Revitalizing previously treated teeth with open apices: a case report and a literature review

Abstract

Revitalizing the root canals of previously treated teeth with open apices is appealing to clinicians and patients. However, there are fundamental differences in the microbiome and the microenvironment between a canal with a primary endodontic infection and a canal with a persistent endodontic infection. The aims of this report are to report a case where a previously treated tooth with an open apex and a large apical radiolucency was treated successfully using regenerative endodontic treatment (RET), and to review and critically appraise the literature on procedures and outcomes of RET that results in revitalization of canal(s) in previously treated teeth with open apices. A maxillary central incisor with poor quality root filling, a large apical radiolucency and an open apex was retreated using RET using platelet-rich fibrin as the scaffold. After 24 months, there was complete healing of the periapical lesion and obvious radiographic signs of apical root closure. Electronic searches were performed in MEDLINE, Scopus and Embase and the baseline, procedural and outcome data of qualified articles were collected. An assessment tool was developed to rate the quality of evidence reported in these case report/series. Nine articles, three case series and six case reports, with a total of 17 teeth of all types were included in the reports identified. The age of patients ranged from 7-48 years (mean: 19.4 years). The recall period ranged from 12-72 months (mean: 29 months). All 17 teeth survived and were functional with healing/healed outcomes. “Apical closure” was the most common radiographic finding regarding root development. The quality of evidence using the new assessment tool was rated “Excellent” in three case reports but only “Fair” in the other six articles. The present case report as well as the review of the literature suggest that revitalizing the root canal system of teeth with open apices and post-treatment disease using RET is a potentially valid treatment

option. However, more clinical studies with higher levels of evidence and higher quality of evidence are required to confirm the viability of this treatment approach.

Introduction

Revitalizing the root canal system of immature teeth with pulp necrosis using regenerative endodontic treatments (RET) is a biologically-based approach to address endodontic disease (Hargreaves *et al.* 2013). As with all root canal treatments, the main goal of RET is to control canal infection in order to induce healing of periapical disease (Torabinejad *et al.* 2017). In addition to healing of periapical lesions, RET stimulates the development of immature roots (Chrepa *et al.* 2020), where the increase in the volume of mineralized tissue in the root structure reported in histological studies (Becerra *et al.* 2014; Nosrat *et al.* 2015) has the potential to improve the structural integrity of the tooth. However, there is no direct evidence on how or whether the root development that occurs as a consequence of RET results in greater fracture resistance (load capacity) in immature human teeth or any tooth with an open apex. It has been reported in an animal study that immature teeth treated with RET had greater 'fracture resistance' compared to untreated immature teeth (Zhou *et al.* 2017). To support that finding, Finite Element Analysis on an immature maxillary central incisor revealed that apposition of simulated hard tissue (dentine or cementum) on the root canal walls reduced mechanical stress in the root following biting or trauma (Bucchi *et al*. 2019). If these findings are applicable to human teeth, RET could potentially lead to longer tooth survival as compared to other treatment options where no root development is induced, such as apical plugs with hydraulic cements (Pace *et al.* 2007, 2014). Indeed, RET of immature teeth with pulp necrosis has been reported to have comparable outcomes to MTA apical plugs in terms of resolution of clinical symptoms, healing of periapical lesions and survival rates (Torabinejad *et al*. 2017).

The main focus of research in the field of RET has been on immature teeth with pulp necrosis (Tong *et al.* 2017). Although the biological basis of RET in previously treated teeth might have similarities to teeth with pulp necrosis, there are fundamental differences between these two scenarios. Performing RET in previously treated teeth with open apices and infected root canals is more challenging as the microenvironment of the root canal space is different from that without previous treatment. The effects of previous procedures on dentinal walls, the impact of intra-canal debris, and the presence of different bacterial flora (Gomes *et al.* 2004, Siqueira & Rôças 2009; Siqueira *et al*. 2007) may all have an impact on the outcome of RET in previously treated teeth. The possibility of root canal retreatment using RET was first introduced by Nevins & Cymerman (2015) and there are now several reports describing successful canal revitalization of previously treated teeth.

The aims of this report are to:

1) Report the successful retreatment of a previously treated maxillary central incisor with a large apical radiolucent lesion and an open apex using RET;
2) Review and critically appraise the literature on the procedures and outcomes of RET in previously treated teeth with open apices; and assess the level and quality of evidence in reports.

Case report

An 18-year-old non-Caucasian white female was referred to the Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences for assessment and treatment of tooth 21. The patient complained of "frequent pus discharge from the gum next to the front tooth." The patient's medical history was non-contributory, and they were not taking any

medications at the initial visit. The patient reported a history of trauma during childhood and episodes of pain and swelling in the anterior maxilla three years ago, for which they had undergone incision and drainage, and root canal treatment by a general dentist.

Clinical examinations revealed a sinus tract adjacent to tooth 21, and no tenderness to percussion or palpation. The periodontal findings were within normal limits (i.e. probing< 3 mm, mobility <1 mm). Periapical radiographic examination of tooth 21 revealed a large radiolucent lesion associated with the tooth with inadequate root filling (Figure 1A). The apex of the tooth was open. The endodontic diagnosis was “previously treated tooth with chronic apical abscess”. Two treatment options were presented to the patient: non-surgical retreatment using an apical plug technique; or revitalizing the tooth using RET. The benefits and risks of each option was discussed with the patient who chose to revitalize the tooth using RET and signed a written consent form.

After local anaesthesia by infiltration of 1.8 mL of 2% lidocaine with 1:80,000 epinephrine (Daru Pakhsh, Tehran, Iran), rubber dam isolation was performed, the previous restoration was removed, and an access cavity prepared. Gutta-percha was removed using Hedström files (Dentsply Sirona, Ballaigues, Switzerland), and working length was determined (radiographic length measured from the incisal edge to the root apex minus 2 mm) (Figure 1B). The root canal space was irrigated with 2.5% NaOCl using a 30-gauge side-vent needle (Canal Clean; Biodent Co. Ltd., Guynggi-do, Korea), inserted 1 mm short of working length. The canal was then mechanically prepared using the Edge Taper Platinum rotary system (EdgeEndo, Albuquerque, NM, USA) followed by passive ultrasonic irrigation using a size 25 K-file while the canal was filled with 2.5% NaOCl. The root canal was dried with sterile paper points and a creamy paste of calcium hydroxide powder (Golchadent, Tehran, Iran), mixed with saline, was delivered into the

canal using a lentulo spiral, and the access cavity was restored with IRM (Reinforced Zinc Oxide; Golchadent).

