

# A critical analysis of clinical research methods to study regenerative endodontics

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## Abstract

Regenerative endodontic treatment such as revitalization provides a treatment option for immature teeth with pulp necrosis. The main difference to the alternative procedure, the apical plug, is the induction of a blood clot inside the canal as a scaffold for healing and new tissue formation. Due to the biology-based and minimally-invasive nature of the treatment, revitalization has raised considerable interest in recent years. Whereas the procedure is fairly new and recommendations from endodontic societies have been in place only for a few years, the treatment protocol has evolved over the past two decades. Evidence has been created, not only from laboratory and animal work, but also from clinical studies including case reports, cohort studies and eventually prospective randomized controlled clinical trials, systematic reviews and meta-analyses. However, the research methods and clinical studies with subsequent reports oftentimes present with methodical limitations, which makes it difficult to objectively assess the value of this treatment modality. Several open questions remain, including the need for a more differentiated indication of revitalization after different traumatic injuries, the long-term prognosis of treated teeth and the true benefits for the patient. Therefore, this review aims to identify and reflect on such limitations, scrutinizing study design, diagnostic tools, procedural details and outcome parameters. A core outcome set is also proposed in this context, which can be considered in future clinical investigations. These considerations may lead to a more detailed and stringent planning and execution of future studies in order to create high-quality evidence for the treatment modality of revitalization and thus provide more robust data, create a larger body of knowledge for clinicians and further specify current recommendations.

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## INTRODUCTION

The treatment modalities of regenerative endodontics have raised enormous interest in recent years. Whereas the terminology includes “revascularization” (Iwaya, Ikawa & Kubota, 2001), “regenerative endodontic procedures” (Garcia- Godoy & Murray, 2012; Petrino *et al.*, 2010), “revitalization” (ESE, 2016) and “guided endodontic repair” (Diogenes *et al.*, 2016), these terms refer to a biology-based treatment option for immature teeth with pulp necrosis and thus to an alternative to the apical plug (ESE, 2016). The procedure includes thorough disinfection by means of irrigation and intracanal medication with minimal instrumentation of the root canal walls, followed by the provocation of bleeding into the canal. The blood clot, which is subsequently covered with a hydraulic calcium silicate cement, forms a scaffold for wound healing and new tissue formation (Diogenes *et al.*, 2016). Observations of a completion of root formation in teeth treated by revitalization raised expectations to achieve true regeneration of the dentine-pulp-complex with this procedure. Early hypotheses proposed the involvement of stem cells of the apical papilla (Lovelace *et al.*, 2011), which are present around immature teeth and drive the formation of root and dental pulp tissue by cell differentiation. As a consequence of the pooling of blood in the canal, an influx of these stem cells was suggested and substantiated by detection of increased stem cell markers in blood from root canals during treatment compared to blood drawn for the arm vein of the same patient (Lovelace *et al.*, 2011).

However, data from animal studies (Silva *et al.*, 2010; Wang *et al.*, 2010) as well as from clinical cases (Lin *et al.*, 2014) has revealed that the newly formed tissues were ectopic tissues such as soft connective tissues, cementum or bone, thus the theory of true regeneration after revitalization was contested quite early on. Today, it is agreed that revitalization generates similar success rates as compared to the apical plug in terms of healing (Torabinejad *et al.*, 2017), but a continuation of root formation is not predictable and there is a variation in outcomes in this regard (Kahler *et al.*, 2014). The induction of bleeding results in repair rather than regeneration in most of the cases, where only the presence of remnants of the original pulpal tissue may give rise to new pulp cells and therefore lead to true regeneration (Austah *et al.*, 2018).

With many questions in terms of outcome and long-term prognosis of teeth treated with revitalization, continuously produced data, in particular from clinical trials, sheds light on more and more aspects. Whereas evidence exists from laboratory studies all the way to randomized controlled clinical trials, systematic reviews and meta-analyses, it has to be appreciated that the quality of studies varies considerably and that some of the current recommendations (CONSORT, PRIRATE) are difficult to implement in this field. Therefore, the aim of this report is to present the methodological diversity of clinical studies on revitalization in order to show the variety of parameters and criteria used. Based on clinical trials in which revitalization was investigated exclusively or in comparison with other interventions, parameters related to the study design, the included cases, the diagnostic measures, the conduct of the treatment as well as the outcome evaluation will be identified and critically discussed. Finally, recommendations and guidance for the implementation of clinical revitalization studies are to be deduced.

## **MATERIALS AND METHODS**

### *Literature search and inclusion criteria*

Initially, MEDLINE (Ovid) was searched for controlled trials and evidence syntheses of regenerative endodontics including the use of platelet-rich plasma in endodontics (inception to July 2021). In order to identify only relevant study types, study filters were employed, in particular the tools "Filter for Systematic Reviews/Meta-Analysis/Health Technology Assessment - OVID Medline, Embase, PsycINFO" (CADTH, 2021), the "Cochrane Highly Sensitive Search Strategy for identifying randomized trials in MEDLINE: sensitivity- and precision-maximizing version (2008 revision); Ovid format" (Higgins & Green, 2011) and the "Filter for controlled non-randomized studies with best sensitivity Ovid MEDLINE" by Waffenschmidt *et al.* (2020). The search strategy is displayed in Appendix 1. In addition to the electronic search, a hand search of reference lists of included papers and published systematic reviews was performed.

### *Screening and data extraction*

For further assessment, all clinical trials were included in which revitalization treatments had implemented a blood-clot therapy (ESE, 2016) and had systematically evaluated the outcome of more than 10 treatment cases, thus case reports and case series were excluded. Studies from other dental specialties, preclinical studies or laboratory investigations as well as narrative and systematic reviews were excluded, however, the latter were considered during hand search.

Two reviewers (TA, MW) screened the articles in two stages (1. abstracts and titles, 2. full texts) using the software Rayyan (Ouzzani *et al.*, 2016). Controversies were discussed and



solved by vote of a third reviewer (KMG). Finally, 49 of 1513 potentially relevant articles were identified after full text screening.

A data extraction form was used to compile broad information on study parameters, case-specific details, diagnostic landmarks, treatment-related features and outcome measures (Appendix 2). For better overview, selected parameters that were relevant for the assessment and discussion of revitalization studies were summarized in Table 1. Other aspects relevant to the results are listed and explained in the running text.

## RESULTS

### *Study parameters*

The 49 included studies were published between 2008 and 2021 (Table 1). All trials were initiated with at least 10 patients and at least one treated tooth per patient, with the total number of patients per study ranging from 12 to 118. Out of 49 studies, 35 studies were conducted prospectively and 14 retrospectively (Fig. 1a). Furthermore, 20 studies reported randomization procedures (Fig. 1b), but only 20 used control or comparative groups (Fig. 1c) such as apexification or apical (MTA) plug (8 studies), conventional root canal treatment (2 studies), platelet concentrates (12 studies) or a gelatine-scaffold with fibroblast growth factor (1 study). Further studies made comparisons regarding treatment protocols (3), bioactive restoration materials (2), materials to cover the coagulum (2), age groups (1), apical preparation sizes (1) or intracanal medicaments (1).

