63-8.

22. Woo SM, Kim WJ, Lim HS, Choi NK, Kim SH, Kim SM, et al. Combination of Mineral Trioxide Aggregate and Platelet-rich Fibrin Promotes the odontoblastic differentiation and mineralization of human dental pulp cells via BMP/Smad signalling pathway. J Endod 2016;42:82-8.

23. Petrino JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB. Challenges in regenerative endodontics: A case series. J Endod 2010;36:536-41.

24. Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B. Regenerative endodontics: A comprehensive review. Int Endod J 2018;51:1367-98.

25. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: The potential role in pulp/dentin regeneration and bioroot engineering. J Endod 2008;34:645-51.

26. Nosrat A, Li KL, Vir K, Hicks ML, Fouad AF. Is pulp regeneration necessary for root maturation? J Endod 2013;39:1291-5.

27. Nosrat A, Homayounfar N, Oloomi K. Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic immature teeth; A literature review and report of a case. J Endod 2012;38:1428-34.