The patient returned two weeks later complaining of continuous pus discharge from the mucosa adjacent to tooth 21. Clinical examination revealed that the sinus tract had not healed. After local anaesthesia and rubber dam isolation the temporary restoration was removed, and the calcium hydroxide medicament was removed by hand filing using a size 80 K-file with passive ultrasonic irrigation with 2.5% NaOCl. Double antibiotic paste (DAP; 500 mg/mL) with a creamy consistency was prepared by using equal amounts of 250 mg ciprofloxacin powder (Aria, Tehran, Iran) and 250 mg metronidazole powder (Daru Pakhsh) mixed with 1 mL of saline. The DAP was delivered into the canal, and the tooth was restored with IRM.

Three weeks later the patient returned and there was no pain on palpation or percussion and the sinus tract had healed. After local anaesthesia with 1.7 mL of Mepivacaine plain (Daru Pakhsh) and rubber dam isolation, the root canal was rinsed with 20 mL of 17% EDTA solution (Sigma Aldrich, St. Louis, MO, USA) and dried with sterile paper points. Nine mL of the patient's whole blood was drawn from the cubital vein and collected in a sterile tube. The tube was then centrifuged (DUO Quattro, Process for PRF, Nice, France) at 208 g for 8 min (Figure 1C). The platelet-rich fibrin (PRF) clot was separated from the acellular plasma and red blood cell layer and compressed in a compression box (Figure 1C). The liquid obtained by compression of the PRF clot was used for root canal irrigation, and the PRF membrane was rolled, delivered into the canal, and condensed apically using an endodontic plugger. OrthoMTA (bioMTA, Daejeon, Korea) was prepared and placed over the PRF in the coronal third of the root canal. A moist cotton pellet was placed over the OrthoMTA and the access cavity was restored with light-cure

glass ionomer (Figure 1D). After 1 week, the setting of OrthoMTA was confirmed, and the tooth was permanently restored with composite resin.

The patient was recalled 6 months after the procedure (Figure 1E) and at 2 years (Figures 1F-H). Clinically, there was no pain on percussion/palpation, probing depths were within normal limits (≤3 mm), mobility was normal (<1 mm), and there was no sinus tract. Radiographically, complete healing of the periapical lesion occurred over time (Figure 1F). At the 2-year follow up a mineralized bridge was observed in the apical third of the canal (Figure 1F). At the 2-year recall, a CBCT of the anterior maxilla was obtained (Figures 1G, H) to determine the status of the root development and apical closure, as recommended in the protocol published by the American Association of Endodontists (https://f3f142zs0k2w1kg84k5p9i1o-wpengine.netdna-ssl.com/specialty/wpcontent/uploads/sites/2/2018/06/ConsiderationsForRegEndo_AsOfApril2018.pdf). The CBCT sections revealed complete healing of the periapical lesion, closure of the apex, and formation of a mineralized bridge in the apical third of the root canal (Figure 1G).

Literature review

Inclusion criteria

All clinical studies that attempted to revitalize the root canal system of a previously treated tooth with an open apex were included. The definition of previous treatment was any type of root canal treatment, apexification or regenerative endodontic treatment.

Exclusion criteria

- Reports on revitalizing the root canal system of teeth with pulp necrosis and open apices;

- Studies on revitalizing the root canal system of mature teeth with closed apices;
- Animal studies.

Search strategy

Electronic searches were undertaken in MEDLINE, Scopus, and Embase databases to identify appropriate reports. Three combinations of keywords were used:

A) “Regenerative” AND “Endodontic” AND “Retreatment”;

B) “Pulp” AND “Regeneration” AND “Retreatment”;

C) “Revitalization” AND “Tooth” AND “Retreatment”;

The search strategy was not limited to immature teeth to ensure the inclusion of all possible scenarios associated with a tooth having an open apex. The Medical Subject Heading (MeSH) was as follow for combination A: ("regenerative endodontics"[MeSH Terms] OR ("regenerative"[All Fields] AND "endodontics"[All Fields]) OR "regenerative endodontics"[All Fields] OR ("regenerative"[All Fields] AND "endodontic"[All Fields]) OR "regenerative endodontic"[All Fields]) AND ("retreatment"[MeSH Terms] OR "retreatment"[All Fields]). The Mesh for strategy B was as follow: The Mesh for combination B was as follow: ("dental pulp"[MeSH Terms] OR ("dental"[All Fields] AND "pulp"[All Fields]) OR "dental pulp"[All Fields] OR "pulp"[All Fields]) AND ("regeneration"[MeSH Terms] OR "regeneration"[All Fields]) AND ("retreatment"[MeSH Terms] OR "retreatment"[All Fields]). The Mesh for combination C was as follow: revitalization[All Fields] AND ("tooth"[MeSH Terms] OR "tooth"[All Fields]) AND ("retreatment"[MeSH Terms] OR "retreatment"[All Fields]).

Two authors (AN and OD) independently reviewed the title and abstract of articles to determine if they met the inclusion criteria. Disagreements were resolved by reviewing the

inclusion/exclusion criteria and discussion to achieve consensus. All included manuscripts were then subjected to full text review and analysis.

Data extraction

Three categories of data were extracted from each study:

- Baseline data: sample size (i.e. number of teeth); age; tooth type; periapical diagnosis; type of previous endodontic treatment; apical diameter of canal;
- Procedural data: irrigant(s); type of root canal medicament; duration of medicament; type of scaffold; type of material used as an orifice plug;
- Outcome data: range and average duration of recall; clinical outcome measures; radiographic outcome; radiographic changes in the root structure.