### *Case-specific details*

Overall, 25 studies reported patient age as both mean or median value and also as age range. Twenty two studies reported only one of these values and 2 studies did not report any data with regards to patient age. Furthermore, 15 out of 30 studies specified the age for the individual groups investigated.

In all studies, treatment was indicated by pulp necrosis due to trauma, tooth anomaly or caries (Fig. 2a). Hereby, the studies included different aetiologies or combinations as follows: only trauma (13 studies); trauma, caries and anomaly (10 studies); trauma and anomaly (9 studies); trauma and caries (6 studies); trauma and defective restorations (1 study); only anomaly (1 study). One study included cases caused by trauma, caries and anomaly as well as undocumented reasons. Interestingly, 8 studies did not report the aetiology for pulp necrosis at all.

With regard to the revitalization treatments, 37 studies included only single-rooted teeth (Fig. 2b). In contrast, 7 studies included incisors, premolars as well as molars, and 5 studies did not report tooth type or number of roots. Most studies, except for 3, included only immature teeth, however, the width of the apical foramen was only reported in 13 studies.

Periapical lesions were a strict inclusion criterion in 15 studies, but optional in 32 studies and not reported in 2 studies (Fig. 2c). Of the latter, only 1 study exclusively included teeth without any signs of periapical lesions.

#### *Diagnostic landmarks*

In the course of the preoperative diagnostic assessment, pulp sensibility was evaluated by using both thermal and electric pulp tests in 19 studies, in one study only cold and in four studies only electric pulp tests were reported (Fig. 3a). Furthermore, sensibility tests were not specified in 5 studies and 20 studies did not report using any sensibility tests.

For postoperative evaluation of pulp sensibility, 19 studies used both methods, one study reported only using cold tests and seven studies only electric pulp tests. 5 studies did not specify vitality tests and 17 studies gave no information in this regard.

Periapical radiographs were used for radiological assessment of teeth in 46 studies. Out of these, 7 studies combined them with cone beam computed tomography (CBCT; 6 studies) or magnetic resonance imaging (MRI; 1 study). One study reported using computed tomography (CT) and CBCT, and 2 provided no information about the type of radiographs.

In order to ensure a certain standardization of the periapical radiographs, 7 studies used individualized bite blocks or registration (Fig. 3b). Thirteen studies attempted to improve the alignment and comparison of radiographs by using paralleling devices or positioning aids. Twenty nine studies did not report any measure to avoid distortion or differences in scale.

Since the standardization of radiographs are technically limited, digital approaches to align sequential radiographs can be helpful for quantification purposes (Fig. 3c). With this regard, most studies resorted to the TurboReg plugin within ImageJ (18 studies) (Schindelin *et al.*, 2012). However, 9 studies used ImageJ without the TurboReg plugin to correct images and another 2 studies used ImageJ with a viewer software (Digora). In 5 Studies, the analyses were carried out only in the viewer software (Digora, Dolphin, Sopro and Infinitt). Likewise, three-dimensional radiographs were analysed with the respective software package (EzD2009 software, One Volume Viewer, OnDemand 3D Application, MeVisLab, 3matic, CS 3D imaging software, Owandy and RadiAnt Viewer). Fourteen studies did not provide information regarding analysing software.

#### *Treatment-related features*

All studies provided information on root canal disinfection, where different concentrations of sodium hypochlorite (NaOCl) were used. Fifteen studies reported concentrations below or equal to 1.5 % sodium hypochlorite, 17 studies between 1.5 % and 3 %, and 12 studies over 3 % (Fig. 4a). Five studies used variable concentrations of sodium hypochlorite. 44 studies provided additional information on final rinse prior to the revitalization procedure. EDTA was reported as part of final irrigation in 28 studies.

As an interappointment canal dressing, the use of antibiotic mixtures was most common (32 studies), followed by calcium hydroxide preparations (9 studies) or other types of dressings (8 studies). Among them were two studies that used formocresol as intracanal medication (Fig. 4b).

In regard to the induction of bleeding into the root canal, 19 studies stated that the blood clot was covered by a collagen matrix, 27 studies did not use any kind of matrix and 3 studies did not report using one. For bioactive restorations, the majority of studies used MTA (34 studies) followed by glass ionomer cement (2 studies), Portland cement (2 studies), Biodentine (1 study) or a tricalcium silicate-based putty (1 study). Five studies used various materials within the study and 4 studies did not provide information in this regard (Fig. 4c).

Unfortunately, most studies did not provide information regarding the number of operators (27). A single operator was involved in 15 studies, and in respectively one study 2, 3 or 8 operators performed treatments. At least four studies took place in teaching facilities and were thus performed by several operators.

#### *Outcome measures*

Similar to the issue “operators”, most studies did not provide information on the number of evaluators (17). One or two evaluators were involved in 13 studies respectively, 4 studies involved three evaluators and 2 studies involved even four evaluators.

According to the ESE position statement, follow-up appointments are recommended after 6, 12, 18 and 24 month and annually afterwards. In the evaluated studies, follow-up times ranged from 4 to 96 month. While most cases were only followed up for at least 12 months (23 studies), 6 studies evaluated participants for 18 months and only 6 studies for at least 24 months (Fig. 5a). Eleven studies evaluated the outcome after 6 months only and one study even after 4 months. Two studies did not report the follow up periods at all.

Obviously, regular evaluations are of great importance especially in the first year. Clinical data was collected at least every two months (1 study), every three months (19 studies) or every six months (7 studies). In 9 studies, follow-up intervals varied, and no further specifications were given in 13 studies.

Success is mostly defined as absence of symptoms and healing of apical periodontitis (45 studies) and included parameters of root growth in length (42 studies) and thickness (41 studies) especially in the apical third (35 studies). Interestingly, only 27 studies also tested sensibility by cold or electric pulp tests (Fig. 5b).

## DISCUSSION

### *Study parameters*

In regard to the level of evidence, prospective and randomized clinical studies are desirable. The prospective design to test a hypothesis which determines all relevant procedural details allows for a deduction of cause and effect in a much more stringent way as compared to retrospective studies or case series. Randomization is clearly recommended to reduce bias. The literature search performed for this review yielded 49 clinical trials, where 35 were prospective studies and only 22 introduced randomizations into the protocol.

The question of the appropriate number of patients to be included needs to be addressed, ideally by means of a power analysis prior to the conduct of the study. Since revitalization is indicated in immature teeth, it should be defined and discussed, which age group of patients to include, ideally followed by a defined diameter at the apical foramen, e.g. based on the classification by Cvek (1992). Accordingly, a study protocol might state to include teeth at stages 1 to 3 of root formation (1 = less than half root length; 2 = half root length; 3 = under two-thirds root length, but exclude teeth at stage 4 and 5 (4 = nearly completed root length but wide apical foramen; 5 = completed root development with closed apical foramen) (Kim *et al.*, 2018). Questions arise in regard to an adequate control group, where the apical plug appears to be most suitable for revitalization procedures. Out of the clinical studies screened for this review, surprisingly, only 10 (20 %) compared revitalization to apexification, the apical plug or conventional root canal treatment. In turn, the question whether revitalization itself may be a control group for more novel procedures such as injection of platelet-rich fibrin or tissue-engineering approaches becomes obvious. Blinding of operators or patients is an additional tool to increase the quality of a study, however, in this context it is nearly impossible to realize such a scenario, as the protocol and materials used are different. Accordingly, none of the clinical studies on revitalization used a protocol for blinding. Still, the conductors of clinical studies should be aware of this tool and discuss this issue.

