European Archives of Paediatric Dentistry (2021) 22:527–534
https://doi.org/10.1007/s40368-020-00572-4

CASE SERIES STUDY

# Regenerative endodontics: a promising tool to promote periapical healing and root maturation of necrotic immature permanent molars with apical periodontitis using platelet-rich fibrin (PRF)

**M. Yoshpe**[1,2] · **A. Y. Kaufman**[3,4] · **S. Lin**[4,5] · **M. Ashkenazi**[6,7]

Received: 13 May 2020 / Accepted: 10 October 2020 / Published online: 27 October 2020
© European Academy of Paediatric Dentistry 2020

## Abstract

**Purpose** Currently, mineral trioxide aggregate (MTA) apexification is recommended as the preferred treatment for permanent anterior immature necrotic teeth. Apexification treatment does not enable further development and maturation of the teeth, resulting in short roots with thin root canal walls, that often are prone to cervical fractures. This study presents the regenerative endodontic procedure (REP) as an alternative treatment for immature necrotic molars with apical periodontitis or a chronic apical abscess. REP enables periapical healing as well as root lengthening and widening of the dentinal root canal walls.

**Case series results** Six immature first molars teeth (five mandibular, one maxillary) with apical periodontitis or chronic apical abscess were treated with REP. Patients underwent periodic follow-up visits every 3 months the first year and twice a year thereafter. The final clinical examination revealed no symptoms, no gingival pockets, and no sensitivity to percussion. Cold sensitivity tests were negative. Radiographs revealed full periapical healing in all the treated molars, remarkable root lengthening, and dentinal wall thickening.

**Conclusion** REP with PRF is feasible and may have some advantages over MTA apexification since it facilitates root elongation, dentinal thickening of the root canals walls, and narrowing of the apical foramen.

**Keywords** Immature · Molars · Necrosis · Open apex · PRF · Regenerative endodontics

## Introduction

The most frequent causes of endodontic treatment in immature permanent molars are deep occlusal caries and/or severe molar incisor hypomineralization (MIH) (Weerheijm 2003) in contrast to anterior teeth were traumatic injuries are the main reason for endodontic treatment. The endodontic treatment of molar teeth is very challenging because the roots of these teeth are short with thin walls and they have wide-open apices (Andreasen et al. 2002). Such teeth, that need endodontic treatment, were traditionally treated by apexification with long-term calcium hydroxide medication (Andreasen et al. 2002). Torabinejad and Chivian (1999) suggested the use of MTA for a single appointment apexification. Recently, the EAPD in its guidelines recommended replacing calcium hydroxide $(Ca(OH))_2$ by MTA for anterior teeth. The use of REP was recommended only for anterior teeth with a hopeless prognosis even with the application of MTA (Duggal et al. 2017). However, no REP treatment protocol was suggested as an option for molars due to caries or MIH.