The outcome of treatments was determined. A positive outcome was defined as lack of clinical signs/symptoms (no pain on percussion/palpation/function or sinus tract) (as reported) and radiographic reduction or complete resolution in the size of the periapical lesion (healed or healing) (examined objectively by AN and OD). Survival was defined as the tooth being present in the mouth at the time of follow-up.

Level and quality of evidence

For each report, the study design was determined, and a score for the level of evidence (LoE) was assigned based on study design, as described previously (Torabinejad *et al.* 2005, Shamszadeh *et al.* 2019).

To determine the quality of evidence (QoE) presented in the reports an assessment tool for case reports and case series was developed (Figure-2). Some items of the tool were adapted from the

NIH tool for assessment of case series (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools) and from the PRICE 2020 guidelines (Nagendrababu *et al.* 2020). The tool was customized to assess the "quality of evidence" rather than "quality of reporting" by focusing on three areas: case selection/definition; procedure; and outcome (Figure 2).

The "Case selection/definition" section aimed to assess the QoE regarding the patients' demographics, medical history, description of symptoms, description of clinical and radiographic signs detected by clinician, description of diagnostic methods used by clinician, and the accuracy of diagnosis based on clinical and radiographic findings. A maximum score of 5-7 is allocated to this section. The rater may opt to exclude the questions on demographic data and medical history based on whether they are associated with the presentation of symptoms and/or the outcome (Figure 2).

The "Procedure" section aimed to assess the QoE regarding the details of the clinical procedures. The procedures must be described in detail so that other clinicians can replicate them. One score is allocated to this section (Figure 2).

The 'Outcome" section aimed to assess the QoE in terms of the length of follow up, presentation of clinical findings, presentation of radiographic findings, details of diagnostic methods used to determine the clinical and radiographic findings, and the outcome presented by the authors. A maximum score of 5 is allocated to this section (Figure 2).

The assessment tool results in a maximum score of 11 to 13. The score-based rating is illustrated in Figure 2.

The clarity and reproducibility of the scoring processes were then tested by one of the co-authors who was not involved in the development of the tool. Two authors scored and rated the studies.

Disagreements on ratings were discussed and resolved and where necessary resulted in improvements to the assessment tool.

Results

After removal of duplicates, the keyword combinations resulted in the identification of 38 articles in MEDLINE, 19 articles in Scopus and 40 articles in Embase (total of 97). After review of the titles and abstracts, 9 articles met the inclusion criteria and were subjected to full text review and analysis. The reports were published between 2015 to 2020. An article was considered a case report if only one tooth was included. Three reports were case series (LoE 4) and six were case reports (LoE 5) (Table 1). No clinical trials or cohort studies were found.

Descriptive statistics

Baseline data

Overall, there were 17 teeth treated in 13 patients in the nine reports; eleven teeth were pooled from the case series and 6 teeth from case reports (Table 1). Three teeth were excluded from the reports of Nevins & Cymerman (2015) because they were also included in Cymerman & Nosrat (2020) with longer follow-up periods.

The ages of patients ranged from 7 to 48 years with an average of 19.4 years. Maxillary anterior teeth constituted 82% (n=14) of the treated teeth followed by mandibular molars (n=2; 12%) followed by mandibular premolars (n=1; 6%) (Table 1).

The periapical diagnoses were determined based on clinical and radiographic findings of the case, regardless of the terminologies used by the authors. The most reported diagnosis was acute apical abscess (n=4; 23%) and the least reported diagnosis was asymptomatic apical periodontitis

(n=2; 12%) (Table 1). Previous treatments were either a root canal treatment (n=14; 82%) or an RET (n=3; 18%) with post-treatment endodontic disease (Table-1). The data regarding apical diameter of canals was presented only in one study (Cymerman & Nosrat, 2020), where all included teeth (n=5; 29%) had an apical canal diameter of ≥1 mm (mean: 1.84 mm; range:1-3.7 mm), as determined in pre-operative CBCT images.

Procedural data

In all studies, NaOCl with variable concentrations (1.5%-6%) was used as the irrigant (Table 2). In previously root canal treated teeth the root filling material was removed using heat or mechanical instrumentation alone except in two teeth where a solvent was also used. Two studies used calcium hydroxide as the inter-appointment medicament (Table 2), the remainder of the studies used antibiotic pastes (Table 2). The period of medicament placement ranged from 2 weeks to 6 months (Table 2). In one study, the treatment was performed in one visit and no inter-appointment dressing was used (Table 2).

Several scaffolds were used: SynOss Putty (Collagen Matrix Inc, Oakland, NJ, USA) (n=6; 35%), blood clot (with or without collagen) (n=6; 35%), platelet-rich plasma (PRP) (n=3; 18%) and platelet-rich fibrin (PRF) (n=2; 12%). The orifice plugs were mainly calcium silicate-based cements: mineral trioxide aggregate, Biodentine (Septodont, Saint-Maur-des-Fossés, France), and Bioceramic putty (Brasseler USA, Savannah, GA, USA) (Table 2).

Outcome data

The follow-up of cases ranged from 12 to 72 months (mean: 29.2 months) (Table 3). Clinically, all teeth survived and were reported to be functional and asymptomatic. Radiographic assessment of the periapical lesions revealed ongoing healing in 3 (18%) or complete healing in

14 (82%) teeth (Table 3). Assessments of root development revealed varying degrees of canal calcification and apical closure. The most common finding regarding root development that was reported was “apical closure” (Table 3). Radiographic assessment of the outcome was achieved using cone beam computed tomography (CBCT) imaging in 12 teeth (70%) (Table 3).

Study ratings

The QoE in three case reports (n=3) was rated “Excellent” and only “Fair” in the remaining six studies (n=14). Details of assessments and ratings are illustrated in Table 4.