### *Case-specific details*

After thorough analysis of the studies that are published, it appears obvious that information on important details is often lacking. Thus, a consistent flow of information is relevant. Patient age is an important parameter in regards to outcome, both in test and control groups. Several studies report on advanced patient age which does not fit with the status of an immature tooth, except if a traumatic impact led to arrested root development some time in the past. In 17 out of the 49 studies, patients of age  $\geq 18$  years were included and in 2 studies there was no specification.

Of particular importance is furthermore the aetiology of pulp necrosis, which needs to be documented and interpreted in the discussion. Outcomes may vary in respect to the cause

of the problem. Traumatic impact in its various forms may damage Hertwig's epithelial root sheath (HERS) or the apical papilla, both of which are structures that drive root maturation (Huang *et al.*, 2008). Luxation injuries, in particular intrusive luxations, will severely damage the periodontium (Tsilingaridis *et al.*, 2012). If the cause of pulp necrosis is caries or infection due to a developmental anomaly, e.g. *dens evaginatus*, outcomes have shown to be quite different from those after a dental trauma (Austah *et al.*, 2018; Banchs & Trope, 2004; Chen, Huang & Bakland, 2020; Nazzal *et al.*, 2018). In this context, tooth type is furthermore relevant, as dental trauma affects mostly (maxillary) incisors (Lauridsen *et al.*, 2012), whereas *dens evaginatus* is predominant in premolars (Levitan & Himel, 2006). Therefore, this information is critical and needs to be reported, and a distinction between outcomes according to the cause of pulp necrosis might be feasible. Whereas most studies reported on the presence or absence of periapical radiolucencies and defined the presence of an apical lesion as one of the inclusion criteria, other studies did not comment on this issue or even decided to do the opposite, meaning only teeth without signs of apical infection were included. The presence of an apical lesion is considered an important prognostic factor as the inability to sufficiently eliminate bacteria within the root canal appears to be a critical step in particular in regenerative approaches (Fouad, 2020). This was also demonstrated in a recent animal study, where persisting bacteria as detected histologically were clearly associated with a lack of mineral deposition along the root walls, even in the absence of radiographically visible periapical lesions (Verma *et al.*, 2017).

#### *Diagnostic landmarks*

It is noticeable in several studies on revitalization that preoperative diagnostics or documentation of their results are sparse. In young patients, pulp sensibility testing has limited validity (Krastl *et al.*, 2021). Nevertheless, baseline cold or electric pulp tests need to be documented, and the possibility of false-positive results have to be taken into account. Not only at baseline, but also during follow-ups, diagnostics is important. Similar criteria for evaluation should be applied for pre- and postoperative diagnostics, in particular response to cold and/or electric pulp test, tenderness to percussion, tooth mobility, probing depth, ankylotic percussion tone, pain on palpation, swelling, sinus tract and tooth discolouration (ESE, 2016; ESE, 2021). Radiographically, the diameter of the apical lesion (if present), diameter of the apical foramen as well as the root length and thickness should be reported (ESE, 2016).

Most groups use periapical radiographs for postoperative follow-up. An evaluation of root length and thickness without objective measurement tools should no longer be accepted. However, currently available tools to align radiographs for comparative measurements have drawbacks. The commonly used software tool TurboReg (Image J) was reported in 18

clinical studies screened here. Whereas TurboReg offers easy handling, it also has limitations, since the user can define only 3 reference points on a pair of radiographs, which may result in inadequate alignment. The consequence is either repeated alignment to the point of sufficient fit or, if unnoticed, faulty measurements and thus questionable results. Whereas individualized film holders may represent a valid option to compare radiographs from the follow-up period in adults, there is a particular challenge with young patients, who will literally outgrow these devices. With the use of CBCT, additional information, in particular on the development of root length and thickness, can be gained (Meschi *et al.*, 2018). Certainly, in young patients, the benefits of 3D diagnostics have to be carefully balanced against the exposure of an increased dose of radiation (ESE, 2019).

#### *Treatment-related features*

As recommendations with precise procedural details have been available from the European Society of Endodontology (ESE, 2016) as well as from the American Association of Endodontists (AAE, 2021.) for several years, it may be postulated to follow these recommendations in order to have similar treatment protocols between studies, which enables a structured augmentation of evidence, also via systematic reviews. For example, earlier studies reported on the use of high concentrations of sodium hypochlorite or of chlorhexidine while not using EDTA. These variations can be minimized by stricter implementation of the existing recommendations. In addition, an understanding of the slightly different preconditions of revitalization compared to conventional root canal treatment will be beneficial. Whereas higher concentrations of sodium hypochlorite are more toxic to stem cells of the apical papilla and reduce their differentiation capabilities (Martin *et al.*, 2014), EDTA can expose the collagen network on the dentine surface as well as growth and differentiation factors (Galler *et al.*, 2016), which may positively affect the adhesion and differentiation of cells present in the root canal after provocation of bleeding. Similarly, the use of different intracanal medicaments will affect surrounding cells (Althumairy *et al.*, 2014) and should thus follow published recommendations. Whereas recommendations for many of the procedural steps are clear, the effects are not fully understood for others. Hydraulic calcium silicates are recommended to cover the blood clot due to their ability to set in the presence of moisture, however, other suitable materials are lacking and thus active research is needed to develop adequate alternatives. Mineral trioxide aggregate is most commonly applied onto the clot, but discoloration may be an undesirable side effect, in particular if the radiopacifier is bismuth oxide (Marciano *et al.*, 2015). Biodentine, a laboratory grade tricalcium silicate that uses zirconium oxide as radiopacifier appears to evoke less discoloration, however, the contact with blood is a critical factor (Slaboseviciute *et al.*, 2021). Discoloration of teeth, especially of front teeth, should be reported as one of the outcome

parameters after revitalization (Kahler & Rossi-Fedele, 2016). Furthermore, the number of operators who perform the treatment during the course of clinical studies may be worth discussing. However, the actual procedure for a revitalization treatment is less challenging as the alternative treatment of the apical plug and may thus be less sensitive in terms of operator skills.

#### *Outcome measures*

Following the quality guidelines for other clinical studies, the evaluation of outcome parameters should be performed by more than one evaluator and follow a methodical protocol. Inter- and intra-examiner reliability should ideally be assessed with statistical tools.

