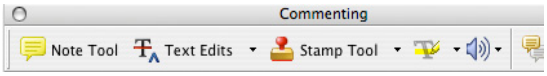
ELSEVIER

INSTRUCTIONS ON THE ANNOTATION OF PDF FILES

To view, print and annotate your content you will need Adobe Reader version 9 (or higher). This program is freely available for a whole series of platforms that include PC, Mac, and UNIX and can be downloaded from http://get.adobe.com/reader/. The exact system requirements are given at the Adobe site: http://www.adobe.com/products/reader/tech-specs.html.

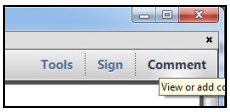
Note: ***Please do NOT make direct edits to the PDF using the editing tools as doing so could lead us to overlook your desired changes.*** *Rather, please request corrections by using the tools in the Comment pane to annotate the PDF and call out the changes you are requesting. If you opt to annotate the file with software other than Adobe Reader then please also highlight the appropriate place in the PDF file.*

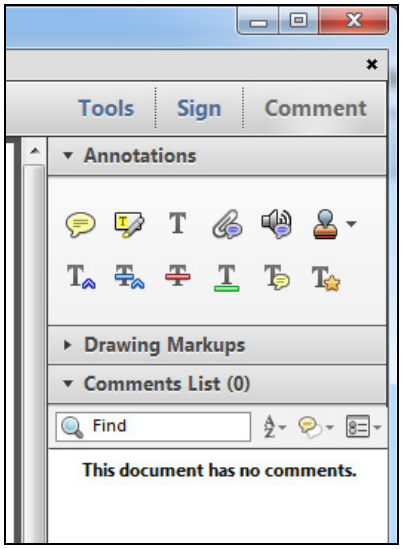
<table>
<tr><th colspan="2">PDF ANNOTATIONS</th></tr>
<tr><th>Adobe Reader version 9</th><th>Adobe Reader version X and XI</th></tr>
<tr><td>When you open the PDF file using Adobe Reader, the Commenting tool bar should be displayed automatically; if not, click on ‘Tools’, select ‘Comment & Markup’, then click on ‘Show Comment & Markup tool bar’ (or ‘Show Commenting bar’ on the Mac). If these options are not available in your Adobe Reader menus then it is possible that your Adobe Acrobat version is lower than 9 or the PDF has not been prepared properly.
 

(Mac)
PDF ANNOTATIONS (Adobe Reader version 9)

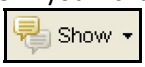
The default for the Commenting tool bar is set to ‘off’ in version 9. To change this setting select ‘Edit | Preferences’, then ‘Documents’ (at left under ‘Categories’), then select the option ‘Never’ for ‘PDF/A View Mode’.
 

(Changing the default setting, Adobe version 9)</td><td>To make annotations in the PDF file, open the PDF file using Adobe Reader XI, click on ‘Comment’.

If this option is not available in your Adobe Reader menus then it is possible that your Adobe Acrobat version is lower than XI or the PDF has not been prepared properly.
 

This opens a task pane and, below that, a list of all Comments in the text. These comments initially show all the changes made by our copyeditor to your file.
 
</td></tr>
</table>

HOW TO...		
Action	**Adobe Reader version 9**	**Adobe Reader version X and XI**
Insert text	Click the 'Text Edits' button  on the Commenting tool bar. Click to set the cursor location in the text and simply start typing. The text will appear in a commenting box. You may also cut-and-paste text from another file into the commenting box. Close the box by clicking on 'x' in the top right-hand corner.	Click the 'Insert Text' icon  on the Comment tool bar. Click to set the cursor location in the text and simply start typing. The text will appear in a commenting box. You may also cut-and-paste text from another file into the commenting box. Close the box by clicking on '_'  in the top right-hand corner.
Replace text	Click the 'Text Edits' button  on the Commenting tool bar. To highlight the text to be replaced, click and drag the cursor over the text. Then simply type in the replacement text. The replacement text will appear in a commenting box. You may also cut-and-paste text from another file into this box. To replace formatted text (an equation for example) please Attach a file (see below).	Click the 'Replace (Ins)' icon  on the Comment tool bar. To highlight the text to be replaced, click and drag the cursor over the text. Then simply type in the replacement text. The replacement text will appear in a commenting box. You may also cut-and-paste text from another file into this box. To replace formatted text (an equation for example) please Attach a file (see below).
Remove text	Click the 'Text Edits' button  on the Commenting tool bar. Click and drag over the text to be deleted. Then press the delete button on your keyboard. The text to be deleted will then be struck through.	Click the 'Strikethrough (Del)' icon  on the Comment tool bar. Click and drag over the text to be deleted. Then press the delete button on your keyboard. The text to be deleted will then be struck through.
Highlight text/ make a comment	Click on the 'Highlight' button  on the Commenting tool bar. Click and drag over the text. To make a comment, double click on the highlighted text and simply start typing.	Click on the 'Highlight Text' icon  on the Comment tool bar. Click and drag over the text. To make a comment, double click on the highlighted text and simply start typing.
Attach a file	Click on the 'Attach a File' button  on the Commenting tool bar. Click on the figure, table or formatted text to be replaced. A window will automatically open allowing you to attach the file. To make a comment, go to 'General' in the 'Properties' window, and then 'Description'. A graphic will appear in the PDF file indicating the insertion of a file.	Click on the 'Attach File' icon  on the Comment tool bar. Click on the figure, table or formatted text to be replaced. A window will automatically open allowing you to attach the file. A graphic will appear indicating the insertion of a file.
Leave a note/ comment	Click on the 'Note Tool' button  on the Commenting tool bar. Click to set the location of the note on the document and simply start typing. Do not use this feature to make text edits.	Click on the 'Add Sticky Note' icon  on the Comment tool bar. Click to set the location of the note on the document and simply start typing. Do not use this feature to make text edits.

HOW TO...		
Action	**Adobe Reader version 9**	**Adobe Reader version X and XI**
Review	To review your changes, click on the 'Show' button  on the Commenting tool bar. Choose 'Show Comments List'. Navigate by clicking on a correction in the list. Alternatively, double click on any mark-up to open the commenting box.	Your changes will appear automatically in a list below the Comment tool bar. Navigate by clicking on a correction in the list. Alternatively, double click on any mark-up to open the commenting box.
Undo/delete change	To undo any changes made, use the right click button on your mouse (for PCs, Ctrl-Click for the Mac). Alternatively click on 'Edit' in the main Adobe menu and then 'Undo'. You can also delete edits using the right click (Ctrl-click on the Mac) and selecting 'Delete'.	To undo any changes made, use the right click button on your mouse (for PCs, Ctrl-Click for the Mac). Alternatively click on 'Edit' in the main Adobe menu and then 'Undo'. You can also delete edits using the right click (Ctrl-click on the Mac) and selecting 'Delete'.

SEND YOUR ANNOTATED PDF FILE BACK TO ELSEVIER

Save the annotations to your file and return as instructed by Elsevier. Before returning, please ensure you have answered any questions raised on the Query Form and that you have inserted all corrections: later inclusion of any subsequent corrections cannot be guaranteed.

FURTHER POINTS

- Any (grey) halftones (photographs, micrographs, etc.) are best viewed on screen, for which they are optimized, and your local printer may not be able to output the greys correctly.
- If the PDF files contain colour images, and if you do have a local colour printer available, then it will be likely that you will not be able to correctly reproduce the colours on it, as local variations can occur.
- If you print the PDF file attached, and notice some 'non-standard' output, please check if the problem is also present on screen. If the correct printer driver for your printer is not installed on your PC, the printed output will be distorted.

AUTHOR QUERY FORM

ELSEVIER	**Journal:** YMED **Article Number:** 101400	**Please e-mail your responses and any corrections to:** **E-mail:** corrections.esch@elsevier.tnq.co.in

Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list. **It is crucial that you NOT make direct edits to the PDF using the editing tools as doing so could lead us to overlook your desired changes.** Note: if you opt to annotate the file with software other than Adobe Reader then please also highlight the appropriate place in the PDF file. To ensure fast publication of your paper please return your corrections within 48 hours.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof.

Location in article	Query / Remark: Click on the Q link to find the query's location in text Please insert your reply or correction at the corresponding line in the proof
	If there are any drug dosages in your article, please verify them and indicate that you have done so by initialing this query
Q1	Please provide professional degrees (eg, PhD, MD) for the author(s) "Simay Koc, Massimo Del Fabbro".
Q2	Please provide a conflict of interest statement.
Q3	As per style abstract subheads should be "Objective(s), Methods, Results, Conclusion(s)". Please check and revise the headings.
Q4	Please provide the expansion, if any, for "ROBINS-1" in the sentence "The risk-of-bias assessment …" in the abstract and also in its one occurrence within the main text.
Q5	Do not use virgule or slash "/" in running text as a shorthand for *and/or* (eg, *he/she* to indicate *he and/or she*), which is inaccurate and imprecise as per journal style. Please replace them with either "and" or "or" where applicable, but not "and/or", throughout the article.
Q6	The abbreviation "PC" has been defined as "platelet concentrate" as well as "Platelet concentrated". Please check if the representation should be made consistent in the article.
Q7	Please provide the expansion for "MTAD" in the sentence "They showed that …"
Q8	Please provide the expansion for "ESE" in the sentence "Considering all the disadvantages …"
Q9	Refs. [22] and [54] were identical, the latter has been removed from the reference list and subsequent references have been renumbered.
Q10	Please provide publisher name and location for the ref. 28.
Q11	Please provide the expansion for "JOE" and "IEJ" in Tables 2 and 3, and expansion for "J Int Med Res" and "J Clin Pediatr Dent" in Table 2.