✉ M. Yoshpe
Rita.yoshpe@gmail.com

1 Endodontic Department, Faculty of Dental Medicine, Hebrew University-Hadassah, Jerusalem, Israel

2 Smile Medical Services Ltd., ha Prahim 28, Ashdod, Israel

3 Department of Endodontology, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel

4 Department of Endodontics, School of Graduate Dentistry, Rambam Health Care Campus, Haifa, Israel

5 Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

6 Center of Oral and Dental Health, Sheba Medical Center, Tel Hashomer, Israel

7 Pediatric Dentistry, Multidisciplinary Dental Clinic, Petach-Tikva, Israel

The great advantage of MTA apexification over $Ca(OH)_2$ is that it shortens the duration of the treatment and enables completion of treatment in two sessions (Duggal et al. 2017). The REP is a relatively new therapeutic approach that enables further maturation of the roots. Iwaya et al. (2001) were the first to describe this procedure. In 2004, Banchs and Trope (2004) proposed a treatment protocol for the management of such teeth called "revascularization". This protocol was adopted by the American Association of Endodontics (AAE 2016), and by the European Society of Endodontology (Galler et al. 2016) with minor changes. The treatment was called "regenerative endodontics". The indication for this treatment includes teeth with necrotic pulp or with irreversible pulpitis. Moreover, in the case of pulpitis PRF maybe used for pulpotomy (Keswani et al. 2014). The decision on how to treat teeth depends very much on the cooperation ability of the child during the treatment. The most important part of this REP is the thorough disinfection of the root canals. Both protocols suggest irrigating the root canals with sodium hypochlorite and medicating them with $Ca(OH)_2$ or triple antibiotic paste comprising of metronidazole, ciprofloxacin, and minocycline. During the second appointment, after EDTA irrigation, apical bleeding is induced into the root canal to produce a blood clot to recruit stem cells and growth factors (GF). The blood clot serves as a scaffold for the stem cells and contains GF that facilitates further root development (AAE 2016; Galler et al. 2016). Duggal et al. (2017) emphasized that a blood clot is not the ideal scaffold and suggested to look for alternatives. Recently, the use of different scaffolds has increased. Platelet-rich plasma (PRP) has been suggested as an ideal biomaterial for improving treatment results because it does not act only as a scaffold, it contains stem cells and GF too (Bezgin et al. 2015; Dohan et al. 2006; Murray 2018; Sachdeva et al. 2015; Torabinejad and Turman 2011; Ulusoy et al. 2019; Zhu et al. 2012). Platelet-rich fibrin (PRF) is a second-generation scaffold of PRP developed by Choukroun (Dohan et al. 2006). The PRF compared to PRP releases the GF slowly over a 2-week period and thus increases its effectiveness. Its use in regenerative endodontics was first described by Keswani and Pandey (2013). The clinical advantages of using PRF over blood clots and PRP in REP were described in a meta-analysis comprising 222 cases from 12 studies conducted by Murray (2018). He concluded that in comparison to blood clots and PRP, PRF induced a higher rate of apical closure (100%) and root lengthening after 1 year.

Currently, REP is an accepted treatment for anterior teeth and there are many articles and random clinical studies describing its outcome (Arslan et al. 2019; Jeeruphan et al. 2012; Tong et al. 2017; Duggal et al. 2017; Nazzal et al. 2020). Only 25 clinical studies and a few case reports have been published addressing REP as a treatment option for immature molars (Nosrat et al. 2011; Roghanizadeh and Fazlyab 2018; Schmoeckel et al. 2017; Ulusoy and Cehreli 2017; Tzanetakis et al. 2020). The objective of this case study is to demonstrate the potential of REP using PRF as a scaffold to promote healing of periapical lesions, apical closure, and continuation of root development in permanent immature molars diagnosed with necrotic pulp and apical periodontitis.

## Case series description and results

Six healthy children, ages 7–11 years old, were referred to endodontics specialized clinic for endodontic treatment of their carious immature permanent molars. All the patients suffered from pulp necrosis associated with apical periodontitis (which was confirmed by a periapical radiograph). Three of these children received previous endodontic treatment in other dental offices. All the teeth were slightly sensitive to percussion but not to palpation and had normal periodontal pockets. One tooth was associated with a sinus tract. Radiographic examinations confirmed immature roots and open apices. Accordingly, regenerative endodontic treatment with PRF was offered, and written informed consent was received.

## Clinical procedure

All cases were treated by a single endodontist (M.Y.). Medical and dental history was obtained for all patients, followed by a thorough oral and dental examination. Mobility and cold sensitivity, electric pulp, probing and percussion tests were carried out. Two-dimensional diagnostic radiographs were taken, and a diagnosis was provided. Some teeth were initially treated by Ledermix (Haupt Pharma GmbH, Wolfratshausen, Germany) or Ca(OH)2 as emergency treatment. In cases where applicable, a treatment plan recommending REP was proposed to the guardians and they were asked to sign an informed consent. The distribution details of each treated tooth/patient are summarized in Table 1. During the first visit, all the patients received local anesthesia: inferior alveolar nerve block in the lower arch and infiltration in the upper arch. Each injection included 1 cartridge of 2% Lidocaine & 1:100,000 epinephrine (Safco, USA). The treatment was conducted under a surgical operating microscope (DOM; Labomed, Prima, USA) as described by Yoshpe et al. (2020). After tooth isolation and access opening, all cases were treated by means of REP according to the AAE protocol (Sachdeva et al. 2015). Working length was established, each tooth was irrigated with 20 ml of 1.5% hypochlorite (NaOCl), dried with paper points, and dressed with a creamy triple antibiotic paste (TAP),