Follow-ups need to be performed regularly and exceed minimum time spans, where follow-up periods of 24 months and higher are desirable in order to more clearly distinguish successes and failures. Of critical importance is the differentiation between various clinical and radiological outcome parameters (Diogenes & Ruparel, 2017) as the terms “success” and “failure” may not be adequately precise in regards to revitalization. Accordingly, discussion is justified as to whether a case is a success if a periapical lesion reduces in size but there is no mineralized tissue accretion and thus no increase in root length and thickness. Thus, more specific success criteria for revitalization have to be defined. A useful and practical proposal is made by Chugal *et al.* (2017) where clinical success is described by the absence of pain, swelling and sinus tract (primary goal) and by a response to pulp vitality tests (tertiary goal). Radiologically, success is defined by resolution of apical radiolucency (primary goal) and root growth in length and thickness (secondary goal). Failure is precisely defined by the authors as the non-achievement of the primary goals (Chugal *et al.*, 2017).

While diagnostics after revitalization in young patients is challenging for a variety of reasons, including compliance, limited value of sensibility testing, lack of standardization of radiographs, and inability to determine the nature of the newly formed tissue by clinical and radiographic examination, a “core outcome set” as a check-list, which may include

- tooth survival,
- absence of signs and symptoms of inflammation,
- healing of periapical lesions,
- root thickening and lengthening,
- response to sensibility testing and
- tooth discoloration

could be a helpful tool for the time being. The assessment of relevant core outcomes is on the one hand conducive to the quality of individual studies and on the other hand improves comparability with other trials. This is an important basis for the summary in systematic

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reviews and the analysis of pooled data. Nevertheless, it must be acknowledged that an innovative field such as regenerative endodontics, in particular, benefits from answering further questions and reporting relevant observations.

## **CONCLUSION**

Although a large number of studies on revitalization is available, these have often limited informative value due to inconsistent methodology or bias. High-quality clinical trials are necessary to provide suitable evidence for systematic reviews and meta-analysis to enable more definitive recommendations. On the one hand, general quality criteria for clinical studies should be taken into account, and on the other hand, the described core outcomes should be documented in order to improve comparability among studies.

## **FUNDING**

None.

## **CONFLICT OF INTEREST**

The authors state explicitly that there is no conflict of interests related to this review.



## FIGURE LEGENDS

**Figure 1** Study parameters. (a) Proportion of data collected prospectively or retrospectively. (b) Use of randomisation procedures. (c) Selection of control/comparative groups for revitalisations in the studies.

**Figure 2** Case-specific details. (a) Documented aetiology. (b) Proportion of single-rooted teeth or inclusion of multi-rooted teeth; (c) Presence of periapical lesions prior to revitalisation.

**Figure 3** Diagnostic landmarks. (a) Use of sensibility tests in the course of postoperative control. (b) Tools used to improve the reproducibility of radiographs; (c) Software used to process and measure radiographs.

**Figure 4** Treatment-related features. (a) Concentration of sodium hypochlorite used for canal disinfection. (b) Intracanal dressing applied before revitalisation; (c) Materials described to cover the blood clot.

**Figure 5** Outcome measures. (a) Documented follow-up times in the studies. (b) Selection of applied criteria to describe the treatment success of revitalisation.

## **TABLES**

**Table 1** List of all included studies with selected parameters in chronic order. If any of the fields are empty, the aspect was not fulfilled or no clear information was available. Under control/comparative groups, those methods were listed with which the revitalisation was compared or with regard to which aspect a comparison was sought within the revitalisation studies. The aetiologies (anomaly, caries, trauma) were each abbreviated by their initial letters. Anomaly was bracketed in conjunction with trauma if the trauma occurred because of an anomaly. The number of patients was recorded at the end of the studies and at baseline (in brackets). Patient age was listed as range, however, substitute data was added in brackets if age range was not documented (mean and standard deviation, maximum).

## LITERATURE

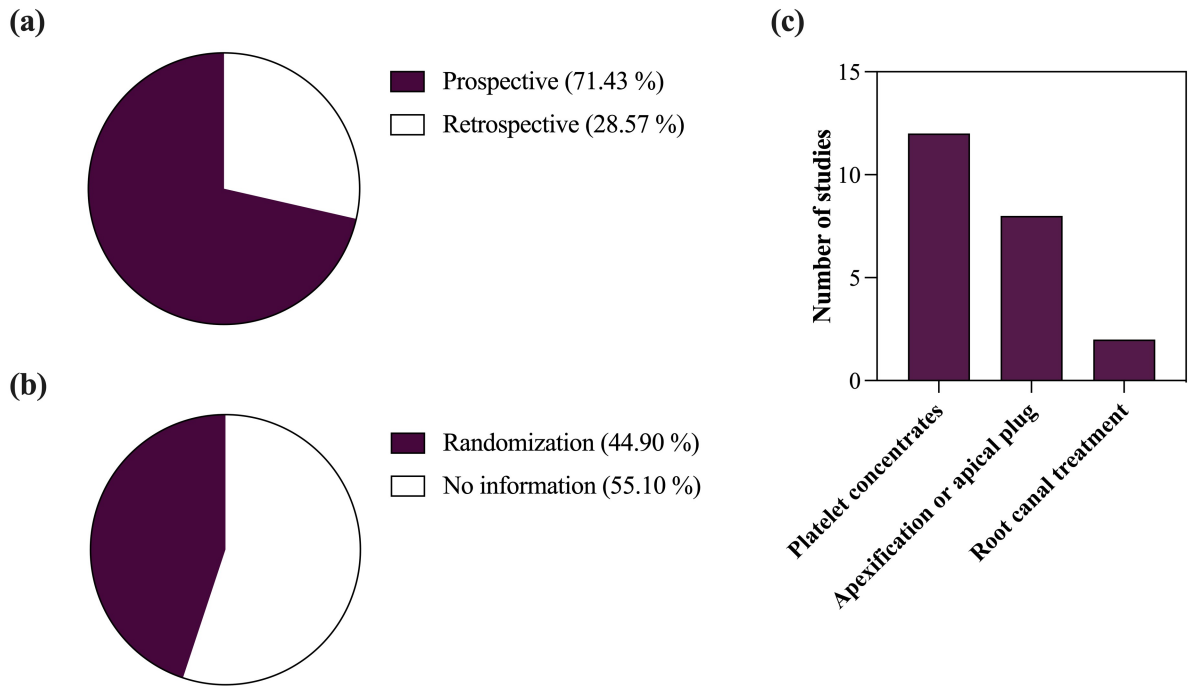
- American Association of Endodontists (2021) Clinical considerations for a regenerative procedure [Internet: 2021-07-30]. Available from: <https://f3f142zs0k2w1kg84k5p9i1o-wpengine.netdna-ssl.com/specialty/wp-content/uploads/sites/2/2021/08/ClinicalConsiderationsApprovedByREC062921.pdf>
- Austah O, Joon R, Fath WM *et al.* (2018) Comprehensive characterization of 2 immature teeth treated with regenerative endodontic procedures. *Journal of Endodontics* **44**, 1802–11.
- Banchs F, Trope M (2004) Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? *Journal of Endodontics* **30**, 196–200.
- CADTH (2021) Strings attached: CADTH Database Search Filters [Internet: 2021-07-30]. Ottawa: CADTH. Available from: <https://www.cadth.ca/resources/finding-evidence/strings-attached-cadths-database-search-filters>
- Chen J-W, Huang GT-J, Bakland LK (2020) Dens Evaginatus Current Treatment Options. *The Journal of the American Dental Association* **151**, 358–67.
- Chugal N, Mallya SM, Kahler B, Lin LM (2017) Endodontic treatment outcomes. *Dental Clinics of North America*, 61, 59–80.
- Cvek M (1992) Prognosis of luxated non - vital maxillary incisors treated with calcium hydroxide and filled with gutta - percha. A retrospective clinical study. *Dental Traumatology* **8**, 45–55.
- Diogenes AR, Ruparel NB (2017) Regenerative endodontic procedures: clinical outcomes. *Dental Clinics of North America* **61**, 111–25.
- Diogenes AR, Ruparel NB, Shiloah Y, Hargreaves KM (2016) Regenerative endodontics: a way forward. *Journal of the American Dental Association* **147**, 372–80.
- ESE (2016) European society of endodontology position statement: revitalization procedures. *International Endodontic Journal* **49**, 717–23.
- ESE (2019) European society of endodontology position statement: use of cone beam computed tomography in endodontics. *International Endodontic Journal* **52**, 1675–8.
- ESE (2021) European society of endodontology position statement: endodontic management of traumatized permanent teeth. *International Endodontic Journal* **54**, 1473–81.
- Fouad AF (2020) Contemporary microbial and antimicrobial considerations in regenerative endodontic therapy. *Journal of Endodontics* **46**, S105–14.
- Galler KM (2016) Clinical procedures for revitalization: current knowledge and considerations. *International Endodontic Journal* **49**, 926–36.