(*continued on next page*)

Q12	A description explaining the designator “a” is provided in the legend section of Table 4 but the same is not cited anywhere within the table. Please check and provide the citation.
Q13	Please confirm that given names and surnames have been identified correctly and are presented in the desired order and please carefully verify the spelling of all authors' names.
	Please check this box or indicate your approval if you have no corrections to make to the PDF file ☐

Thank you for your assistance.

FEATURE ARTICLE

DOES THE ETIOLOGY OF PULP NECROSIS AFFECT REGENERATIVE ENDODONTIC TREATMENT OUTCOMES? A SYSTEMATIC REVIEW AND META-ANALYSES

SIMAY KOÇ[a], AND MASSIMO DEL FABBRO[b,c]

[a]Department of Endodontics, Faculty of Dentistry, Akdeniz University, Antalya, Turkey
[b]Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy
[c]IRCCS Orthopedic Institute Galeazzi, Milan, Italy

ABSTRACT

Aim

To evaluate if there is a connection between the causes of pulp necrosis (eg, caries, trauma, dental anomaly) and the success of regenerative endodontic treatment.

Materials and Methods

Electronic databases (PubMed, Scopus, Web of Science, Cochrane Central Register of Controlled Trials, Embase) were searched for studies on regenerative endodontic treatment, which used both clinical and radiographic evaluation of root maturation after at least 6 months of follow-up. The search terms "necrotic pulp", "regenerative endodontic treatment", "revascularization", and "revitalization" were combined using Boolean operators. The main Journals on endodontics and dental traumatology were additionally hand-searched. Studies were included if they specified the causes of pulp necrosis. The primary question under review was, "Does the cause of pulp necrosis affect the outcome of regenerative endodontic treatment?" Other factors such as tooth type, intracanal medicament, irrigation protocol, use of a collagen matrix, and the type of scaffold were evaluated for possible relation with the outcome. The risk-of-bias assessment for randomized and nonrandomized studies was performed separately, using a modified Cochrane Collaboration's tool and ROBINS-I tool, respectively. Meta-analysis was performed, when possible, between studies comparing treatment outcomes of teeth whose pulp necrosis had different etiology. The search strategy yielded 1197 items. After screening, 18 studies reporting 445 regenerative endodontic treatment cases were included.

Results

The overall success rate for 274 teeth with trauma etiology was 94.8%, for 95 teeth with dens evaginatus etiology was 93.1%, and for 24 teeth with caries etiology was 96%. No significant difference was found between the results of regenerative endodontic treatment among teeth with trauma, dens evaginatus, and caries etiology ($P = .055$). Meta-analysis of studies comparing teeth with caries vs dens evaginatus and those with trauma vs caries confirmed that there was no evidence for difference in outcomes.

Conclusion

Further randomized studies specifically testing such hypothesis are needed to confirm the preliminary results of this review.

CORRESPONDING AUTHOR:
Massimo Del Fabbro, Department of Biomedical, Surgical, and Dental Sciences, Università degli Studi di Milano, IRCCS Istituto Ortopedico Galeazzi, Via Riccardo Galeazzi 4, 20161 Milano, Italy.
E-mail: massimo.delfabbro@unimi.it

KEYWORDS
Pulp necrosis, Regenerative endodontic treatment, Trauma, Caries, Dental anomaly, Systematic review

Source of Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Conflict of Interest: ■.

Received 25 August 2019; revised 1 December 2019; accepted 17 January 2020

J Evid Base Dent Pract 2020: [101400]
1532-3382/$36.00

doi: https://doi.org/10.1016/j.jebdp.2020.101400

INTRODUCTION

Regenerative endodontic treatment (RET) has become a popular method for the treatment of immature teeth which have become nonvital because of some insults such as trauma, deep caries, and developmental dental anomalies.[1]

This treatment has been introduced in the past years as an alternative method to traditional apexification techniques. The aim of RET is not only to induce healing of apical periodontitis but also to regain the normal pulpal physiological functions. These include continued root development, immune competency, and normal nociception. Thus, providing the regeneration of the components and normal function of the pulp-dentin complex is the ultimate goal of this procedure.[2]

Caries is a multifactorial disease that can cause inflammation and fibrosis of the pulp tissue by penetrating the pulp with bacteria and their noxious products. Caries can cause chronic inflammation of the pulp tissue, which can progressively lose its viability. Prolonged inflammation or repeated insults reduce the ability of the pulp to repair itself, and necrosis spreads along the entire root canal space.[3,4]

Dental trauma is another etiological factor that may cause partial or complete interruption of apical blood flow in the traumatized tooth. When the apical blood flow is not restored or is inadequate, pulp necrosis can easily occur. The incidence of trauma to permanent teeth in adults is about 33%. In children, the root development of permanent teeth is usually incomplete, so pulp necrosis may also critically affect root development.[5,6]

In cases of dens evaginatus (DE), usually only a thin layer of hard tissue protects the pulp, and dental caries can easily exceed this structure and affect the pulp tissue, causing necrosis. A hard-tissue projection (enamel-covered tubercle) extends from the occlusal surface or cingulum of the affected tooth in cases of dens evaginatus. Pulp tissue extending to tubercle is present in 43% of these cases. Occlusal trauma that may cause tubercle fracture may affect the pulp tissue.[7,8] Exposure of pulp tissue can result in pulp necrosis of immature, permanent teeth, leading to impairment of root development.[8]

Apexification with calcium hydroxide ($Ca(OH)_2$) dressings is a conventional method for treatment of necrotic immature permanent teeth.[9] Calcium hydroxide, a material that promotes the placement of a root canal filling material by supporting the formation of a calcified apical barrier, also has several disadvantages such as formation of calcified barrier without root elongation and persistence of thin and short root canal walls.[10–12]

The apical barrier technique or mineral trioxide aggregate (MTA) apical plug is another apexification method which has been used more recently.[13,14] One limitation of this technique with MTA is that the root-to-crown ratio does not allow continuous root development.[15] Short and thin roots will remain in an immature permanent tooth, and MTA does not provide reinforcement of the tooth.[8]

Regenerative endodontic procedures, which were first developed by Nygaard Östby in 1961, although with limited success, are defined as biology-based procedures designed to predictably replace the lost structures of the root canal system.[13,16,17] RET comprises the following steps. Disinfection of root canal system with various recommended irrigation solutions and intracanal medicaments such as triple or double antibiotic paste (DAP), or $Ca(OH)_2$ is the first step of RET. After successful disinfection protocol, the intracanal medicament paste is removed, and apical bleeding is induced to produce a blood clot as a scaffold. Finally, coronal restoration is completed after the canal orifice is sealed with MTA.[18]

Resolution of clinical and radiographical signs and symptoms is the evidence of periapical healing, and they are assumed as the primary indicators of successful endodontic regenerative treatment.[3] There are some additional goals of this procedure for a high level of success such as increased thickness of dentin in the root canal walls and/or increase in the length of the immature root as well as positive response to the vitality test.[19]

The most important advantage of RET is the continued root development, an outcome that cannot be provided by any apexification technique.[12]

Through a preliminary literature search, it was realized that evidence about the assessment of whether the cause of pulp necrosis affects the clinical outcomes of RET is missing. Therefore, the aim of this study was to evaluate by means of a systematic review of the recent literature if there is a relation between the causes of pulp necrosis and the success of the RET.

MATERIALS AND METHODS

This systematic review and meta-analysis was carried out based on preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines.[20] The protocol of this systematic review was registered on the international prospective register of systematic reviews (PROSPERO) with registration number CRD42019140292.

Research Question

Articles that addressed the following PICO question were selected: "In patients having necrotic tooth with immature

root, which is treated with regenerative endodontic treatment, and does the cause of pulp necrosis (dental trauma, dental anomalies, and caries) effect the outcomes of regenerative endodontic treatment?" (Table 1).

Literature Search Strategy

A systematic electronic search limited to English language publications was performed in PubMed, Scopus, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), and Embase databases using a series of search terms combined with the Boolean Operators "AND" and "OR", covering the period from January 2014 to June 2019. This narrow range of dates was chosen to select only studies performed with the most updated techniques and materials and to avoiding large discrepancies in protocols, which are common when comparing studies performed a long time ago. The key words used in the electronic research were "necrotic pulp", "regenerative endodontic therapy", "revascularization", and "revitalization". The following search string was developed with the combination of relevant keywords: "(Pulp necrosis) AND (((Pulp regeneration) OR Pulp revitalization) OR Pulp revascularization).