**Table 1** Patients' detailed data

| Case # | Age | Gender | Tooth # | Diagnosis | Duration of intracanal medication (days) | Follow up (months) |
|---|---|---|---|---|---|---|
| 1 | 10 | F | 18 | NP; CAA | 14—$Ca(OH)_2$<br>25—TAP | 24 |
| 2 | 7.5 | M | 30 | PIT; SAP | 32—TAP | 30 |
| 3 | 10.5 | F | 195 | PIT; SAP | 14—"Ledermix"<br>14—TAP | 23 |
| 4 | 7.5 | F | 19 | PIT; SAP | 23—TAP | 24 |
| 5 | 7 | F | 30 | PIT; SAP | 23—TAP | 24 |
| 6 | 7 | F | 14 | PIT; SAP | 28—"Ledermix"<br>35—TAP | 16 |

*NP* necrotic pulp, *CAA* chronic apical abscess, *PIT* previously initiated therapy, *SAP* symptomatic apical periodontitis, *TAP* triple antibiotic paste

comprising a mixture of three equal parts (250 mg each) of metronidazole (Sanofi-aventis, France), ciprofloxacin (Dexcel, Israel) and cefuroxime (Zinnat,-GSK, UK). The antibiotics were dissolved in saline to obtain a creamy paste consistency and inserted into the root canal by Centrix AccuDose® 20ga Needle Tubes (Shelton, CT, USA) and an endodontic plugger to control the insertion depth. The teeth were then temporarily restored using interim restorative material (Dentsply/Caulk, York, PA). During the second visit, following ensuring that the patients were asymptomatic, PRF was prepared chairside by drawing 40 ml of blood by a qualified nurse using a butterfly needle (BC12; Zhejiang Kindly Medical Devices Co, Longwan, China). The blood sample was immediately centrifuged for 8 min at 1300 rpm (Dohan et al. 2006). Following centrifugation, the medium phase (buffy coat, about four ml in each tube) which contained PRF was extracted by forceps, cut into small pieces, and stored in sterile condition at room temperature until used. The patients were anesthetized by one cartridge of 3% Scandonest (Septodont, Ontario, Canada) without vasoconstrictor, to allow blood flow into the tooth. The temporary restorative material was removed under rubber dam isolation. The root canal was irrigated with 20 ml of 17% EDTA and dried with paper points (Murray 2018). A file (#20–25) bended at its tip was introduced one mm beyond the apical foramen to induce bleeding from the periapical area into the pulp. The prepared PRF, as mentioned above, was inserted into the root canal orifice and plugged into the premeasured length of the root canal using endodontic pluggers up to 1 mm below the root canal orifice. A resorbable collagen plug (Collagen Matrix, New Jersey, USA) was inserted above the PRF and covered with MTA (Angelus, Londrina, Brazil). MTA was placed in half of the pulp chamber. The coronal part was sealed with glass Ionomer (Fuji IX-GC, Japan) and composite (3 M). Follow up appointments were scheduled every 3 months for the first year. During all the follow-up examinations the sensitivity test results were negative.

## Patients' description (Table 1)

### Individual details of case series

#### Case No. 1

A 10-year-old girl was referred to the clinic for endodontic treatment on her permanent lower left first molar. Clinical examination revealed a deep carious lesion associated with buccal vestibular swelling. The periodontal pockets were less than three mm. The tooth was sensitive to percussion. Radiographic examination revealed extensive radiolucency and open apexes of both mesial and distal roots. The




**Fig. 1** Permanent lower left second molar. **a** Diagnostic X ray. **b** Radiograph taken upon completing treatment. **c** Three-month follow-up. **d** 24-month follow up radiograph

diagnostic X-ray demonstrated a tracing of the sinus tract (Fig. 1a). Accordingly, the diagnosis of pulp necrosis and the chronic apical abscess was confirmed. Following explanations and receiving written informed consent, the tooth was treated by REP, which was performed as described above (Fig. 1b). Following 3 months, the tooth was asymptomatic to percussion and to palpation and radiograph examination revealed impressive healing and diminishing of the radiolucency around the roots. Moreover, the apical foramen of the mesial root began to close (Fig. 1c). No changes were noted regarding the length of the roots. During the 24-month follow-up examination, the tooth was still asymptomatic with no sensitivity to percussion or palpation. The X-ray radiograph demonstrated complete healing, apical closure, and thickening of the dentinal walls of the mesial root. The distal root demonstrated an increase in its length and dentinal root canal wall width. A normal PDL surrounding the roots (Fig. 1d).