- Galler KM, Widbiller M, Buchalla W *et al.* (2016) EDTA conditioning of dentine promotes adhesion, migration and differentiation of dental pulp stem cells. *International Endodontic Journal* **49**, 581–90.
- Garcia-Godoy F, Murray PE (2012) Recommendations for using regenerative endodontic procedures in permanent immature traumatized teeth. *Dental Traumatology* **28**, 33–41.
- Higgins JPT, Green S (2011) Cochrane highly sensitive search strategy for identifying randomized trials in medline: sensitivity- and precision-maximizing version (2008 revision); Ovid format [Internet: 2021-07-30]. The Cochrane Collaboration. Available from: [https://handbook-5-1.cochrane.org/chapter\\_6/box\\_6.4.d\\_cochrane\\_hsss\\_2008\\_sensprec\\_ovid.htm](https://handbook-5-1.cochrane.org/chapter_6/box_6.4.d_cochrane_hsss_2008_sensprec_ovid.htm)
- Huang GT-J, Sonoyama W, Liu Y, Liu H, Wang S, Shi S (2008) The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. *Journal of Endodontics* **34**, 645–51.
- Iwaya SI, Ikawa M, Kubota M (2001) Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. *Dental Traumatology* **17**, 185–7.
- Kahler B, Mistry S, Moule A *et al.* (2014) Revascularization outcomes: a prospective analysis of 16 consecutive cases. *Journal of Endodontics* **40**, 333–8.
- Kahler B, Rossi-Fedele G (2016) A review of tooth discoloration after regenerative endodontic therapy. *Journal of Endodontics* **42**, 563–9.
- Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B (2018) Regenerative endodontics: a comprehensive review. *International Endodontic Journal* **51**, 1367–88.
- Krastl G, Weiger R, Filippi A, *et al.* (2021) Endodontic management of traumatized permanent teeth: a comprehensive review. *International Endodontic Journal* **54**, 1221–45.
- Lauridsen E, Hermann NV, Gerds TA, Kreiborg S, Andreasen JO (2012) Pattern of traumatic dental injuries in the permanent dentition among children, adolescents, and adults. *Dental Traumatology* **28**, 358–63.
- Levitan ME, Himel VT (2006) Dens evaginatus: literature review, pathophysiology, and comprehensive treatment regimen. *Journal of Endodontics* **32**, 1–9.
- Lin LM, Shimizu E, Gibbs JL, Loghin S, Ricucci D (2014) Histologic and histobacteriologic observations of failed revascularization/revitalization therapy: a case report. *Journal of Endodontics* **40**, 291–5.
- Lovelace TW, Henry MA, Hargreaves KM, Diogenes AR (2011) Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. *Journal of Endodontics* **37**, 133–8.
- Marciano MA, Duarte MAH, Camilleri J (2015) Dental discoloration caused by bismuth oxide in mta in the presence of sodium hypochlorite. *Clinical Oral Investigations* **19**, 2201–9.

- Martin DE, Almeida JFAD, Henry MA *et al.* (2014) Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. *Journal of Endodontics* **40**, 51–5.
- Meschi N, EzEldeen M, Garcia AET, Jacobs R, Lambrechts P (2018) A retrospective case series in regenerative endodontics: trend analysis based on clinical evaluation and 2- and 3-dimensional radiology. *Journal of Endodontics* **44**, 1517–25.
- Nazzal H, Kenny K, Altimimi A, Kang J, Duggal MS (2018) A prospective clinical study of regenerative endodontic treatment of traumatized immature teeth with necrotic pulps using bi-antibiotic paste. *International Endodontic Journal* **51** Suppl 3, e204–15.
- Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A (2016) Rayyan—a web and mobile app for systematic reviews. *Systematic Reviews* **5**, 210.
- Petrino JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB (2010) Challenges in regenerative endodontics: a case series. *Journal of Endodontics* **36**, 536–41.
- Schindelin J, Arganda-Carreras I, Frise E *et al.* (2012) Fiji: An open-source platform for biological-image analysis. *Nature Methods* **9**, 676–82.
- da Silva LAB, Nelson-Filho P, da Silva RAB *et al.* (2010) Revascularization and periapical repair after endodontic treatment using apical negative pressure irrigation versus conventional irrigation plus triantibiotic intracanal dressing in dogs' teeth with apical periodontitis. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **109**, 779–87.
- Slaboseviciute M, Vasiliauskaite N, Drukteinis S, Martens L, Rajasekharan S (2021) Discoloration potential of biodentine: a systematic review. *Materials* **14**, 6861.
- 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.
- Tsilingaridis G, Malmgren B, Andreasen JO, Malmgren O (2012) Intrusive luxation of 60 permanent incisors: a retrospective study of treatment and outcome. *Dental Traumatology* **28**, 416–22.
- Verma P, Nosrat A, Kim JR *et al.* (2017) Effect of residual bacteria on the outcome of pulp regeneration in vivo. *Journal of Dental Research* **96**, 100–6.
- Waffenschmidt S, Navarro-Ruan T, Hobson N, Hausner E, Sauerland S, Haynes RB (2020) Development and validation of study filters for identifying controlled non - randomized studies in PubMed and Ovid MEDLINE. *Research Synthesis Methods* **11**, 617–26.
- Wang X, Thibodeau B, Trope M, Lin LM, Huang GT-J (2010) Histologic characterization of regenerated tissues in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis. *Journal of Endodontics* **36**, 56–63.