Criteria for Study Selection

Studies were included in this systematic review if they met the following inclusion criteria:

- in vivo clinical studies (randomized clinical trials, controlled clinical trials, prospective case series, and retrospective studies) related to the outcome of regenerative endodontic therapy for immature necrotic permanent tooth with or without radiographic signs of apical pathology;
- the cause of pulp necrosis was clearly indicated;
- size of sample was given (more than 5 cases);
- the outcome was based on both clinical examination and radiographic interpretation;
- the success of the treatment was evaluated by both preoperative and postoperative comparable radiographs of each cases and clinical examination;
- follow-up of at least 6 months.

Studies in other languages, studies performed in animals, case reports and case series including less than 5 teeth, studies not involving immature necrotic permanent teeth treated with RET, or those not including the cause of pulp necrosis were not included.

Evaluation of Selected Studies

After an initial electronic search, both abstracts and titles were evaluated by 2 investigators (S.K. and M.D.F.), and in case of uncertainty, full text was read and a decision was made by joint discussion. After the initial assessment of the title and abstract, full-text evaluation of the relevant articles was performed, and the articles that were not considered eligible to inclusion criteria were excluded from the study. Disagreements concerning the inclusion of a study were discussed until a decision was obtained by consensus.

Table 1. Framework of PICO question.

Population	Intervention	Comparison	Outcome
In patients having necrotic tooth with immature root	Which is treated with regenerative endodontic treatment	Does the cause of pulp necrosis (dental trauma, dental anomalies, and caries)	Effect the clinical and radiographic outcomes of regenerative endodontic treatment?

The following information was specified for each study and recorded on a data-extraction form: author(s), journal, year of publication, study settings, study design, ethical approval, sponsor, age, sample size, diagnosis, tooth number, cause of necrosis, irrigation protocol, intracanal medication protocol, use of scaffold, use of matrix, number of visits, capping material, follow-up time, and treatment outcomes.

The methodological parameters such as the randomization method in randomized studies and the precise definition of outcomes assessment, for all studies, were also recorded.

The authors of the included studies were contacted if further information was needed for clarification of some data.

Assessment of the Risk of Bias of the Studies

The methodological quality of the selected studies was independently evaluated by 2 reviewers (S.K. and M.D.F.). The risk of bias for randomized studies was assessed using modified Cochrane Collaboration's tool. The criteria chosen were randomization, allocation concealment, blinding of the participants, examiner and outcome assessment, attrition bias, and reporting bias. Furthermore, comparability and the homogeneity of group(s) were the additional criteria specified for the assessment of bias. The risk of bias was estimated low when all criteria were met, and no more than 1 criterion was judged unclear; moderate risk of bias was defined if 2 or more criteria were judged unclear and other criteria were met. High risk of bias was considered when 1 or more criteria were not met.

For nonrandomized studies the ROBINS-I tool was used.[21] This tool evaluates 7 different domains for each study. Two

REV 5.6.0 DTD ■ YMED101400_proof ■ 14 February 2020 ■ 1:17 am ■ ce

"Pre-intervention" (bias due to confounding and bias in selection of participants into the study), 1 "At intervention" (bias in classification of interventions), and 4 "Post-intervention" (bias due to deviations from intended interventions, bias due to missing data, bias in measurement of outcomes, and bias in selection of the reported result). Each domain comprises some "signaling questions" that guide in making a decision on the risk of bias. Response options for each signaling question are as follows: "Yes", "Probably yes", "Probably no", "No", and "No information". Such responses provide the basis for domain-level judgements about risk of bias, which in turn provides the basis for an overall risk-of-bias judgment regarding a specific outcome. The latter is categorized as "Low risk", "Moderate risk", "Serious risk" and "Critical risk" of bias. The score "Low risk" corresponds to the risk of bias associated to a high-quality randomized study and is very rarely assigned to a nonrandomized study of intervention because of the many confounding factors usually present.[21]

Outcome Variables

Success of RET in permanent immature necrotic teeth with different etiology was considered the primary outcome variable. Asymptomatic teeth examined both clinically and radiographically during the follow-up period and teeth not requiring any other endodontic treatment after RET protocol were accepted as successful cases.

The secondary outcome variables included the assessment of RET success according to tooth type, disinfection procedure during the treatment such as irrigation protocol (sodium hypochlorite [NaOCl], combination of NaOCl and ethylenediaminetetraacetic acid (EDTA), combination of NaOCl and EDTA, and chlorhexidine [CHX]), intracanal medicaments (calcium hydroxide [$Ca(OH)_2$], combination of calcium hydroxide and 2% chlorhexidine gel [CHP], DAP, triple antibiotic paste [TAP]), scaffold (blood clot alone, platelet concentrate, none), and matrix used (collagen, none).

Statistical Analysis

Descriptive statistics of the included studies was performed by summarizing the total number of cases treated with each RET and the percentage of successful cases.

A meta-analysis was performed if 2 or more studies comparing the results of the treatment of necrotic teeth with different etiology were found.

The weighted mean difference between necrotic teeth caused by trauma and caries, trauma and dens evaginatus, and caries and dens evaginatus were aimed to be estimated using a random-effect model using the software RevMan (version 5.3; The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark, 2014). The results of meta-analysis were graphically represented by means of Forest plots using RET cases as the analysis unit.

Pearson's chi square or Fisher's Exact test, as appropriate, was used to perform statistical comparisons among the test groups for main outcome variables such as causes of necrosis, type of scaffold, type of intracanal medicaments, matrix type, tooth type, and irrigation procedure when meta-analysis was not practicable.

RESULTS

Study Selection

Electronic search of databases provided a total of 1197 articles. After the removal of duplicated studies and screening of titles and abstract, 26 studies were considered as eligible for this systematic review, and the full text was evaluated. Eight of them were excluded with reasons shown in the systematic flow chart (Figure 1). The general information and the study characteristics of the 18 included studies[22–39] are shown in detail in Table 2.

Four of the articles included in this study reported outcomes of teeth with necrotic pulp that had different causes of necrosis and had been treated using blood clot as scaffold. These articles that had similar study design were found eligible for meta-analysis.

Study Characteristics

In all the articles included in this study, treatments were performed at a university hospital setting, and none of them declared to have sponsors. Of the 18 articles included in the study, 8 were randomized controlled trials, 5 were prospective case series including at least 5 cases, and 5 were retrospective studies. All the cases were completed in multiple visits.

In the study with the highest sample size, 73 necrotic teeth were included,[38] while the studies with the least sample size were case series with 5 necrotic teeth treated.[28,37] A total of 445 teeth represented the population evaluated in this review. The majority of the participants were children, and the minimum follow-up period was 8 months.[28] Detailed information regarding the features of the sample and the treatment protocol for each included study is shown in Table 3.

The risk-of-bias summary of included articles is described in Figure 2 and Figure 3. As mentioned before, risk-of-bias assessment was evaluated separately for randomized studies (Figure 2) and nonrandomized studies, which included prospective case series and retrospective studies (Figure 3). As a result of this evaluation, of the 8 randomized studies, 3 studies were judged at low risk of bias, and 5 studies at moderate risk of bias. On the other

Figure 1. The systematic flow chart of the study selection process.