### Case no. 2

A seven and a half-year-old boy previously underwent pulp extirpation of his permanent lower right first molar to resolve acute pain. The child was referred to our clinic for the completion of the endodontic treatment. Clinical examination revealed slight sensitivity to percussion, but no sensitivity to palpation, with probing depths less than three mm and the soft tissue was normal with no pathological signs. Radiographic examination revealed immature roots associated with 2 × 4 mm radiolucency. In addition, external root resorption at the distal aspect of the mesial root was also observed (Fig. 2a). Previously initiated endodontic therapy with symptomatic apical periodontitis was diagnosed. Clinical procedures for REP were performed as previously described (Fig. 2b). During the 30-month follow-up, the tooth was asymptomatic. Radiographic examination revealed slight root elongation, closure of the apices, and intact PDL surrounding the roots (Fig. 2c). Interestingly, the outline of the apex of the mesial root recovered and demonstrated a normal shape.

### Case no. 3

A ten and a half-year-old girl was referred to the clinic due to pain in her permanent lower left first molar. Clinical tests revealed a negative response to cold test and sensitivity to percussion and palpation. Probing depths were less than three mm. Radiographic examination revealed an open apex in both the mesial and distal roots and apical radiolucency around the distal root (Fig. 3a). A diagnosis of necrotic pulp with asymptomatic apical periodontitis was determined. Following written informed consent, REP




**Fig. 2** Permanent lower right first molar. **a** Diagnostic X ray. **b** Radiograph taken upon completing treatment. **c** 30-month follow-up radiograph




**Fig. 3** Permanent lower left first molar. **a** Diagnostic X ray. **b** Radiograph taken upon completing treatment. **c** 23-month follow-up radiograph

was performed as described above (Fig. 3b). During the 23-month follow-up examination, the tooth was asymptomatic with no sensitivity to percussion or palpation. Radiographic examination revealed no radiolucency, and the apexes of both roots were closed with a thicker dentin walls.

### Case no. 4

A seven and a half-year-old girl was referred to our clinic following an unsuccessful partial pulpotomy in her permanent lower left first molar. Clinical examination revealed normal soft tissues. The tooth was a sensitive mesial root and 4 × 6 mm around its distal root (Fig. 4a). Previously initiated endodontic therapy with symptomatic apical periodontitis was diagnosed. The following explanation and written informed consent, the tooth was treated by REP (Fig. 4b). During the 4-month follow-up examination, the tooth was asymptomatic. Moreover, a remarkable resolution of the periapical lesion occurred. The mesial radiolucency disappeared completely, while the size of the distal radiolucency also diminished (Fig. 4c). During the 24-month follow-up examination, the tooth continued to be asymptomatic. Radiographic examination showed complete periapical healing, closed apices, and narrowing of the root canal associated with diffuse obliteration of the distal root pulp (Fig. 4d).




**Fig. 4** Permanent lower left first molar. **a** Diagnostic X ray. **b** Radiograph taken upon completing treatment. **c** Four-month follow-up radiograph. **d** 24-month follow-up radiograph

### Case no. 5

A 7-year-old girl was referred to the clinic after pulp extirpation due to severe pain of the permanent lower right first molar. Clinical examination revealed pocket depths less than 3 mm, with normal soft tissue. Slight tenderness to percussion was observed. Radiographic examination revealed immature short roots with open apices. The root canal walls were very thin, especially in the distal root. Periapical radiolucency measuring 7 × 12 mm surrounding the distal root and 3 × 3 mm radiolucency around the mesial root were noted (Fig. 5a). The tooth was diagnosed as previously initiated treatment with symptomatic apical periodontitis. REP was performed as described in the Clinical Procedure section (Fig. 5b). Throughout all the follow-up examinations the tooth was asymptomatic. The radiograph taken after the 24-month follow-up, revealed improved healing, closure of the apices, further root elongation, intact PDL surrounding the roots and thickening of the dentinal walls, especially of the distal root (Fig. 5c).