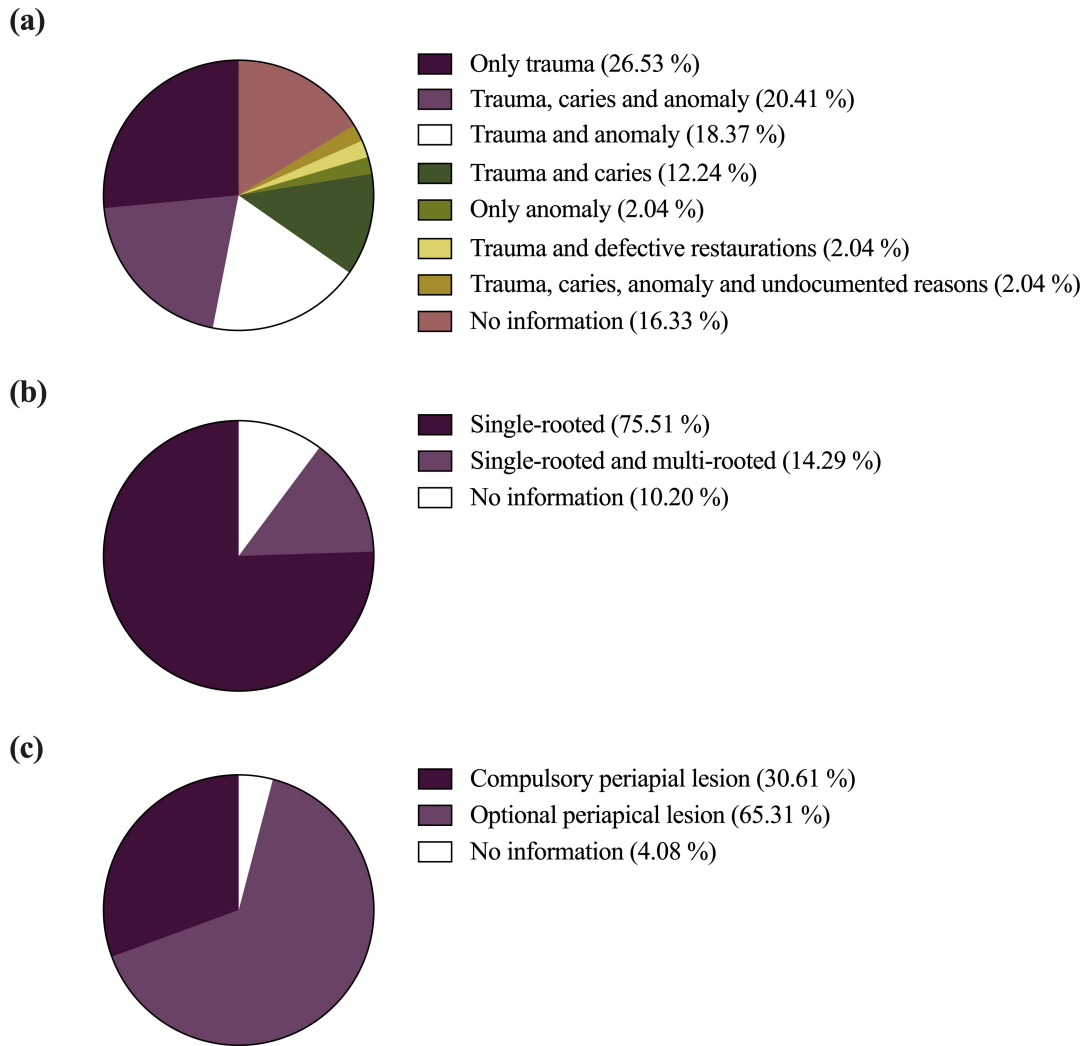
Autor	Year	Prospective	Randomization	Control/comparative groups	Etiology	Patient number	Age range	Mature teeth	Inclusion of molars	Recall time	Additional 3D-Imaging	Individ. X-ray holders	Image processing (TurboReg)
Shah et al.	2008	×			t	14	8-17			≥6			
Ding et al.	2009	×			a, t	3 (12)	8-11			≥12			
Bose et al.	2009			Apex, RCT		88 (94)							×
Lovelace et al.	2011	×			a, t	8 (12)	7-16						
Jadhav et al.	2012	×	×	PRP		20	15-28			12			
Jeeruphan et al.	2012			Apex, MTA-plug	a, c, t	61	(12.9±5.07)			>10			
Chen et al.	2012	×			a, c, t	20	7-13			≥6			
Nagata et al.	2014	×	×	Canal dressings	t	23	10-17			≥9		×	
Nagy et al.	2014	×	×	Apex, Scaffold		29 (36)	9-13			18			
Kahler et al.	2014	×			a, t	12	7-12			≥18			×
Saoud et al.	2014	×			t	17	(11.3)			12		×	×
Alobaid et al.	2014			Apex	a, c, t	31	(8.8±1.6)			(14.5±8.5)			×
Bezgin et al.	2015	×	×	PRP	c, t	18 (20)	7-12			18			×
Narang et al.	2015	×	×	Apex, PRP, PRF		20	(<20)			18			
Estefan et al.	2016	×		Age groups		35 (40)	9-18			≥10			
El Ashiry et al.	2016	×			c, t	16 (20)				24			
Linsuw anont et al.	2016				a, c, t	15 (17)	8-46		×	96	×		×
Bukhari et al.	2016				a, c, t	28	8-31		×	≥7			
Alagl et al.	2017	×	×	PRP	c, t	15 (16)	8-11			12	×		
Botero et al.	2017	×	×	Protocols	a, c, t	19 (25)	(10.5)		×	12		×	×
Jiang et al.	2017	×	×	Matrix	(a), t	40 (43)	(9.82±1.5)			≥6			×
Lin et al.	2017	×	×	Apex	a, t	103 (118)	8-16			12	×		
Shivashankar et al.	2017	×	×	PRP, PRF	c, t	54 (60)	6-28			12			
Chan et al.	2017	×			a, c, t	22	(9.23±2.36)		×	30			×
Li et al.	2017	×			a	20	8-12			12			×
Peng et al.	2017			Restauracion material	a, c, t	57	(10.8±1.9)			≥13			×

Silujjai et al.	2017			Apex	a, c, t	43	8-46		×	≥12		
Song et al.	2017				a, c, t, u	29	8-18		×	≥12		×
Nazzal et al.	2018	×			t	12 (15)	7-10			≥18		
Neelamurthy et al.	2018	×				13 (15)	12-35		×	10		
Lv et al.	2018			PRF	a, t	10	10-12			12		
Aly et al.	2019	×	×	Restauracion material	c, t	23 (24)	(9±0.89; 8.92±1.26)			12		
Ragab et al.	2019	×	×	PRF	t	22	7-12			12		
Rizk et al.	2019	×	×	PRP	t	13	8-14			12		×
Ulusoy et al.	2019	×	×	PRP, PRF, PP	t	65 (77)	8-11			≥10		×
Arslan et al.	2019	×	×	RCT		36 (49)	(20.58±2.53)		×	12		
EISheshtaw y et al.	2020	×	×	PRP	a, t	22 (26)	(12.69±3.99)			12		×
Ramachandran et al.	2020	×	×	PRP		28 (40)	15-54			12		
Rizk et al.	2020	×	×	PRF	t	12 (13)	8-14			12		×
Nazzal et al.	2020	×			t	12 (15)	7-10			≥27		
Pereira et al.	2020				t	15	7-18			≥9		×
Mittmann et al.	2020				t	12 (13)	6-11			≥4		×
Chrepa et al.	2020				a, c, t	51	7-26		×	≥12		×
El Kateb et al.	2020	×	×	Preparation sizes	t, r	18	20-34		×	12		×
Cerqueira-Neto et al.	2021	×		Protocols	t	17	7-15			24		
Jiang et al.	2021	×	×	Matrix	(a), t	76 (80)	7-15			≥6		×
Zizka et al.	2021			Protocols	a, t	16 (18)	(<13)			24		×
Pereira et al.	2021			Apex	t	37	6-18			≥12		
Shetty et al.	2021	×			c, t	31 (42)	9-38			≥18		×