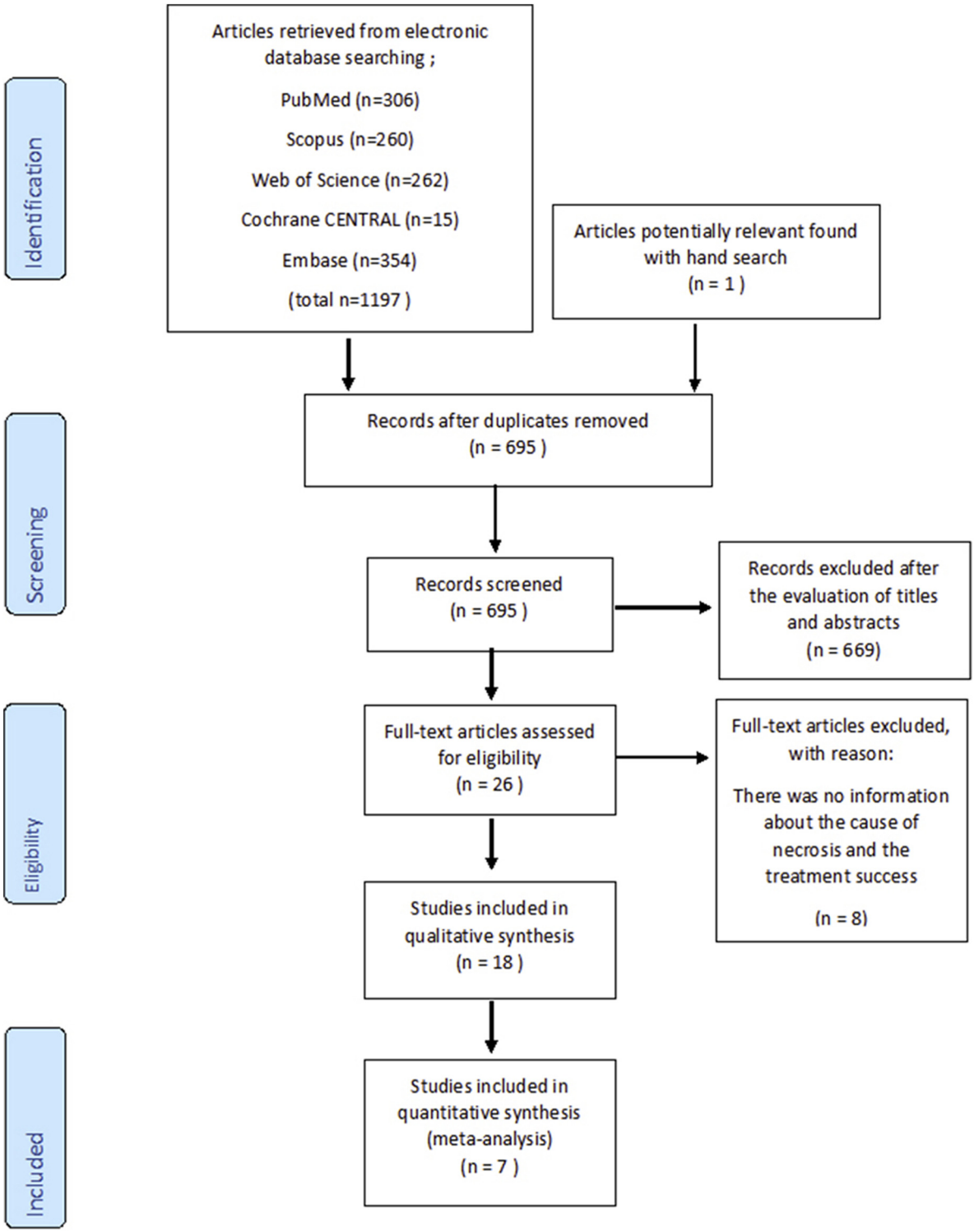


Table 2. Preoperative factors and general information of RET cases included in this study.

Author and journal	Year	Study setting	Study design	Ethical approval	Sponsor	Age	Sample size	Follow-up duration
Nagata et al., JOE[22]	2014	University	Randomized controlled study	Yes	No	7-17	23	15 mo
Kahler et al., JOE[23]	2014	University	Prospective case series	Yes	No	10.5	16	15 mo
Saoud et al., JOE[24]	2015	University	Prospective case series	No	No	11.3	20	12 mo
Bezgin et al., JOE[25]	2015	University	Randomized controlled study	Yes	No	9.95	20	18 mo
Sharma and Mittal, Saudi Endod J[26]	2016	University	Randomized controlled study	Yes	No	10-25	16	12 mo
Lin et al., JOE[27]	2017	University	Randomized controlled study	Yes	No	10.5 ± 1.8	69	12 mo
Carmen et al., Hindawi[28]	2017	University	Prospective case series	No	No	6.5-8.5	5	8-12 mo
Alagl et al., J Int Med Res[29]	2017	University	Randomized controlled study	Yes	No	9-11	30	12 mo
Silujai and Linsuwanont, JOE[30]	2017	University	Retrospective study	Yes	No	8-23	17	44-46 mo
Li et al., JOE[31]	2017	University	Prospective case series	No	No	10.6 ± 0.99	20	16.1 ± 8.8 mo
Linsuwanont et al., IEJ[32]	2017	University	Retrospective study	Yes	No	7-23	15	Min. 12 mo
Jiang et al., JOE[33]	2017	University	Randomized controlled study	Yes	No	9.8 ± 1.5 (control), 10.3 ± 1.9 (test)	43	16.1 ± 8.8 mo (control), 15.0 ± 5.8 mo (test)
Bukhari et al., JOE[34]	2016	University	Retrospective study	No	No	8-31	28	12 mo
Nazzal et al., IEJ[35]	2018	University	A prospective case series	Yes	No	8.3	12	22 mo
Lv et al., BMC Oral Health[36]	2018	University	Retrospective study	Yes	No	9-14	11	12 mo
Meschi et al., JOE[37]	2018	University	Retrospective study	Yes	No	8-12	5	38.2 ± 9.3 mo
Ulusoy et al., JOE[38]	2019	University	Randomized controlled study	No	No	8-11	73	28.25 ± 1.20 mo
Ragab et al., J Clin Pediatr Dent[39]	2019	University	Randomized controlled study	Yes	No	9.86 ± 1.55	22	12 mo

Table 3. Intraoperative information of RET cases included in this study.

Author and journal	Sample	Tooth	Cause of necrosis	Irrigation protocol	Intracanal medication	Scaffold	Use of matrix	Number of visit	Capping material	Success	Failure
Nagata, JOE[22]	12	Maxillary incisors	Trauma	6% NaOCl (20 ml.), 2% CHX (10 ml), 17% (3 ml) EDTA	TAP	BC	Collagen barrier	Multiple	MTA	12	0
	11	Maxillary incisors	Trauma	6% NaOCl (20 ml), 2% CHX (10 ml), 17% (3 ml) EDTA	CHP	BC	Collagen barrier	Multiple	MTA	10	1
Kahler-JOE[23]	13	Maxillary central incisors	Trauma	1% NaOCl	TAP	BC	No	Multiple	MTA	13	0
	3	Mandibular second premolar	Dens evaginatus	1% NaOCl	TAP	BC	No	Multiple	MTA	3	0
Saoud-JOE[24]	20	Anterior teeth	Trauma	2.5% NaOCl	TAP	BC	No	Multiple	MTA	20	0
Bezgin-JOE[25]	4	Premolars	Caries	2.5% NaOCl (20 ml), 0.12% CHX (10 ml), 5% EDTA (20 ml)	Metron + cipro + cefaclor	PRP	No	Multiple	MTA	4	0
	6	Incisors	Trauma	2.5% NaOCl (20 ml), 0.12% CHX (10 ml), 5% EDTA (20 ml)	Metron + cipro + cefaclor	PRP	No	Multiple	MTA	6	0
	2	Premolars	Caries	2.5% NaOCl (20 ml), 0.12% CHX (10 ml), 5% EDTA (20 ml)	Metron + cipro + cefaclor	BC	No	Multiple	MTA	2	0
	8	Incisors	Trauma	2.5% NaOCl (20 ml), 0.12% CHX (10 ml), 5% EDTA (20 ml)	Metron + cipro + cefaclor	BC	No	Multiple	MTA	7	1
Sharma, Saudi Endod J[26]	4	Maxillary incisors	Trauma	2.5% NaOCl	TAP	BC	No	Multiple	Glass ionomer	4	0
	4	Maxillary incisors	Trauma	2.5% NaOCl	TAP	PRF	No	Multiple	Glass ionomer	4l	0
	4	Maxillary incisors	Trauma	2.5% NaOCl	TAP	BC + collagen	No	Multiple	Glass ionomer	4	0

(continued)

REV 5.6.0 DTD ■ YMED101400_proof ■ 14 February 2020 ■ 1:17 am ■ ce

Table 3. (continued)

Author and journal	Sample	Tooth	Cause of necrosis	Irrigation protocol	Intracanal medication	Scaffold	Use of matrix	Number of visit	Capping material	Success	Failure
	4	Maxillary incisors	Trauma	2.5% NaOCl	TAP	BC + Poly-Lactic-co-glycolic acid	No	Multiple	Glass ionomer	4	0
Lin, JOE[27]	48	Premolar	Dens evaginatus	1.5% NaOCl (20 ml) and 17% (20 ml) EDTA	TAP	BC	Collagen barrier	Multiple	MTA	47	1/
	21	Central incisor	Trauma	1.5% NaOCl (20 ml) and 17% (20 ml) EDTA	TAP	BC	Collagen barrier	Multiple	MTA	15	6
Carmen, Hindawi[28]	1	Mandibular first molar	Caries	5% NaOCl	TAP	BC	No	Multiple	MTA	1	0
	1	Maxillary central incisor	Trauma	5% NaOCl	TAP	BC	No	Multiple	MTA	1	0
	1	Mandibular first molar	Caries	6% NaOCl	TAP	BC	No	Multiple	MTA	1	0
	1	Mandibular first molar	Caries	7% NaOCl	TAP	BC	No	Multiple	MTA	1	0
	1	Mandibular first molar	Caries	8% NaOCl	TAP	BC	No	Multiple	MTA	1	0
Alagl, J Int Med Res[29]	12	Incisor	Trauma	2.5% NaOCl (20 ml), 0.12 % CHX (10 ml), 17% (20 ml) EDTA	TAP	PRP	No	Multiple	MTA	12	0
	3	Premolar	Caries	2.5% NaOCl (20 ml), 0.12 % CHX (10 ml), 17% (20 ml) EDTA	TAP	PRP	No	Multiple	MTA	3	0
	12	Incisor	Trauma	2.5% NaOCl (20 ml), 0.12 % CHX (10 ml), 17% (20 ml) EDTA	TAP	BC	No	Multiple	MTA	12	0
	3	Premolar	Caries	2.5% NaOCl (20 ml), 0.12 % CHX (10 ml), 17% (20 ml) EDTA	TAP	BC	No	Multiple	MTA	3	0
Silujai, JOE[30]	5	Maxillary incisors	Trauma	1.5-2.5% NaOCl, 17% EDTA	$Ca(OH)_2$ or TAP	BC	No	Multiple	MTA	4	1