Discussion

The concept of “revitalizing” the root canal system of a tooth sometime after a previous attempt at endodontic treatment and when there is post-treatment disease is appealing. RET is a biologically-based treatment, which unlike other forms of endodontic treatment, aims to revitalize the pulp space and, in teeth with open apices, stimulate further root development. The present review revealed that the overall quality of evidence in the majority of reports of cases involving RET in previously treated teeth with open apices was low. There are no analytic clinical studies (i.e. clinical trials or observational studies) on this subject and the evidence is limited to case series and case reports. A major bias in these reports is that the authors only describe successful cases. In other words, the “possibility” of a successful treatment is introduced in these reports, but the real success rate of the treatment cannot be determined because there were no control groups, and no negatives cases were reported. Also, due to lack of control groups, the outcome of alternative treatments cannot be compared to the new treatment.

As a consequence, there is a need for clinical studies with higher level of evidence to understand the outcome of RET in previously treated teeth.

In this study, a customized tool to assess the quality of case report/series articles in the field of Endodontics was developed. The resources available for quality assessment of case report/series studies are scarce and are mainly applied in the field of Medicine, but not Dentistry or Endodontics (Murad *et al.* 2018). Quality assessment is a critical step to understand the quality of evidence. While LoE is determined based on elements of the study design, the QoE is determined based on technical details of the reports. Papers with same study design (for instance case reports) provide the same LoE but different QoE depending on the details within the manuscript. A combination of LoE and QoE creates a clearer picture about a report and the value of the associated outcomes. The assessments revealed that most of articles on revitalization of previously treated teeth with open apices were of only 'fair' quality. Only three case reports were categorized as having 'excellent' quality. Also, the LoE in most articles was 5, which is the lowest level. It is worth noting that the current review is not a systematic review, and unlike systematic reviews the approach taken provides an overall low level of evidence (LoE 5). This is due to lack of comparative clinical studies in this field. Clearly, clinical studies with far better quality and higher level of evidence are essential in this field.

Successful disinfection of the root canal system is the most critical and the most challenging step in RET (Fouad *et al.* 2013, Fouad 2020). It has been reported that residual bacteria in the root canal space are detrimental during tissue engineering (Verma *et al.* 2017). Antibiotic pastes with creamy consistency (1000 mg/mL) have reported to be effective in completely eradicating intra-canal bacteria (Windley *et al.* 2005, AlSaeed *et al.* 2018). However, this high concentration of antibiotics might be toxic to stem cells from the apical papilla (SCAP) (Ruparel *et al.* 2012), and

makes the dentinal walls less inducive to stem cell proliferation (Althumairy *et al.* 2014). Reduced concentrations of antibiotics (i.e. 1 mg/mL) have been reported to be non-toxic to stem cells (Ruparel *et al*. 2012) and have no detrimental effects on canal walls (Althumairy *et al*. 2014). However, the reduced concentration might not be as effective as a full-strength concentration in eradicating bacteria (AlSaeed *et al*. 2018). An important piece of information that could have provided a better understanding about the efficacy of disinfection strategies is the comparison between disinfection strategies in primary and retreatment cases. This information was only provided in one study (Chaniotis *et al*. 2017) where the initial RET was completed in a single visit. The RET for retreatment was completed in two visits (6 months apart) with a calcium hydroxide dressing. The irrigation protocol was the same for both procedures.

The bacterial population in root canals associated with teeth with failed RET have not been studied. There is a difference in the nature and resistance of bacterial populations in teeth with pulp necrosis as compared to teeth with post-treatment endodontic disease following endodontic treatment. While the bacteria in teeth with pulp necrosis (i.e. primary infections) are mainly gram-negative strict anaerobes, the bacteria in teeth with post-treatment disease (i.e. persistent infections) are mainly gram positive facultative anaerobes (Gomes *et al*. 2004; Siqueira & Rôças 2009; Siqueira *et al*. 2007). Even though gram negative strict anaerobic bacteria are susceptible to the effect of disinfectants such as NaOCl, the gram-positive facultative anaerobes are not as susceptible (Siqueira *et al*. 2007). In other words, disinfection of the root canal system for RET in teeth with persistent infection might be more challenging compared to teeth with primary infections. The present review revealed that high concentration antibiotic pastes were the most common type of root canal dressing used in teeth with open apices retreated with RET. Further

ex vivo and *in vivo* studies are recommended to determine the optimal canal disinfection protocol for RET in previously treated teeth with open apices.

Another challenge in achieving optimal infection control in previously root canal treated teeth is the effective removal of debris and material from canal walls. Various removal techniques have been reported to result in different levels of remaining debris on canal walls (Mollo *et al*. 2012), which can interfere with the disinfection of the root canal space. In the current review, all cases with "healing" outcome were previously root canal treated (Tables 1 and 3). Blood clots, SynOss Putty (Collagen Matrix Inc, Oakland, NJ, USA) and platelet-derived scaffolds were the three main scaffolds used for teeth with open apices retreated with RET. Blood clots are the most popular scaffold for RET procedures in teeth with primary root canal infections (Torabinejad *et al*. 2017). SynOss Putty has been reported to induce formation of mineralized tissue in the root canal space of human teeth when used in RET (Nosrat *et al.* 2019). Platelet-derived scaffolds such as PRP and PRF are rich in growth factors and can induce proliferation and differentiation of stem cells (Vogel *et al.* 2006). In other words, they can facilitate formation of new tissues in the root canal micro-environment (Zhu *et al.* 2013). Recent clinical trials reported successful outcomes for these scaffolds (PRP and PRF) when used for RET in human teeth (Ragab *et al.* 2019, Ulusoy *et al.* 2019, ElSheshtawy *et al.* 2020). A systematic review reported that most human teeth treated with platelet-derived scaffolds had continued radiographic root development in the form of thickening of the root canal wall and closure of the apex (Metlerska *et al.* 2019). On the other hand, animal studies have reported an association between the use of platelet-derived scaffolds and formation of bony islands in the root canal space (Torabinejad *et al.* 2014) and also an overall greater volumes of soft tissue formation in the root canal space (Zhu *et al*. 2013). The case report presented here revealed radiographic evidence of apical closure and