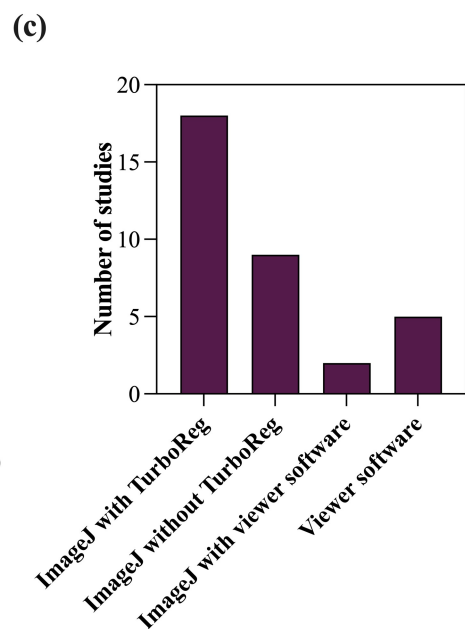
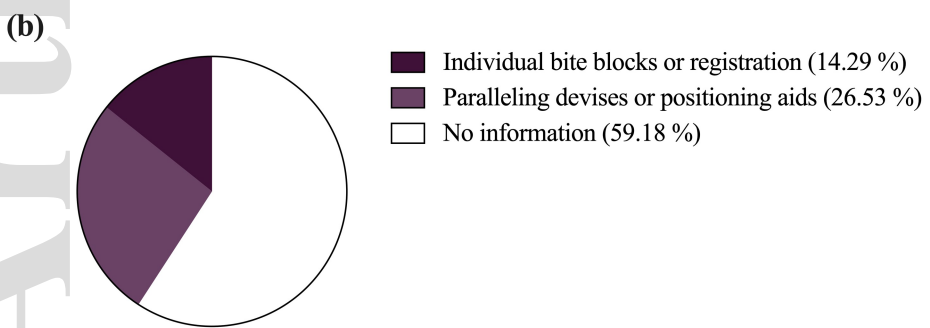
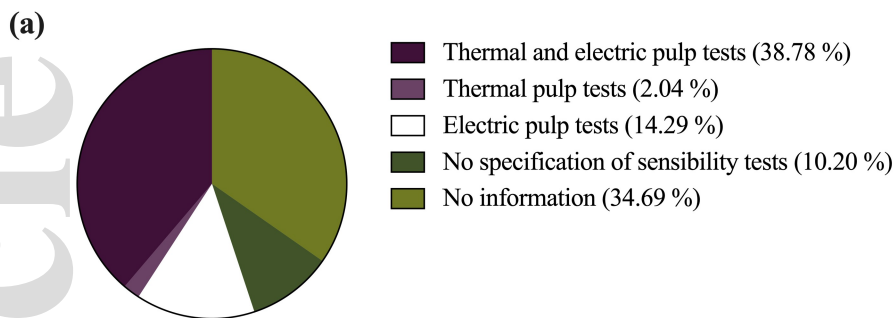