	10	Mandibular second premolar	Dens evaginatus	1.5-2.5% NaOCl, 17% EDTA	$Ca(OH)_2$ or TAP	BC	No	Multiple	MTA	7	3
	2	Mandibular first molar	Caries	1.5-2.5% NaOCl, 17% EDTA	$Ca(OH)_2$ or TAP	BC	No	Multiple	MTA	2	0
Li, JOE[31]	20	Premolars	Dens evaginatus	2.5% NaOCl	$Ca(OH)_2$	BC	No	Multiple	MTA	20	0
Linsuwanont, IEJ[32]	8	Mandibular premolars	Dens evaginatus	NaOCl and EDTA	$Ca(OH)_2$ or TAP	BC	No	Multiple	MTA	5	3
	5	Maxillary incisors	Trauma	NaOCl and EDTA	$Ca(OH)_2$ or TAP	BC	No	Multiple	MTA	4	1
	2	Mandibular first molar	Caries	NaOCl and EDTA	$Ca(OH)_2$ or TAP	BC	No	Multiple	MTA	2	0
Jiang, JOE[33]	9	Anterior teeth	Trauma	1.25% NaOCl (20 ml), 17% (20 ml) EDTA	$Ca(OH)_2$	BC	No	Multiple	MTA	9	0
	5	Anterior teeth	Trauma	1.25% NaOCl (20 ml), 17% (20 ml) EDTA	$Ca(OH)_2$	BC	Bio-Gide	Multiple	MTA	5	0
	13	Premolars	Broken central cusp	1.25% NaOCl (20 ml), 17% (20 ml) EDTA	$Ca(OH)_2$	BC	No	Multiple	MTA	13	0
	16	Premolars	Broken central cusp	1.25% NaOCl (20 ml), 17% (20 ml) EDTA	$Ca(OH)_2$	BC	Bio-Gide	Multiple	MTA	16	0
Bukhari, JOE[34]	5	Molars	Caries	3% NaOCl + 17% EDTA	TAP	No	Collagen barrier or not	Multiple	MTA or bioceramic	4	1
	3	Premolars	Dental Anomaly DE, talon	3% NaOCl + 17% EDTA	TAP	No	Collagen barrier or not	Multiple	MTA or bioceramic	3	0
	20	Anterior teeth	Trauma	3% NaOCl + 17% EDTA	TAP	No	Collagen barrier or not	Multiple	MTA or bioceramic	18	2
Nazzal, IEJ[35]	12	Maxillary incisors	Trauma	0.5% NaOCl	DAP	BC	No	Multiple	Portland cement	12	0

(continued)

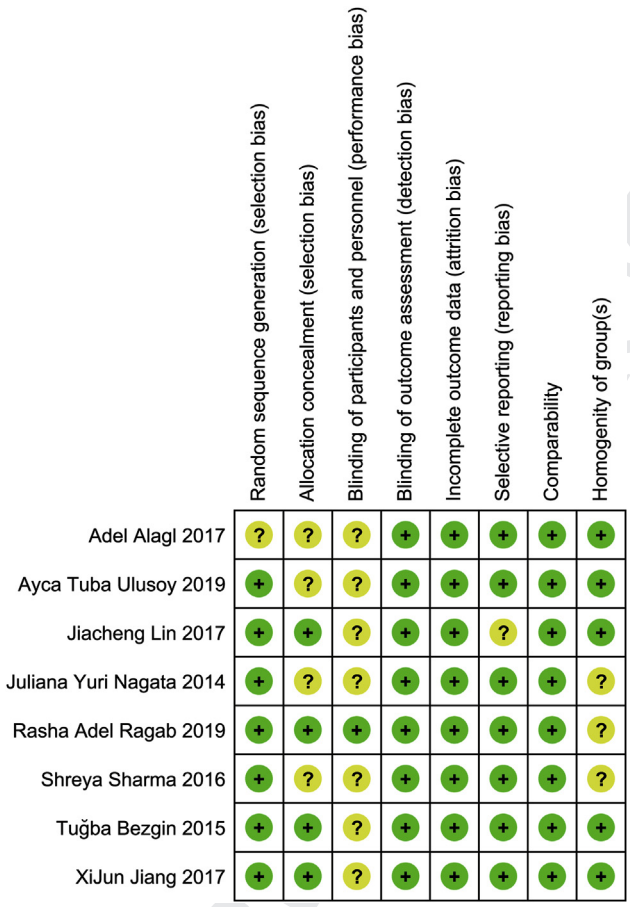
REV 5.6.0 DTD ■ YMED101400_proof ■ 14 February 2020 ■ 1:17 am ■ ce

Table 3. (continued)

Author and journal	Sample	Tooth	Cause of necrosis	Irrigation protocol	Intracanal medication	Scaffold	Use of matrix	Number of visit	Capping material	Success	Failure
Lv, BMC Oral Health[36]	5	Mandibular premolars	Dens evaginatus	1% NaOCl (20 ml.), 17% (10 ml) EDTA	TAP	BC	Collagen barrier	Multiple	MTA	5	0
	1	Central incisors	Tooth fracture	1% NaOCl (20 ml), 17% (10 ml) EDTA	TAP	PRF	No	Multiple	MTA	1	0
	4	Mandibular premolars	Dens evaginatus	1% NaOCl (20 ml), 17% (10 ml) EDTA	TAP	BC	Collagen barrier	Multiple	MTA	4	0
	1	Central incisors	Tooth fracture	1% NaOCl (20 ml), 17% (10 ml) EDTA	TAP	PRF	No	Multiple	MTA	1	0
Meschi, JOE[37]	1	Maxillary incisors	Dens evaginatus	2.5% NaOCl (20 ml), 17% (5 ml) EDTA	DAP	BC	Collagen barrier	Multiple	MTA	1	0
	4	Maxillary incisors	Trauma	2.5% NaOCl (20 ml), 17% (5 ml) EDTA	DAP	BC	Collagen barrier	Multiple	MTA	3	1
Ulusoy, JOE[38]	18	Maxillary incisors	Trauma	1.25% NaOCl (20 ml), 17% (10 ml) EDTA	TAP	PRP	No	Multiple	MTA	18	0
	17	Maxillary incisors	Trauma	1.25% NaOCl (20 ml), 17% (10 ml) EDTA	TAP	PRF	No	Multiple	MTA	16	1
	17	Maxillary incisors	Trauma	1.25% NaOCl (20 ml), 17% (10 ml) EDTA	TAP	PP	No	Multiple	MTA	17	0
	21	Maxillary incisors	Trauma	1.25% NaOCl (20 ml), 17% (1 ml) EDTA	TAP	BC	No	Multiple	MTA	20	1
Ragab, J Clin Pediatr Dent[39]	11	Maxillary incisors	Trauma	5% NaOCl (20 ml)	DAP	BC	No	Multiple	Gray MTA	11	0
	11	Maxillary incisors	Trauma	5% NaOCl (20 ml)	DAP	PRF	No	Multiple	Gray MTA	11	0

BC, blood clot; $Ca(OH)_2$, calcium hydroxide; CHP, combination of calcium hydroxide and 2% chlorhexidine gel; CHX, chlorhexidine; DAP, double antibiotic paste; EDTA, ethylenediaminetetraacetic acid; MTA, mineral trioxide aggregate; NaOCl, sodium hypochlorite; PP, platelet pellet; PRF, platelet-rich fibrin; PRP, platelet-rich plasma; TAP, triple antibiotic paste.

Figure 2. Risk-of-bias summary: review authors' judgements about each risk-of-bias item for randomized studies.



hand, of the 10 nonrandomized studies, 4 were judged at critical risk of bias and 6 at serious risk of bias.

Etiology of the Pulp Necrosis

Out of a total of 445 samples included in this review, RET was successful in 422 cases (94.5%). In 289 cases (64.94%), the etiology was dental trauma, in 102 cases (22.92%), the necrosis was caused by DE, in 25 cases (5.61%) by dental caries, and in 29 cases (6.51%) by broken central cusp (Table 4).

When evaluating the success rate of RET for each etiology, trauma, DE, and caries had 94.8%, 93.1%, and 96% success rate, respectively (Table 4). All the teeth with broken central cusp were found successful at the end of the follow-up period.

There was not a significant difference between the results of RET among the teeth with trauma, DE, and caries etiology ($P = .055$). The teeth with broken central cusp were not included to this statistical analyze because this etiology was reported in only 1 study, and detailed information could not be obtained from the author of the article.

Type of Teeth With Pulp Necrosis

Of the 290 anterior teeth treated with RET, 275 (94.8%) were successful and 15 were unsuccessful. Of the other teeth, 142 were premolar (135 [95.1%] successful, 7 unsuccessful) and 13 were molar (12 [92.3%] successful, 1 unsuccessful). No significant difference in success rate was found between tooth type ($P = .063$).

Disinfection Protocol Used in RET Cases

All the RET cases reported the use of sodium hypochlorite (NaOCl) as the main irrigation solution with a concentration ranging from 1% to 5% (Table 2). In 111 cases, NaOCl was the only irrigation solution without any failed case. NaOCl and EDTA combination was used in 261 cases, of which 240 (92%) were successful at the end of follow-up period.

Figure 3. Risk-of-bias summary: review authors' judgements about each risk-of-bias item for nonrandomized studies.