### Case no. 6

A 7-year-old girl was referred to the clinic after pulp extirpation of the permanent upper right first molar due to severe pain. Soft tissue was normal, probing was at normal depth, and there was no sensitivity to palpation, but the tooth was sensitive to percussion. Radiographic examination revealed short roots with open apices, especially in the palatal root. Small periapical radiolucency was noted around the mesiobuccal root (Fig. 6a). Previously initiated therapy with symptomatic apical periodontitis was diagnosed. Clinical procedures for REP were performed as described before (Fig. 6b). Because the patient did not adhere to the treatment

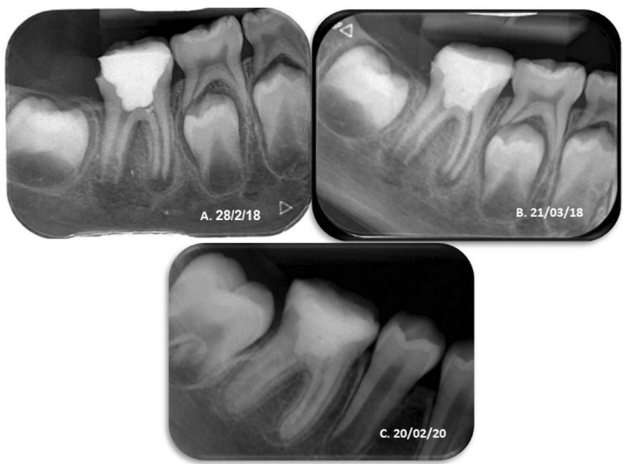



**Fig. 5** Permanent lower right first molar. **a** Diagnostic X ray. **b** Radiograph taken upon completing treatment. **c** Radiograph taken 24 months following the regenerative endodontics




**Fig. 6** Permanent upper right first molar. **a** Diagnostic X ray. **b** Radiograph taken upon completing treatment. **c** 16-month follow-up radiograph

schedule, the intracanal medicament remained in the root canal for 2 months: 1-month Ledermix and 1-month TAP. During the 16-month follow-up examination, the tooth was asymptomatic. Radiographic examination revealed intact PDL surrounding the roots, thickening of dentinal walls of both buccal roots, and apical closure but with no further root elongation (Fig. 6c).

The overall outcome of all cases regarding periapical healing was complete healing and apical closure. Radiographic findings of all cases indicated dentinal wall thickening. Most of the cases showed some root elongation and in some cases, root canal obliteration has occurred. No discoloration was observed in all the treated teeth.

## Discussion

This series of cases demonstrates the successful outcome of REP treatment using PRF in six immature necrotic permanent molar teeth with or without evidence of periapical radiolucency. Clinical and radiographic examinations revealed further root development, apical closure, and complete healing of the periapical lesions, which is a pivotal objective of any endodontic treatment (Murray 2018).

These results correspond with many other randomized control studies and meta-analysis, which show high rates of complete periapical healing following REP in incisors and premolars (Arslan et al. 2019; Jeeruphan et al. 2012; Murray 2018; Ulusoy et al. 2019).

We used TAP according to the AAE and ESE recommendations. Arruda et al. (2018) proved that medication with triple antibiotic solution significantly improved root canal disinfection, and its effects were at least comparable with the calcium hydroxide/chlorhexidine paste.