formation of a calcified bridge in the apical third of the root canal space. These findings are indirect evidence of revitalization and formation of new tissue in the apical root canal and the use of PRF as a scaffold might have played a role in the formation of these new intra-canal tissues. Estefan *et al*. (2016) reported the probability and the amount of root development following RET in immature teeth with pulp necrosis was negatively associated with the age of the patient and positively associated with the diameter of the apical foramen. In other words, younger patients with wider apical foramina (≥1 mm) had greater root development following RET (Estefan *et al*. 2016). Fang *et al.* (2018) reported that narrow (< 0.5 mm) apical foramina were associated with lower probability of success following RET in immature teeth as compared to teeth with wider apical foramina. It is likely that patients who receive root canal treatment due to pulp necrosis in immature teeth are on average younger than those who receive treatment due to post-treatment disease following root canal treatment in teeth with open apices. A systematic review (Torabinejad *et al*. 2017) reported that the age range of patients who received RET due to pulp necrosis in immature teeth was 7-18 years and that patients older than 18 years were exceptions. The present review revealed that the age of patients who received RET on previously treated teeth ranged from 7- 48 years. Except one paper (Cymerman & Nosrat, 2020) the rest of the studies included in the present review did not provide information on the diameter of the apical foramen of the teeth. The most common outcome observed regarding root development in teeth with open apices following retreatment with RET was “apical closure” without increase in root length or wall thickness. The age of the patients might be a contributing factor to this pattern of root development. Further clinical studies are needed to determine whether age and diameter of apical foramina are critical factors in the “probability” and “quantity” of root development following RET in previously treated teeth with open apices.

Conclusions

The level of evidence and quality of evidence in reports of revitalizing previously treated root canals in teeth with open apices was low. Further clinical studies with higher level and quality of evidence are essential to determine the success rate, the optimum disinfection protocol, and the appropriate tissue engineering strategies. The case report and the present review demonstrate the potential of revitalization in previously treated teeth with open apices.

References

Al-Tammami M, Al-Nazhan S (2017) Retreatment of failed regenerative endodontic of orthodontically treated immature permanent maxillary central incisor: a case report. *Restorative Dentistry and Endodontics* **42**, 65-71.

AlSaeed T, Nosrat A, Melo M et al. (2018) Antibacterial Efficacy and Discoloration Potential of Endodontic Topical Antibiotics. *Journal of Endodontics* **44**, 1110-4.

Althumairy R, Teixeira F, Diogenes A (2014) Effect of dentin conditioning with intracanal medicaments on survival of stem cells of apical papilla. *Journal of Endodontics* **40**, 521-5.

Becerra P, Ricucci D, Loghin S, Gibbs J, Lin L (2014) Histologic study of a human immature permanent premolar with chronic apical abscess after revascularization/revitalization. *Journal of Endodontics* **40**, 133-9.

Bucchi C, Marcé-Nogué J, Galler KM, Widbiller M (2019) Biomechanical performance of an immature maxillary central incisor after revitalization: a finite element analysis. *International Endodontic Journal* **52**, 1508-18.

Chaniotis A (2017) Treatment Options for Failing Regenerative Endodontic Procedures: Report of 3 Cases. *Journal of Endodontics* **43**, 1472-8.

Chrepa V, Joon R, Austah O et al. (2020) Clinical Outcomes of Immature Teeth Treated with Regenerative Endodontic Procedures-A San Antonio Study. *Journal of Endodontics*.

Cymerman J, Nosrat A (2020) Regenerative Endodontic Treatment as a Biologically Based Approach for Non-Surgical Retreatment of Immature Teeth. *Journal of Endodontics* **46**, 44-50.

ElSheshtawy A, Nazzal H, El Shahawy O et al. (2020) The effect of platelet-rich plasma as a scaffold in regeneration/revitalization endodontics of immature permanent teeth assessed using 2-dimensional radiographs and cone beam computed tomography: a randomized controlled trial. *International Endodontic Journal* **53**, 905-21.

Estefan B, El Batouty K, Nagy M, Diogenes A (2016) Influence of Age and Apical Diameter on the Success of Endodontic Regeneration Procedures. *Journal of Endodontics* **42**, 1620-5.

Fang Y, Wang X, Zhu J, Su C, Yang Y, Meng L (2018) Influence of Apical Diameter on the Outcome of Regenerative Endodontic Treatment in Teeth with Pulp Necrosis: A Review. *Journal of Endodontics* **44**, 414-31.

Fouad A, Nosrat A (2013) Pulp regeneration in previously infected root canal space. *Endodontic Topics* **28**, 24-37.

Fouad AF (2020) Contemporary Microbial and Antimicrobial Considerations in Regenerative Endodontic Therapy. *Journal of Endodontics* **46**, S105-S14.

Gaviño Orduña J, Caviedes-Bucheli J, Manzanares Céspedes M et al. (2017) Use of Platelet-rich Plasma in Endodontic Procedures in Adults: Regeneration or Repair? A Report of 3 Cases with 5 Years of Follow-up. *Journal of Endodontics* **43**, 1294-301.

Gomes B, Pinheiro E, Gade-Neto C et al. (2004) Microbiological examination of infected dental root canals. *Oral Microbiology and Immunology* **19**, 71-6.

Hargreaves KM, Diogenes A, Teixeira FB (2013) Treatment options: biological basis of regenerative endodontic procedures. *Journal of Endodontics* **39**, S30-43.

Metlerska J, Fagogeni I, Nowicka A (2019) Efficacy of Autologous Platelet Concentrates in Regenerative Endodontic Treatment: A Systematic Review of Human Studies. *Journal of Endodontics* **45**, 20-30 e1.

Miltiadous M, Floratos S (2015) Regenerative Endodontic Treatment as a Retreatment Option for a Tooth with Open Apex - A Case Report. *Brazilian Dental Journal* **26**, 552-6.

Mollo A, Botti G, Prinicipi Goldoni N et al. (2012) Efficacy of two Ni-Ti systems and hand files for removing gutta-percha from root canals. *International Endodontic Journal* **45**, 1-6.

Murad M, Sultan S, Haffar S, Bazerbachi F (2018) Methodological quality and synthesis of case series and case reports. *BMJ Evidence Based Medicine* **23**, 60-3.