Study	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended intervention	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result
Kahler 2014	serious	moderate	serious	serious	low	moderate	moderate
Saoud 2015	moderate	moderate	serious	low	low	moderate	low
Bukhari 2016	serious	critical	moderate	moderate	low	low	low
Li 2017	moderate	moderate	serious	low	low	moderate	low
Linsuwanont 2017	critical	critical	serious	moderate	low	low	low
Lopez 2017	serious	moderate	serious	serious	low	serious	low
Silujjai 2017	critical	critical	moderate	moderate	moderate	low	low
Hongbing Lv 2018	serious	critical	moderate	moderate	low	serious	low
Meschi 2018	serious	serious	serious	low	low	low	low
Nazzal 2018	moderate	moderate	serious	moderate	serious	serious	low

Of the 73 teeth irrigated with NaOCl, EDTA, and CHX combination, 71 (97.3%) were successful and 2 failed. There was a significant difference in success rate among the irrigation protocols (P = .001). The highest success rate was observed in cases where the only irrigation solution was NaOCl. It has been shown that the success rate is the lowest in cases where NaOCl and EDTA are used together.

In addition, when the effect of EDTA use on the success of RET was evaluated, 23 of 311 teeth irrigated with different percentages of EDTA solution failed, but no failures occurred in teeth that were not irrigated with EDTA. A significant difference was found between EDTA(+) and EDTA(−) groups (P = .001). In the EDTA(+) group, the percentage of solution was 17% for 299 teeth, 5% for 20 teeth, and unknown for 15 teeth. There was no significant difference among the various concentrations of EDTA (P = .38).

Various combinations of antibiotics and $Ca(OH)_2$ were used as an intracanal medicament.

A combination of calcium hydroxide and 2% CHX gel (CHP) was used as an intracanal medicament in 11 cases, 10 of which (90.9%) were successful. $Ca(OH)_2$ was used in 63 cases and resulted in 100% success. DAP was used in 39 cases, and TAP was used in 300 cases, of which 38 (97.4%) and 287 (95.7%) cases were successful, respectively. In the remaining 32 cases, the intracanal medicament was not specified in detail. There was a significant difference among all intracanal medicament groups (P = .001), whereas there was no significant difference between antibiotic groups (P = .38). The success rate was shown to be highest in cases where $Ca(OH)_2$ was used as an intracanal medicament.

Scaffold and Matrix Used in RET

Blood clot (BC) was used as a scaffold in 315 (94%) cases. Platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and platelet pellet were the platelet concentrate (PC) scaffolds used in 94 cases with 100%, 97.1%, and 100% success rate, respectively. Blood clot and sterile collagen sponge were used together in 4 cases, and blood clot and poly-lactic-co-glycolic-acid were used together in other 4 cases. Successful results were obtained in all cases of BC + additives. Of 28 teeth in which no scaffold was used, 25 (89.3%) were successful. There was a significant difference among the BC group, PC group, BC + additives group, and the group without any scaffold material (P = .002). Furthermore, there was also a significant difference between BC group and PC group (P = .03), in favor of the latter.

Different brands of collagen matrix were used in 127 teeth, and successful results were obtained from 118 of them (92.9%). In 290 teeth, no matrix was used, and 279 of them (96.2%) were successful. On the other hand, there was no information about the matrix material in 28 teeth. No difference was found between the 2 groups (P = .15).

Meta-analyses of Primary Outcomes

Figure 4 is a forest plot showing the results of a meta-analysis aggregating the data of 4 studies[25,29,30,32] that

REV 5.6.0 DTD ■ YMED101400_proof ■ 14 February 2020 ■ 1:17 am ■ ce

Table 4. Comparison of the factors that effect the outcome of RET.

Factors	Success (n = 422)	Failed (n = 23)	*P* value
Cause of necrosis			
Trauma	274	15	
DE	95	7	.055
Caries	24	1	
Tooth type			
Anterior	275	15	
Premolar	135	7	.067
Molar	12	1	
Intracanal medicament			
$Ca(OH)_2$	63	0	
CHP	10	1	.008
DAP	38	1	
TAP	287	13	
Irrigation protocol			
NaOCl	111	0	
NaOCl + EDTA	240	21	.001
NaOCl + EDTA + CHX	71	2	
EDTA irrigation			
EDTA(+)	311	23	.001
EDTA(−)	111	0	
Matrix			
Collagen	118	9	.15
No	279	11	
Scaffold			
BC	296	19	
Platelet concent.	93	1	.002
BC + additives	8	0	
No	25	3	

BC, blood clot; BC + additives, BC + collagen or BC + Poly-Lactic-co-glycolic acid; $Ca(OH)_2$, calcium hydroxide; CHP, combination of calcium hydroxide and 2% chlorhexidine gel; CHX, chlorhexidine; DAP, double antibiotic paste; DE, dens evaginatus; EDTA, ethylenediaminetetra-acetic acid; EDTA(+), teeth irrigated with EDTA; EDTA(−), teeth irrigated without EDTA; MTA, mineral trioxide aggregate; n, sample size; NaOCl, sodium hypochlorite; TAP, triple antibiotic paste; platelet concent., platelet-rich plasma or platelet-rich fibrin or platelet pellet.
[a]*P* values calculated via the Pearson's chi square or the Fisher's exact test.

provided the outcomes of RET of necrotic teeth that had different etiology (trauma vs caries). No heterogeneity among studies was detected ($P = .98$, $I^2 = 0\%$). There was no evidence of a difference between groups ($P = .74$).

Figure 5 is a forest plot showing the results of a meta-analysis aggregating the data of 2 studies[30,32] that provided the outcomes of RET of necrotic teeth that had different etiology (DE vs caries). No heterogeneity among studies was detected ($P = .92$, $I^2 = 0\%$). There was no evidence of a difference between groups ($P = .44$).

Only 1 study was found in which necrotic teeth with traumatic etiology ($n = 21$) were compared to DE etiology ($n = 48$).[27] In this study, teeth with DE etiology had a significantly better prognosis than teeth in which necrosis was caused by trauma ($P = .009$). Because only that study reported the trauma vs DE comparison, no meta-analysis was performed. Indeed, in another study, there were 4 teeth with etiology trauma (1 failure) and only 1 tooth with etiology DE,[38] but owing to the very low sample size, this study was not considered for meta-analysis.

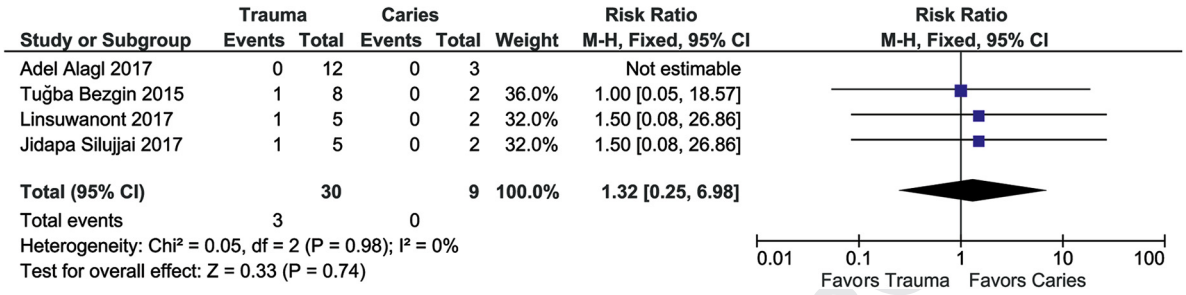
All the aforementioned studies used only blood clot as scaffold. Studies using PCs could not be submitted to meta-analysis because they reported no failures and therefore were considered as "not estimable" by the software.

DISCUSSION

RET has become a widely used method in clinical practice, especially in permanent teeth with open apices and a thin dentin wall. Although many different treatment protocols have been proposed for this treatment method, a consensus has not been reached yet, and there is no standard

REV 5.6.0 DTD ■ YMED101400_proof ■ 14 February 2020 ■ 1:17 am ■ ce

Figure 4. Meta-analysis using a random-effect model for assessing the effect of trauma and caries etiology to RET success in randomized studies. Overall analysis exhibited no significant positive effect of both etiologies in enhancing the success rate in RET cases ($P = .74$). Slight heterogeneity among studies was found ($I^2 = 0\%$, $P = .98$).



treatment protocol that has been proved successful by an adequate number of evidence-based clinical studies.

The present study aimed to investigate the hypothesis that the cause of pulp necrosis might affect the success of RET, through a systematic analysis of the recent published literature. This study may be useful to the clinicians for the management of teeth presenting with necrotic pulp, which are a candidate to RET.

We have included the studies published between January 2014 and June 2019 to evaluate the results of recent RET protocols, which likely are more homogeneous among them than the less recent studies, mainly composed of isolated clinical case reports. This study encountered some limitations, that is, different RET protocols were applied and different success criteria were identified among the included studies. It was decided not to set strict limitations regarding the study design, to consider a wider database. However, all case reports were excluded. In fact, in such articles only successful cases are usually reported, and adding such studies makes it difficult to estimate the actual success rate of regenerative treatments.

Asymptomatic teeth examined both clinically and radiographically during the follow-up period, and teeth not requiring any other endodontic treatment after RET protocol were accepted as successful cases.