Ulusoy et al. (2019) compared the effectiveness of blood clots, PRP, PRF, and plasma platelets (PP), as a scaffold in 88 necrotic immature incisors. They showed similarly high success rates in periapical healing and radiographic root development of these teeth, where 73.9% showed complete apical closure and a greater tendency for conical-shaped apical closure, and 86% showed a positive response to sensitivity tests. In another recent randomized clinical study, Arslan et al. (2019) compared the outcome of REP with conservative root canal treatment in mature teeth (CRCT). Absence or reduction of the radiolucency was observed in 92.3% of the REP group versus 80% in the CRCT group, with no statistically significant differences. In all our cases, we observed apical healing and root elongation. In most of our cases, sensitivity and electric pulp test results were negative. Bose et al. (2009) used a geometrical imaging program to more accurately measure the differences between the preoperative and recall images of the root length in REP treated teeth. They evaluated the outcomes of REPs published cases and compared them to 40 control teeth treated either by apexification (20 teeth) or by conventional root canal treatment (20 teeth). They concluded that REP with triple antibiotic paste or $Ca(OH)_2$ produced significantly greater root length than either MTA apexification or nonsurgical root canal treatment. Tong et al. (2017) performed a systematic review and meta-analysis of the published data on REP. They reported excellent tooth survival rates and resolution of the periapical lesion, although the results for apical closure and continued root development were inconsistent. In 2017 the European Academy of Paediatric Dentistry published guidelines regarding the management of non-vital traumatized immature permanent anterior teeth in children and adolescents (Duggal et al. 2017). They wrote that as the evidence related to the use of a regenerative endodontic technique is currently extremely weak, REP should only be used in very limited situations where the prognosis with other techniques is deemed to be extremely poor (Duggal et al. 2017). Since then, many randomized control studies, meta-analyses, case reports, and case studies have been published (Arslan et al. 2019; Cymerman and Nosrat 2020; Lin et al. 2017; Murray 2018; Tong et al. 2017; Ulusoy et al. 2019; Yoshpe et al. 2020; Nazzal et al. 2020) in addition to previous research studies (Bose et al. 2009; Jeeruphan et al. 2012). These publications have added ample evidence that can serve as a basic proof of the potential of REP to induce healing of periapical lesions, thickening of the dentinal root walls, increasing the root-length and the induction of apical closure, consequently justifying the use of REP in immature teeth with necrotic

pulp and apical periodontitis (Arslan et al. 2019; Bezgin et al. 2015; Bose et al. 2009; Iwaya et al. 2001; Jeeruphan et al. 2012; Lin et al. 2017; Murray 2018; Tong et al. 2017; Torabinejad and Turman 2011; Ulusoy et al. 2019; Ulusoy and Cehreli 2017). In the healing achieved by any type of apexification or conventional endodontic treatment, studies comparing REP and MTA apexification have concluded that the healing achieved with REP is superior or equal to that of MTA apexification (Cymerman and Nosrat 2020; Jeeruphan et al. 2012; Lin et al. 2017). It should be noted that except for the shortening of the treatment duration, MTA has the same shortcomings as those of $Ca(OH)_2$, namely inhibiting further root development (Duggal et al. 2017). Consequently, the long-term prognosis of apexification-treated teeth is questionable, and they may still be susceptible to cervical root fractures. The disadvantage of discoloration that may occur after REP, although we replaced minocycline, is not relevant in molar teeth.

Moreover, currently, the recommended protocol for REP includes only two appointments which are similar to that of apexification by MTA, or by conventional root canal treatment. It should be noted that most of the studies cited evaluated the effectiveness of REP on necrotic permanent incisors or premolars and very few addressed immature permanent molars. The first permanent molars are the most caries-prone teeth in the permanent dentition in young children (Schmoeckel et al. 2017). The first permanent molars have 3–4 times higher probability to develop occlusal caries during the first years following their eruption in the oral cavity (Weerheijm 2003). This is since the erupting molars easily accumulate plaque and are not in contact with the opposing teeth. Moreover, young children usually do not reach these teeth when brushing, because they are under the occlusal plane. In addition, many children over 6 years of age, still use lower concentrations of fluoride in their toothpaste either because of taste preferences or lack of awareness. Another prevalent etiology for the development of deep caries lesion in immature permanent first molars is a disturbance to enamel development such as molar incisor hypomineralization (MIH) (Weerheijm 2003). This is one of the most reported etiological factors that may lead to tooth extraction in young children. The reported prevalence of MIH varies from 2.4 to 40.2% in different countries (Jalevik 2010). Currently, endodontic treatment in severe cases of MIH is not recommended and extraction of the teeth followed by spontaneous or orthodontic alignment of the adjacent teeth is recommended (Elhennawy et al. 2017; Teo et al. 2013). Nevertheless, tooth extraction is still not recommended in patients with a small mandible and with Cl II malocclusion or with a small maxilla and with Cl III malocclusion (Williams and Gowans 2003). Moreover, tooth extraction may not be recommended in children with partial anodontia, or in children with an inability to orthodontically close the space of the extracted tooth, such as in cases of well-developed second molars or a small gonial angle. There is ample evidence that REP for treating molar teeth is a predictable and successful treatment (Tzanetakis et al. 2020). It is recommended that pediatric dentists will consider REP before extracting molars in children.