Nagendrababu V, Chong B, McCabe P et al. (2020) PRICE 2020 guidelines for reporting case reports in Endodontics: a consensus-based development. *International Endodontic Journal* **53**, 619-26.

Nevins AJ, Cymerman JJ (2015) Revitalization of open apex teeth with apical periodontitis using a collagen-hydroxyapatite scaffold. *Journal of Endodontics* **41**, 966-73.

Nosrat A, Kolahdouzan A, Hosseini F, Mehrizi E, Verma P, Torabinejad M (2015) Histologic Outcomes of Uninfected Human Immature Teeth Treated with Regenerative Endodontics: 2 Case Reports. *Journal of Endodontics* **41**, 1725-9.

Nosrat A, Kolahdouzan A, Khatibi A et al. (2019) Clinical, Radiographic, and Histologic Outcome of Regenerative Endodontic Treatment in Human Teeth Using a Novel Collagen-hydroxyapatite Scaffold. *Journal of Endodontics* **45**, 136-43.

Pace R, Giuliani V, Nieri M, Di Nasso L, Pagavino G (2014) Mineral trioxide aggregate as apical plug in teeth with necrotic pulp and immature apices: a 10-year case series. *Journal of Endodontics* **40**, 1250-4.

Pace R, Giuliani V, Pini Prato L, Baccetti T, Pagavino G (2007) Apical plug technique using mineral trioxide aggregate: results from a case series. *International Endodontic Journal* **40**, 478-84.

Prasad J, de Ataide I, Chalakkal P, Likhyani L (2018) Comparison between the Outcomes of Two Platelet-Rich Concentrates on Apexogenesis in Young Permanent Incisors Requiring Endodontic Retreatment. *Contemporary Clininical Dentistry* **9**, S156-S9.

Ragab R, Lattif A, Dokky N (2019) Comparative Study between Revitalization of Necrotic Immature Permanent Anterior Teeth with and without Platelet Rich Fibrin: A Randomized Controlled Trial. *Journal of Clinical Pediatric Dentistry* **43**, 78-85.

Ruparel N, Teixeira F, Ferraz C, Diogenes A (2012) Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. *Journal of Endodontics* **38**, 1372-5.

Saoud T, Huang G, Gibbs J, Sigurdsson A, Lin L (2015) Management of Teeth with Persistent Apical Periodontitis after Root Canal Treatment Using Regenerative Endodontic Therapy. *Journal of Endodontics* **41**, 1743-8.

Shamszadeh S, Asgary S, Nosrat A (2019) Regenerative Endodontics: A Scientometric and Bibliometric Analysis. *Journal of Endodontics* **45**, 272-80.

Siqueira J, Rôças I (2009) Diversity of endodontic microbiota revisited. *Journal of Dental Research* **88**, 969-81.

Siqueira J, Rôças I, Paiva S, Guimaraes-Pinto T, Magalhaes K, Lima K (2007) Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology & Endodontology* **104**, 122-30.

Tong H, Rajan S, Bhujel N, Kang J, Duggal M, Nazzal H (2017) Regenerative Endodontic Therapy in the Management of Nonvital Immature Permanent Teeth: A Systematic Review-Outcome Evaluation and Meta-analysis. *Journal of Endodontics* **43**, 1453-64.

Torabinejad M, Faras H, Corr R, Wright K, Shabahang S (2014) Histologic examinations of teeth treated with 2 scaffolds: a pilot animal investigation. *Journal of Endodontics* **40**, 515-20.

Torabinejad M, Kutsenko D, Machnick T, Ismail A, Newton C (2005) Levels of Evidence for the Outcome of Nonsurgical Endodontic Treatment. *Journal of Endodontics* **31**, 637-46.

Torabinejad M, Nosrat A, Verma P, Udochukwu O (2017) Regenerative Endodontic Treatment or Mineral Trioxide Aggregate Apical Plug in Teeth with Necrotic Pulps and Open Apices: A Systematic Review and Meta-analysis. *Journal of Endodontics* **43**, 1806-20.

Ulusoy A, Turedi I, Cimen M, Cehreli ZC(2019) Evaluation of Blood Clot, Platelet-rich Plasma, Platelet-rich Fibrin, and Platelet Pellet as Scaffolds in Regenerative Endodontic Treatment: A Prospective Randomized Trial. *Journal of Endodontics* **45**, 560-6.

Verma P, Nosrat A, Kim J et al. (2017) Effect of Residual Bacteria on the Outcome of Pulp Regeneration In Vivo. *Journal of Dental Research* **96**, 100-6.

Vogel J, Szalay K, Geiger F, Kramer M, Richter W, Kasten P (2006) Platelet-rich plasma improves expansion of human mesenchymal stem cells and retains differentiation capacity and in vivo bone formation in calcium phosphate ceramics. *Platelets* **17**, 462-9.

Windley W, Teixeira F, Levin L, Sigurdsson A, Trope M (2005) Disinfection of immature teeth with a triple antibiotic paste. *Journal of Endodontics* **31**, 439-43.

Zhou R, Wang Y, Chen Y et al. (2017) Radiographic, Histologic, and Biomechanical Evaluation of Combined Application of Platelet-rich Fibrin with Blood Clot in Regenerative Endodontics. *Journal of Endodontics* **43**, 2034-40.

Zhu W, Zhu X, Huang GT, Cheung G, Dissanayaka W, Zhang C (2013) Regeneration of dental pulp tissue in immature teeth with apical periodontitis using platelet-rich plasma and dental pulp cells. *International Endodontic Journal* **46**, 962-70.

Žižka R, Šedý J, Voborná I (2018) Retreatment of failed revascularization/revitalization of immature permanent tooth - A case report. *Journal of Clinical and Experimental Dentistry* **10**, e185-e8.

Table-1: List of included studies, study design, level of evidence (LoE), and baseline data.