Diogenes et al.[2] reported that in all cases treated with RET, trauma (34%) is the most common etiology for pulpal necrosis, followed by developmental dental anomalies (25%). The most common dental anomaly requiring RET was dens evaginatus (23%), present usually in mandibular premolars, whose incidence is 2.4%-5% in all populations.[2]

In the present study, no relation was found between the success rate of RET and the etiology of pulp necrosis such as trauma, DE, and caries. Lin et al.[27] reported that the prognosis of teeth after RET in the DE group was better than that in the trauma group. They indicated that the

Figure 5. Meta-analysis using a random-effect model for assessing the effect of caries and DE etiology to RET success in randomized studies. Overall analysis exhibited no significant positive effect of both etiologies in enhancing the success rate in RET cases ($P = .44$). Slight heterogeneity among studies was found ($I^2 = 0\%$, $P = .92$).

apical papilla stem cells and Hertwig epithelial root sheath can be damaged due to trauma and longstanding periapical infections which may cause negative treatment outcomes.[40] Although the viability of Hertwig epithelial root sheath, apical papilla, or periodontal ligament cells is thought to be responsible for root development and may affect RET success, the conditions under which they can sustain their viability have not been explained apparently.[41] On the other hand, Linsuwanont et al.,[32] in agreement with our results, found that there is no correlation among the etiology of pulp necrosis, the amount of root dimensional changes, and success rate after RET. To our knowledge, this is the first systematic review and meta-analyses investigating the relation between the outcomes of RET and etiology of pulp necrosis.

The American Association of Endodontists considered that disinfection of root canal system should only be provided by irrigants and intracanal medicament without any mechanical instrumentation to avoid damaging stem cells around periapical tissue.[42] Sodium hypochlorite (NaOCl) is the only irrigation solution used alone or in combination with other solutions such as CHX and EDTA in RET cases, and it is included in 97% of all reported RET cases.[43]

An in vitro study reported that irrigation with a high concentration of NaOCl has a severe negative effect on survival and differentiation of apical papilla stem cells. Low concentrated NaOCl (1.5%) irrigation followed by EDTA (17%) irrigation was suggested to minimize the cytotoxic effect of NaOCl on stem cells[44] and also induce releasing of dentinal signaling molecules.[45] Only in the most recent studies, EDTA was systematically included in the final irrigation protocol. However, in spite of EDTA not being included in any reported RET cases before 2012, most of these studies consistently reported successful outcomes.[43] According to results of the present review, there was no failed cases when EDTA was not used as an irrigation solution, and this is significantly different compared with cases irrigated with EDTA. Mollashahi et al.[46] assessed the effect of some irrigating solutions on stem cells from the human apical papilla (SCAP). They showed that CHX had the lowest cytotoxicity compared with EDTA, MTAD, QMix, and NaOCl, and its cytotoxicity did not change over time compared with other solutions. However, this is completely opposite in respect to what was reported in Trevino et al.'s study.[47] Mollashahi et al.[46] explained this controversy by stating that the culture dishes (compared to in vivo study) were not sufficient to evaluate the interaction effects of host tissues (dentin and cementum) on stem cells. When evaluating this result, it should be considered that EDTA has only recently become a frequently used solution, and cytotoxicity investigations were conventionally performed by in vitro studies. In addition, a study that investigated the effect of residual EDTA on BC formation in RET showed that the final irrigation with saline after EDTA irrigation may effectively promote BC formation.[48]

Antibiotic paste is the most preferred intracanal medicament (53%), and also TAP (51%) is the most commonly used one for the published RET cases.[2] Ruparel et al.[49] in 2012 reported that although all antibiotic mixtures with different concentrations have a deleterious effect on SCAP cells, $Ca(OH)_2$ used as an intracanal medicament at all tested concentrations promotes the survival and proliferation of these cells. The result of that study is consistent with that of the present study, in which the cases treated with $Ca(OH)_2$ displayed the greatest success rate, and there was no significant difference between the cases treated with TAP or DAP. A study reported by Diogenes et al.[2] indicated that $Ca(OH)_2$ can promote the proliferation of SCAP, and based on this evidence, they reformed their RET protocol preferring $Ca(OH)_2$ as a first choice of intracanal medicament. Development of bacterial resistance and clinical side effects such as minocycline-dependent crown coloration are the main disadvantages of the intracanal antibiotic use.[50,51] Perron et al.[52] showed that combined antibiotics used in treatment of mixed microorganisms in a short duration cause the formation of antibiotic resistance. Therefore, one of the route of systemic sensitization is root canal system which can cause severe systemic allergic reaction.[53] Another study was aimed to compare TAP and CHP for RET in traumatized teeth and demonstrated that similar clinical and radiographical results can be obtained for both groups, except that more teeth had crown discoloration in the TAP group.[22]

All the studies that evaluated the effect of combined antibiotic pastes on apical stem cells were in vitro studies. Considering all the disadvantages and uncertainness, the use of antibiotic paste as an intracanal medicament should be avoided until sufficient evidence-based reports are available, as declared in the ESE position statement.[54]

PRP and PRF are PCs that have been recently used in RET cases, under the hypothesis that the high content of growth factors in the alfa granules of platelets may provide a beneficial input to the regenerative process.[39] Therefore, they might increase the success in RET cases. A recent study evaluated the effect of PRP on periapical tissue healing and RET.[39] It reported that, except for no effect on the increase in root length, the treatment with PRP was significantly more successful than the classic protocol using BC.[29] On the other hand, Bezgin et al.[25] did not found any significant difference for treatment outcomes between PRP and BC groups. In a randomized controlled study investigating the efficacy of PRF in RET cases, Ragab et al.[39] reported that PRF might help to improve

the outcomes but was not essential for healing, while another cohort study by Lv et al.[36] consistently found no significant difference between PRF and BC groups in terms of clinical and radiographical outcomes. Ulusoy et al.[38] compared RET protocols using PRP, PRF, platelet pellet, and BC and reported that all treatment groups had similar radiographic outcomes regarding the assessment of all root dimensions. Nevertheless, in another clinical study, PRF and collagen as scaffolds showed better results than BC alone and poly-lactic-co-glycolic-acid for inducing apexogenesis.[26] The authors explained this result with the better bioactivity of PRF and collagen with respect to BC alone, which can only form a weak fibrin mesh and does not contain concentrated growth factors.[26] PRF is also found useful to place MTA properly to an optimal level of root canal applying only a light pressure.[39] Most studies reported that there is no significant difference in clinical outcomes between BC and PC groups. However, this review displayed a significant difference among the groups treated with BC, PC, and BC + additives and treated without any scaffold. Furthermore, when we compared the outcomes of RET for BC and PC, success rate of PC group was significantly higher than that of BC group. When evaluating these results, it should be taken into consideration that studies using PCs represent less than one-third of the total cases included in this study, so any comparison should be made cautiously.

The use of collagen matrix in the RET was shown to facilitate the placement of MTA at the desired level and to assist in the formation of a blood clot that was oozing from periapical tissue when adequate bleeding is not achieved.[55,56] In addition, improvement of revascularization rate and induction of growth factors release are the other opportunities provided by bioactive property of collagen.[57] Jiang et al.[33] reported that the improvement of success rate and maturation of the apical third of root are not significantly different. On the other hand, collagen matrix has an advantage in avoiding cervical root fractures by promoting the dentinal wall thickness increase in the middle third of root. According to our review, the use of collagen matrix did not reveal a remarkable enhancement of the success rate in RET cases. However, the authors indicated that, especially in wide root canals, collagen membrane is very convenient for the placement of the sealing materials.[33]

Limitations

The main limitation of this review is that only a reduced number of studies (4 out of 18) provided comparative results about RET of teeth with necrotic pulp and different etiology. So, the results of meta-analyses are based on a very limited number of cases. Furthermore, in very few studies, the primary aim coincided with that of the present review, meaning that even if the study was a randomized trial, the groups actually might not be randomized according to the etiology of pulp necrosis. The effect of different etiology on RET outcome could not be estimated for cases using platelet concentrate scaffolds because of a small sample size and especially due to lack of failures. Finally, half of the included studies were nonrandomized and were judged at high risk of bias. The low level of evidence on one side suggests that results of the review must be interpreted with caution and, on the other side, calls for more evidence-based studies to provide a reliable answer to the main clinical question of the review.

CONCLUSION

The outcomes of RET of teeth with necrotic pulp have been evaluated through many different protocols and study designs. This systematic review of the current clinical evidence found that there is no evidence to support the hypothesis that the etiology of pulp necrosis may affect the outcome of RET. Further randomized studies specifically testing such hypothesis are needed to confirm the preliminary results of the present review. In addition, the results of this study should be evaluated with caution to these factors because information about the irrigation time for each solution used during the treatment, the presence of periapical lesion, and how long the tooth had been infected is lacking. However, it is an undeniable fact that RET results are highly successful, and this treatment method for immature necrotic teeth is becoming more and more popular among clinicians. Clinicians should closely follow up current RET procedures and keep in mind that RET can be successfully applied to any necrotic immature teeth with RET indication, regardless of the etiology of pulp necrosis.