Although the use of the PRF scaffold has certain advantages over blood clot scaffolds, it has some disadvantages as well. The procedure requires a qualified person to draw the blood from the young child's vein. Some training and experience are needed to prepare and insert the PRF pieces into the root canal. In cases of treating an uncooperative child, usage of $N_2O$, sedation, and collaboration with a pediatric dentist should be considered. From our experience, the ideal timing for blood drawing is after the induction of $N_2O$ and not at the beginning of the treatment.

## Conclusion

REP with PRF is feasible and may have some advantages over MTA apexification since it facilitates root elongation, dentinal thickening of the root canals walls, and narrowing of the apical foramen. Currently, studding REP is a crucial prerequisite for the approval of endodontic graduate programs in the USA and Canada.

Consequently, it is recommended that the relevant future EAPD guidelines and pediatric graduate programs will include REP as a legitimate treatment for necrotic posterior immature teeth.

## References

American Association of Endodontists AAE Clinical Considerations for a Regenerative Procedure. https://www.aae.org/uploadedfiles/publications_and_research/research/currentregenerativeendodonticconsiderations.pdf. Accessed 25 Feb 2016.

Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase the risk of root fracture. Dent Traumatol. 2002;18:134–7.

Arruda MEF, Neves MAS, Diogenes A, Mdala I, Guilherme BPS, Siqueira JF Jr, Rôças IN. Infection control in teeth with apical periodontitis using a triple antibiotic solution or calcium hydroxide with chlorhexidine: a randomized clinical trial. J Endod. 2018;44:1474–9.

Arslan H, Ahmed HMA, Sahin Y, DoganayYildiz E, Gundogdu EC, Guven Y, et al. Regenerative endodontic procedures in necrotic mature teeth with periapicalradiolucencies: a preliminary randomized clinical study. J Endod United States. 2019;45:863–72.

Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? J Endod. 2004;30:196–200.

Bezgin T, Yilmaz AD, Celik BN, Kolsuz ME, Sonmez H. Efficacy of platelet-rich plasma as a scaffold in regenerative endodontic treatment. J Endod. 2015;41:36–44.

Bose R, Nummikoski P, Hargreaves K. A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. J Endod United States. 2009;35:1343–9.

Cymerman JJ, Nosrat A. Regenerative endodontic treatment as a biologically based approach for nonsurgical retreatment of immature teeth. J Endod United States. 2020;46:44–50.

Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi 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 RadiolEndod United States. 2006;101:e37-44.

Duggal M, Tong HJ, Al-Ansary M, Twati W, Day PF, Nazzal H. Interventions for the endodontic management of non-vital traumatised immature permanent anterior teeth in children and adolescents: a systematic review of the evidence and guidelines of the European Academy of Paediatric Dentistry. Eur Arch Paediatr Dent. 2017;18:139–51.

Elhennawy K, Jost-Brinkmann P-G, Manton DJ, Paris S, Schwendicke F. Managing molars with severe molar-incisor hypomineralization: a cost-effectiveness analysis within German healthcare. J Dent England. 2017;65–71.

Galler KM, Krastl G, Simon S, Van Gorp G, Meschi N, Vahedi B, et al. European Society of Endodontology position statement: revitalization procedures. IntEndod J. 2016;49:17–23.

Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol. 2001;17:185–7.

Jalevik B. Prevalence and diagnosis of molar-incisor-hypomineralisation (MIH): a systematic review. Eur Arch Paediatr Dent England. 2010;11:59–64.

Jeeruphan T, Jantarat J, Yanpiset K, Suwannapan L, Khewsawai P, Hargreaves KM. Mahidol study 1: comparison of radiographic and survival outcomes of immature teeth treated with either regenerative endodontic or apexification methods: a retrospective study. J Endod United States. 2012;38:1330–6.

Keswani D, Pandey RK. Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin: a case report. IntEndod J England. 2013;46:1096–104.

Keswani D, Pandey RK, Ansari A, Gupta S. Comparative evaluation of platelet-rich fibrin and mineral trioxide aggregate as pulpotomy agents in permanent teeth with incomplete root development: a randomized controlled trial. J Endod. 2014;40:599–605.

Lin J, Zeng Q, Wei X, Zhao W, Cui M, Gu J, et al. Regenerative endodontics versus apexification in immature permanent teeth with apical periodontitis: a prospective randomized controlled study. J Endod United States. 2017;43:1821–7.

Murray PE. Platelet-rich plasma and platelet-rich fibrin can induce apical closure more frequently than blood-clot revascularization for the regeneration of immature permanent teeth: a meta-analysis of clinical efficacy. Front BioengBiotechnol [Internet]. 2018. https://doi.org/10.3389/fbioe.2018.00139.

Nazzal H, Ainscough S, Kang J, Duggal MS. Revitalisation endodontic treatment of traumatised immature teeth: a prospective long-term clinical study. Eur Arch Paediatr Dent. 2020;21:587–96.

Nosrat A, Seifi A, Asgary S. Regenerative endodontic treatment (revascularization) for necrotic immature permanent molars: a review and report of two cases with a new biomaterial. J Endod. 2011;3:562–7.

Roghanizadeh L, Fazlyab M. Revascularization and apical plug in an immature molar. Iran Endod J Winter. 2018;13(1):139–42.

Sachdeva GS, Sachdeva LT, Goel M, Bala S. Regenerative endodontic treatment of an immature tooth with a necrotic pulp and apical periodontitis using platelet-rich plasma (PRP) and mineral trioxide aggregate (MTA): a case report. IntEndod J England. 2015;48:902–10.

Schmoeckel J, Mourad MS, Splieth CH, Santamaria RM. Management of an immature, partially necrotic permanent molar by pulp revascularization: 2-year follow-up. Quintessence Int Germany. 2017;48:309–13.

Teo TKY, Ashley PF, Parekh S, Noar J. The evaluation of spontaneous space closure after the extraction of first permanent molars. Eur Arch Paediatr Dent England. 2013;14:207–12.

Tong HJ, Rajan S, Bhujel N, Kang J, Duggal M, Nazzal H. Regenerative endodontic therapy in the management of nonvital immature permanent teeth: a systematic review-outcome evaluation and meta-analysis. J Endod United States. 2017;43:1453–64.

Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. J Endod. 1999;25:197–205.

Torabinejad M, Turman M. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: a case report. J Endod. 2011;37:265–8.

Tzanetakis GN, Giannakoulas DG, Papanakou S, Gizani S, Lygidakis N. Regenerative endodontic therapy of immature permanent molars with pulp necrosis: a cases series and a literature review. Eur Arch Paediatr Dent England. 2020. https://doi.org/10.1007/s40368-020-00550-w.

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

Ulusoy AT, Turedi I, Cimen M, Cehreli ZC. Evaluation of blood clot, platelet-rich plasma, platelet-rich fibrin, and platelet pellet as scaffolds in regenerative endodontic treatment: a prospective randomized trial. J Endod United States. 2019;45:560–6.

Weerheijm KL. Molar incisor hypomineralisation (MIH). Eur J Paediatr Dent. 2003;4:114–20.

Williams JK, Gowans AJ. Hypomineralised first permanent molars and the orthodontist. Eur J Paediatr Dent Italy. 2003;4:129–32.

Yoshpe M, Einy S, Ruparel N, Lin S, Kaufman AY. Regenerative endodontics: a potential solution for external root resorption (case series). J Endod United States. 2020;46:192–9.

Zhu X, Zhang C, Huang GTJ, Cheung GSP, Dissanayaka WL, Zhu W. Transplantation of dental pulp stem cells and platelet-rich plasma for pulp regeneration. J Endod. 2012;38:1604–9.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.