Study	Design	LoE	N	Age (mean)	Tooth	PA Dx	Previous Tx
Cymerman & Nosrat (2020)	Case series	4	5	31	Max Ant (4); Mand Mol (1)	AAA (2), AAP (2); CAA (1)	RCT (5)
Prasad et al. (2018)	Case series	4	4	13	Max Ant (4)	SAP (4)	RCT (4)
Žižka (2018)	Case report	5	1	7	Max Ant	SAP	RET
Orduna et al. (2017)	Case report	5	1	24	Max Ant	SAP	RCT
Chaniotis (2017)	Case report	5	1	7	Max Ant	CAA	RET
Al-Tammami et al. (2017)	Case report	5	1	12	Max Ant	CAA	RET
Miltiadous et al. (2015)	Case report	5	1	14	Max Ant	SAP	RCT
Saoud et al. (2015)	Case series	4	2	19	Max Ant (1); Mand Mol (1)	AAA (1), CAA (1)	RCT (2)
Nevins & Cymerman (2015)	Case report	5	1	48	Mand Premol	AAA	RCT

Max: Maxillary; Ant: anterior; Premol: premolar; Mol: molar; PA Dx: periapical diagnosis; AAA: acute apical abscess; AAP: asymptomatic apical periodontitis; SAP: symptomatic apical periodontitis; CAA: chronic apical abscess; Tx: treatment; RCT: root canal treatment; RET: regenerative endodontic treatment.

Table-2: Procedural data of the included studies

Study	Irrigant	Dressing	Duration (mean)	Scaffold	Orifice plug
Cymerman & Nosrat (2020)	NaOCl 6% EndoVac; EDTA 17%	DAP	4 weeks	SynOss Putty (5)	Bioceramic
Prasad et al. (2018)	NaOCl 1.5%	TAP	2 weeks	PRF (2); PRP (2)	MTA
Žižka (2018)	NaOCl 5%	-	-	BC	MTA
Orduna et al. (2017)	NaOCl 5.25%	DAP	2 weeks	PRP	MTA
Chaniotis (2017)	NaOCl 3% EndoVac; EDTA 17%	CH	6 months	BC	Biodentine
Al-Tammami et al. (2017)	NOCl 5.25%; CHX 0.12%	DAP	2 weeks	BC+Collagen	MTA
Miltiadous et al. (2015)	NaOCl 2.5%; EDTA 17%	TAP	2 weeks	BC+Collagen	MTA
Saoud et al. (2015)	NaOCl	CH	5 weeks	BC	MTA
Nevins & Cymerman (2015)	NaOCl 6%; EDTA 17%	DAP	4 weeks	SynOss Putty	MTA

CHX: chlorehexidine; DAP: double antibiotic paste; TAP: triple antibiotic paste; CH: calcium hydroxide; PRP: platelet-rich plasma; PRF: platelet-rich fibrin; BC: blood clot

Table-3: Outcome data of the included studies

Study	Recall (mean)	Clinical success	Radiographic outcome	Changes in root structure	Recall CBCT
Cymerman & Nosrat (2020)	54.4 m	100%	Healed	Calcifications, increased thickness, apical closure	Y (4); N (1)
Prasad et al. (2018)	24 m	100%	Healed	Increased length & thickness, apical closure	Y (4)
Žižka (2018)	15 m	100%	Healed	Increased length & thickness, apical closure	N
Orduna et al. (2017)	48 m	100%	Healing	Calcifications, increased thickness	Y
Chaniotis (2017)	24 m	100%	Healed	Calcification; increased thickness, apical closure	N
Al-Tammami et al. (2017)	36 m	100%	Healed	Calcification, apical closure	Y
Miltiadous et al. (2015)	36 m	100%	Healed	Apical closure	Y
Saoud et al. (2015)	13.5 m	100%	Healing	Increased thickness, apical closure	N (2)
Nevins & Cymerman (2015)	12 m	100%	Healed	Calcification, apical closure	Y

m: month; Y: yes; N: no

Table-4: Details of quality assessments and scoring for the included studies.

Item #	1	2	3	4	5	6	7	8	9	10	11	12	13	Total score	Rating
Cymerman & Nosrat (2020)	0.5	0	0	0	0	0.5	1	1	1	1	1	1	1	8.5	Fair
Prasad et al. (2018)	0.5	0	1	0	1	0	1	1	0.5	0	0.5	0.5	0.5	6.5	Fair
Žižka (2018)	0.5	0	0.5	0.5	1	0	0	1	0.5	1	1	1	1	8	Fair
Orduna et al. (2017)	0.5	0.5	1	1	1	1	0.5	1	1	1	1	0.5	1	11	Excellent
Chaniotis (2017)	0.5	0	1	0.5	1	0.5	0	1	0.5	0.5	1	0.5	1	8	Fair
Al-Tammami et al. (2017)	0.5	0.5	1	1	1	1	0.5	1	0.5	0	0.5	0.5	0.5	8.5	Fair
Miltiadous et al. (2015)	1	0.5	1	1	1	1	1	1	0.5	0	1	0.5	1	10.5	Excellent
Saoud et al. (2015)	0.5	0.5	1	1	1	1	1	1	0.5	0	0.5	0.5	0	8.5	Fair
Nevins & Cymerman (2015)	0.5	0	1	1	0.5	1	1	1	0.5	0.5	1	0.5	1	9.5	Excellent

Figure legend

Figure-1: A) Pre-operative periapical radiograph of tooth #21. This radiograph shows an inadequate root canal treatment associated with a large periapical lesion; B) Intra-operative view, tooth #21. After removal of gutta-percha the opening of the apex is more visible; C) Separating PRF clot from other layers; D) Post-operative view, tooth #21. The PRF is covered with OrthoMTA as a biocompatible orifice plug. The access cavity is temporarily sealed with a wet cotton pellet and light-cure glass ionomer; E) 6-month recall. The periapical lesion is healing, the apex is closing, and there is a calcified barrier forming inside the root canal space; F) 2-year recall; G) Sagittal view of the CBCT image 2 years after the treatment shows complete healing of the periapical lesion, complete closure of the apex and formation of a ~1 mm thick calcified bridge in apical third of the root canal space; H) 3D rendering of the anterior maxilla 2 years after treatment showing normal anatomy of the area and the cortical plate.