REFERENCES

1. Diogenes A, Ruparel NB, Shiloah Y, Hargreaves KM. Regenerative endodontics: a way forward. J Am Dent Assoc 2016;147:372-80.
2. Diogenes A, Henry MA, Teixeira FB, Hargreaves KM. An update on clinical regenerative endodontics. Endod Top 2013;28:2-23.
3. Mohammadi Z. Strategies to manage permanent non-vital teeth with open apices: a clinical update. Int Dent J 2011;61:25-30.
4. Abbott P, Yu C. A clinical classification of the status of the pulp and the root canal system. Aust Dent J 2007;52:S17-31.
5. Chen X, Bao Z-F, Liu Y, Liu M, Jin X-Q, Xu X-B. Regenerative endodontic treatment of an immature permanent tooth at an early stage of root development: a case report. J Endod 2013;39:719-22.
6. Rafter M. Apexification: a review. Dental Traumatol 2005;21:1-8.

7. Hargreaves KM, Diogenes A, Teixeira FB. Treatment options: biological basis of regenerative endodontic procedures. J Endod 2013;35:129-40.

8. Flanagan TA. What can cause the pulps of immature, permanent teeth with open apices to become necrotic and what treatment options are available for these teeth. Aust Dent J 2014;40:95-100.

9. Gaitonde P, Bishop K. Apexification with mineral trioxide aggregate: an overview of the material and technique. Eur J Prosthodont Restor Dent 2007;15:41.

10. Huang GJ. Apexification: the beginning of its end. Int Endod J 2009;42:855-66.

11. Aggarwal V, Miglani S, Singla M. Conventional apexification and revascularization induced maturogenesis of two non-vital, immature teeth in same patient: 24 months follow up of a case. J Conserv Dent 2012;15:68.

12. Shin S, Albert J, Mortman R. One step pulp revascularization treatment of an immature permanent tooth with chronic apical abscess: a case report. Int Endod J 2009;42:1118-26.

13. Jyothi M. Management of immature teeth—A paradigm shift from apexification to apexogenesis. Environment 2001;2:3.

14. McTigue DJ, Subramanian K, Kumar A. Case series: management of immature permanent teeth with pulpal necrosis: a case series. Pediatr Dent 2013;35:55-60.

15. Cehreli ZC, Sara S, Uysal S, Turgut MD. MTA apical plugs in the treatment of traumatized immature teeth with large periapical lesions. Dental Traumatol 2011;27:59-62.

16. Thomson A, Kahler B. Regenerative endodontics—biologically-based treatment for immature permanent teeth: a case report and review of the literature. Aust Dent J 2010;55:446-52.

17. Östby BN. The role of the blood clot in endodontic therapy an experimental histologic study. Acta Odont Scand 1961;19:323-53.

18. Antunes LS, Salles AG, Gomes CC, Andrade TB, Delmindo MP, Antunes LA. The effectiveness of pulp revascularization in root formation of necrotic immature permanent teeth: a systematic review. Acta Odont Scand 2016;74:161-9.

19. Geisler TM. Clinical considerations for regenerative endodontic procedures. Dental Clin 2012;56:603-26.

20. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 2009;6:e1000100.

21. Sterne JAC, Hernan MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ 2016;355:i4919.

22. Nagata JY, de Almeida Gomes BPF, Lima TFR, et al. Traumatized immature teeth treated with 2 protocols of pulp revascularization. J Endod 2014;40:606-12.

23. Kahler B, Sonali M, Moule A, et al. Revascularization outcomes: a prospective analysis of 16 consecutive cases. J Endod 2014;40:333-8.

24. Saoud TM, Martin G, Chen Y-HM, et al. Treatment of mature permanent teeth with necrotic pulps and apical periodontitis using regenerative endodontic procedures: a case series. J Endod 2016;42:57-65.

25. 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.

26. Sharma S, Mittal N. A comparative evaluation of natural and artificial scaffolds in regenerative endodontics: a clinical study. Saudi Endod J 2016;6:9.

27. Lin J, Zeng Q, Wei X, et al. Regenerative endodontics versus apexification in immature permanent teeth with apical periodontitis: a prospective randomized controlled study. J Endod 2017;43:1821-7.

28. Carmen L, Asunción M, Beatriz S, Rosa Y-V. Revascularization in Immature Permanent Teeth with Necrotic Pulp and Apical Pathology: Case Series. Case Reports in Dentistry 2017; 2017.

29. Alagl A, Bedi S, Hassan K, AlHumaid J. Use of platelet-rich plasma for regeneration in non-vital immature permanent teeth: clinical and cone-beam computed tomography evaluation. J Int Med Res 2017;45:583-93.

30. Silujai J, Linsuwanont P. Treatment outcomes of apexification or revascularization in nonvital immature permanent teeth: a retrospective study. J Endod 2017;43:238-45.

31. Li L, Pan Y, Mei L, Li J. Clinical and radiographic outcomes in immature permanent necrotic evaginated teeth treated with regenerative endodontic procedures. J Endod 2017;43:246-51.

32. Linsuwanont P, Sinpitaksakul P, Lertsakchai T. Evaluation of root maturation after revitalization in immature permanent teeth with nonvital pulps by cone beam computed tomography and conventional radiographs. Int Endod J 2017;50:836-46.

33. Jiang X, Liu H, Peng C. Clinical and radiographic assessment of the efficacy of a collagen membrane in regenerative endodontics: a randomized, controlled clinical trial. J Endod 2017;43:1465-71.

34. Bukhari S, Kohli MR, Setzer F, Karabucak B. Outcome of revascularization procedure: a retrospective case series. J Endod 2016;42:1752-9.

35. Nazzal H, Kenny K, Altimini A, Kang J, Duggal MS. A prospective clinical study of regenerative endodontic treatment of traumatized immature teeth with necrotic pulps using bi-antibiotic paste. Int Endod J 2018;51:e204-15.

36. Lv H, Chen Y, Cai Z, et al. The efficacy of platelet-rich fibrin as a scaffold in regenerative endodontic treatment: a retrospective controlled cohort study. BMC Oral Health 2018;18:139.

37. Meschi N, EzEldeen M, Garcia AET, Jacobs R, Lambrechts P. A retrospective case series in regenerative endodontics: trend

REV 5.6.0 DTD ■ YMED101400_proof ■ 14 February 2020 ■ 1:17 am ■ ce

analysis based on clinical evaluation and 2-and 3-dimensional radiology. J Endod 2018;44:1517-25.

38. 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 2019;45:560-6.

39. Ragab RA, Lattif AEAE, Dokky NAEWE. Comparative study between revitalization of necrotic immature permanent anterior teeth with and without platelet rich fibrin: a randomized controlled trial. J Clin Pediatr Dent 2019;43:78-85.

40. Alobaid AS, Cortes LM, Lo J, et al. Radiographic and clinical outcomes of the treatment of immature permanent teeth by revascularization or apexification: a pilot retrospective cohort study. J Endod 2014;40:1063-70.

41. Sonoyama W, Liu Y, Yamaza T, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 2008;34:166-71.

42. American association of endodontics Ccfrp. Available at: www.aae.org/regenerativeendo/. Accessed July 31, 2019.

43. Kontakiotis EG, Filippatos CG, Tzanetakis GN, Agrafioti A. Regenerative endodontic therapy: a data analysis of clinical protocols. J Endod 2015;41:146-54.

44. Martin DE, De Almeida JFA, Henry MA, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod 2014;40:51-5.

45. Galler KM, D'Souza RN, Federlin M, et al. Dentin conditioning codetermines cell fate in regenerative endodontics. J Endod 2011;37:1536-41.

46. Mollashahi NF, Saberi E, Karkehabadi H. Evaluation of cytotoxic effects of various endodontic irrigation solutions on the survival of stem cell of human apical papilla. Iran Endod J 2016;11:293.

47. Trevino EG, Patwardhan AN, Henry MA, 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.

48. Taweewattanapaisan P, Jantarat J, Ounjai P, Janebodin K. The effects of EDTA on blood clot in regenerative endodontic procedures. J Endod 2019;45:281-6.

49. Ruparel NB, Teixeira FB, Ferraz CC, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod 2012;38:1372-5.

50. Lenherr P, Allgayer N, Weiger R, Filippi A, Attin T, Krastl G. Tooth discoloration induced by endodontic materials: a laboratory study. Int Endod J 2012;45:942-9.

51. Reynolds K, Johnson J, Cohenca N. Pulp revascularization of necrotic bilateral bicuspids using a modified novel technique to eliminate potential coronal discolouration: a case report. Int Endod J 2009;42:84-92.

52. Perron GG, Kryazhimskiy S, Rice DP, Buckling A. Multidrug therapy and evolution of antibiotic resistance: when order matters. Appl Environ Microbiol 2012;78:6137-42.

53. Barnes GW, Langeland K. Antibody Formation in Primates Following Introduction of Antigens into the Root Canal. Los Angeles, CA: SAGE Publications Sage CA; 1966.

54. Galler KM, Krastl G, Simon S, et al. European Society of Endodontology position statement: revitalization procedures. Int Endod J 2016;49:717-23.

55. Jung I-Y, Lee S-J, Hargreaves KM. Biologically based treatment of immature permanent teeth with pulpal necrosis: a case series. J Endod 2008;34:876-87.

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

57. Jadhav G, Shah N, Logani A. Revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: a pilot clinical study. J Endod 2012;38:1581-7.