iej\_13734\_f1.png

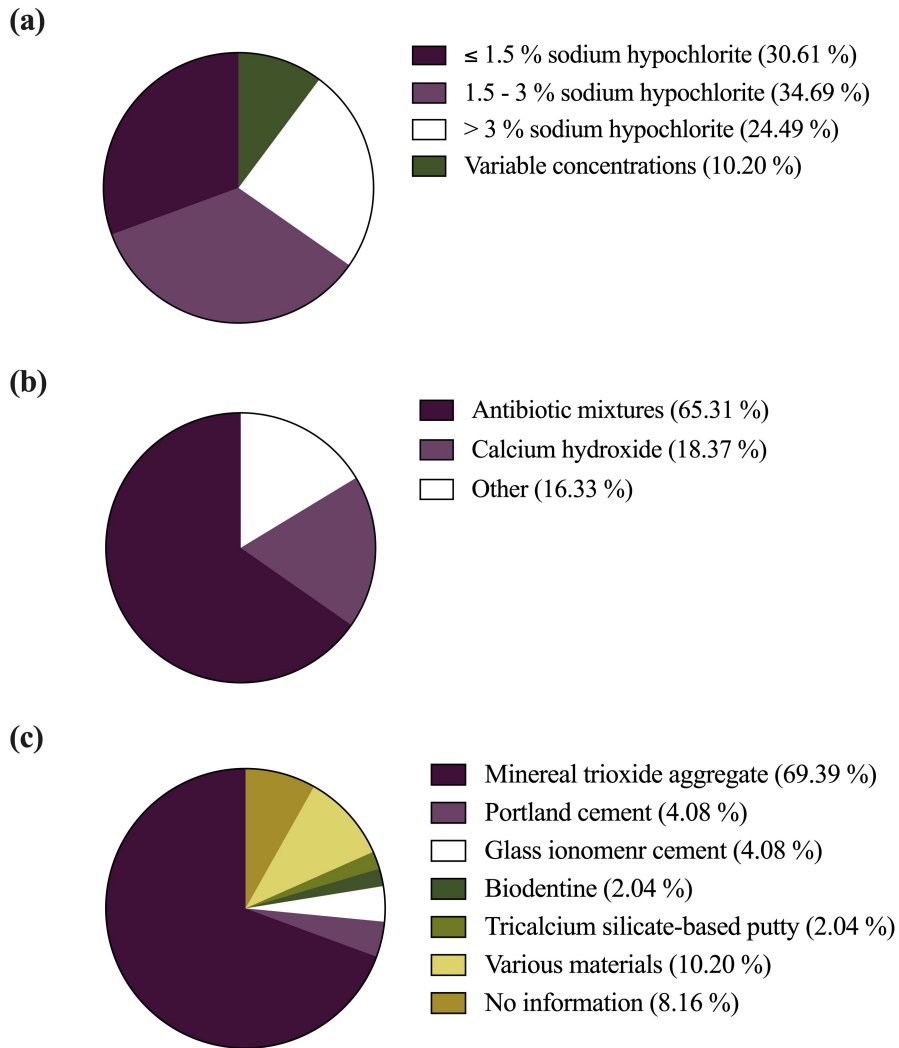




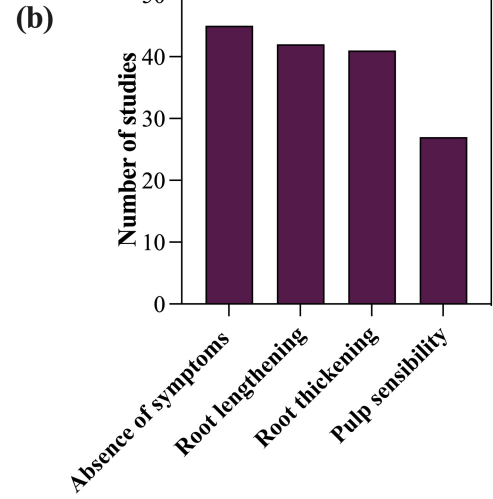
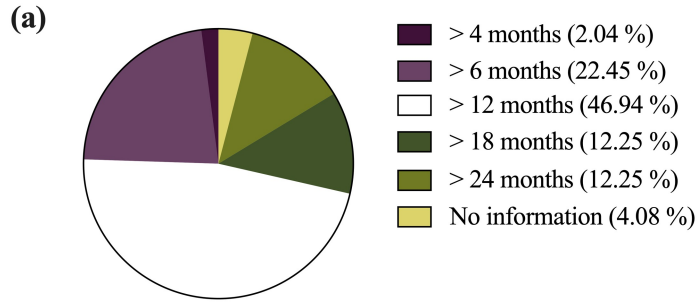
iej\_13734\_f2.png



iej\_13734\_f3.png



iej\_13734\_f4.png



iej\_13734\_f5.png

## Appendix 1

- 1 exp Platelet-Rich Plasma/ 5134
- 2 Endodontics/ or endodont\*.jw. or Root Canal Therapy/ or (Dental Pulp/ or Dental Pulp Cavity/ or exp Tooth Root/) or exp Tooth/ 132808
- 3 (1 and 2) or Regenerative Endodontics/ or ((RET or REP) and endodont\*).ti,ab,kf. or ((revitali#ation or revasculari#ation or regenerat\* or blood clot\* or platelet-rich plasma or platelet-rich fibrin) adj9 (endodont\* or pulp\* or "root" or tooth or teeth or dens or dentes or canin\$ or incisor\$ or incisivi or cuspid\$ or bicuspid or premolar\$ or molar\$)).ti,ab,kf. [Regenerative Endodontics] 5848
- 4 meta-analysis.pt. or (meta-analysis/ or systematic review/ or meta-analysis as topic/ or "meta analysis (topic)"/ or "systematic review (topic)"/ or exp technology assessment, biomedical/) or ((systematic\* adj3 (review\* or overview\*)) or (methodologic\* adj3 (review\* or overview\*))).ti,ab,kf,kw. or ((quantitative adj3 (review\* or overview\* or synthes\*)) or (research adj3 (integrati\* or overview\*))).ti,ab,kf,kw. or ((integrative adj3 (review\* or overview\*)) or (collaborative adj3 (review\* or overview\*)) or (pool\* adj3 analy\*)).ti,ab,kf,kw. or (data synthes\* or data extraction\* or data abstraction\*).ti,ab,kf,kw. or (handsearch\* or hand search\*).ti,ab,kf,kw. or (mantel haenszel or peto or der simonian or dersimonian or fixed effect\* or latin square\*).ti,ab,kf,kw. or (met analy\* or metanaly\* or technology assessment\* or HTA or HTAs or technology overview\* or technology appraisal\*).ti,ab,kf,kw. or (meta regression\* or metaregression\*).ti,ab,kf,kw. or (meta-analy\* or metaanaly\* or systematic review\* or biomedical technology assessment\* or biomedical technology assessment\*).mp,hw. or (medline or cochrane or pubmed or medlars or embase or cinahl).ti,ab,hw. or (cochrane or (health adj2 technology assessment) or evidence report).jw. or (comparative adj3 (efficacy or effectiveness)).ti,ab,kf,kw. or (outcomes research or relative effectiveness).ti,ab,kf,kw. or ((indirect or indirect treatment or mixed-treatment) adj comparison\*).ti,ab,kf,kw. [CADTH Search Filter for Systematic Reviews/Meta-Analysis/Health Technology Assessment OVID Medline, Embase] 542837

5 randomized controlled trial.pt. 538895  
6 controlled clinical trial.pt.94314  
7 randomized.ab. 528339  
8 placebo.ab. 220017  
9 clinical trials as topic.sh. 196802  
10 randomly.ab. 362499  
11 trial.ti. 244465  
12 5 or 6 or 7 or 8 or 9 or 10 or 11 1383144  
13 exp animals/ not humans.sh. 4867563  
14 12 not 13 [Cochrane Highly Sensitive Search Strategy 2008 sensitive and precise  
MEDLINE Ovid] 1272821  
15 exp cohort studies/ or exp epidemiologic studies/ or exp clinical trial/ or exp evaluation  
studies as topic/ or exp statistics as topic/ 5943004  
16 ((control and (group\* or study)) or (time and factors) or program or survey\* or ci or cohort  
or comparative stud\* or evaluation studies or follow-up\*).mp. 7776641  
17 or/15-16 10235315  
18 (animals/ not humans/) or comment/ or editorial/ or exp review/ or meta analysis/ or  
consensus/ or exp guideline/ 8879786  
19 hi.fs. or case report.mp. 646563  
20 or/18-19 9440989  
21 17 not 20 [Filter for controlled non-randomized studies with best sensitivity Ovid  
MEDLINE. Waffenschmidt et al. (2020) <https://doi.org/10.1002/jrsm.1425>] 7939575  
22 3 and 4 [Regenerative Endodontics AND Systematic Reviews, Meta-Analyses] 186  
23 3 and 14 [Regenerative Endodontics AND RCTs] 402  
24 3 and 21 [Regenerative Endodontics AND Controlled Non-Randomized Studies] 1284  
25 or/22-24 [Regenerative Endodontics AND Study Filters] 1513

## Appendix 2

### Data extraction sheet

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	Author:
	PubMed-ID:
<b>Study parameters</b>	Year of publication:
	Study type:
	Randomization procedure:
	Control/comparative groups:
	Patient number:
	Tooth number:
<b>Case specific details</b>	Age range:
	Age (regarding individual groups):
	Specific tooth type:
	Apical closure before treatment:
	Aetiology of pulp necrosis:
	Presence of periapical lesions:
<b>Diagnostic landmarks</b>	Pulp tests before treatment:
	Pulp tests after treatment:
	Type of radiograph:
	Individualized radiographs:
	Standardized paralleling technique:
	Image processing:
<b>Treatment-related features</b>	Use of NaOCl:
	Use of EDTA:
	Interappointment canal dressing:
	Matrix:
	Restoration material:
	Number of operators:
<b>Outcome measures</b>	Number of evaluators:
	Follow-up time:
	Follow-up intervals:
	Absence of symptoms/healing of apical periodontitis:
	Root growth in length/thickness:
	Apical closure after treatment:

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