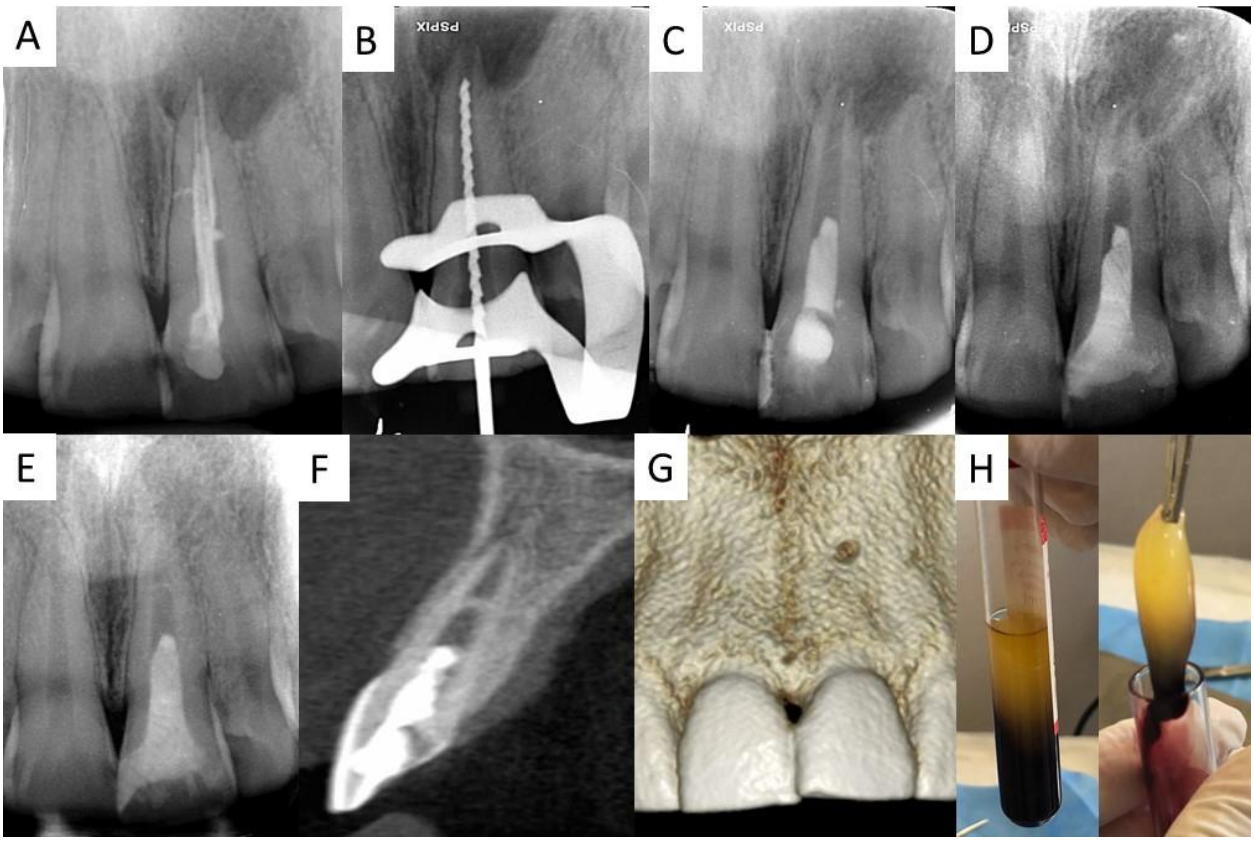
A
B
C
D
E
F
G
H

Figure-2: Assessment tool developed to rate the quality of evidence in case report and case series articles in the field of Endodontics.

Scoring instructions

The rater(s) should assign a score to each question as follow:
- 0: If the item is not reported
- 0.5: if the item is poorly/partially reported
- 1: if the item is fully reported

Score-based quality rating
- Poor: below or equal to 1/3 of the total score
- Fair: more than 1/3 and less than 2/3 of the toal score
- Excellent: equal or more than 2/3 of the totals score

**For case series, if each case is reported separately, the average score for each item must be calculated and used for final scoring.

Case selection/definition

1- Were the patient demographics (*i.e.* tooth number, age, gender, and race) clearly and fully described, if relevant? ***(remove the item if it is irrelevant)***

2- Was the medical history (including systemic diseases and medications taken at the time of treatment) described in detail, if relevant? ***(remove the item if it is irrelevant)***

3- Were the symptoms clearly and fully described?

4- Were the clinical signs / features clearly and fully described (pain on percussion, palpation, biting; periodontal exams [probing depths and mobility])?

5- Were the radiographic signs / features (presence/absence of periapical lesion; root/canal anatomy; quality of previous treatment) clearly and fully described?

6- Were the applicable diagnostic methods (*i.e.* vitality tests, percussion, palpation, probing, radiographs, tissue/bacterial/fungal staining, laboratory tests, bacterial samples) and the tools used to arrive at the diagnosis clearly and fully described?

7- In the context of the clinical and radiographic findings, was the diagnosis correct?

Procedure

8- Were the procedures/interventions described with sufficient detail to allow other investigators to replicate the clinical and radiographic procedures?

Outcome

9- Was the follow up period sufficient (normally 4 years and more) to demonstrate the long-term outcome? (calculate the average follow up in case series; consider score of 0.5 for 1-3 years, a score of zero for less than one year)

10- Were the clinical outcomes (including signs and symptoms) clearly and fully described (signs: results of percussion, palpation, periodontal exams [probing depths, mobility])?

11- Were the radiographic outcomes clearly and fully described?

12- Were the results of the applicable diagnostic methods used to determine the outcome (vitality tests, percussion, palpation, probing, radiographs, tissue/bacterial/fungal staining, laboratory tests, bacterial samples) clearly and fully described?

13- Was the interpretation of the author(s) in terms of the outcome consistent with the evidence presented?

Rater's initials:
Final score:
Quality: