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REVIEW ARTICLE

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.

KEYWORDS

clinical study, dental pulp necrosis, immature teeth, regenerative endodontics, revitalization

INTRODUCTION

The treatment modalities of regenerative endodontics have raised enormous interest in recent years. Whereas the terminology includes "revascularization" (Iwaya et al., 2001), "regenerative endodontic procedures" (Garcia-Godoy & Murray, 2012; Petrino et al., 2010), "revitalization" (Galler et al., 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) have 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

TABLE 1 List of all included studies with selected parameters in chronic order

Author	Year	Prospective	Randomization	Control/ comparative groups	Aetiology	Patient number	Age range	Mature teeth	Inclusion of molars	Recall time	Additional 3D imaging	Individual 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			
Linsuwanont 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			Restauration material	a, c, t	57	(10.8 ± 1.9)			≥13			×

(Continues)

TABLE 1 (Continued)

Author	Year	Prospective	Randomization	Control/ comparative groups	Aetiology	Patient number	Age range	Mature teeth	Inclusion of molars	Recall time	Additional 3D imaging	Individual X-ray holders	Image processing (TurboReg)
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	×	×	Restauration 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			
ElSheshtawy 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			×
Žižka 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	×		×

Note: 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 were added in brackets if age range was not documented (mean and standard deviation, maximum).

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 (Figure 1a). Furthermore, 20 studies reported randomization procedures (Figure 1b), but only 20 used control or comparative groups (Figure 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 restauration 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 (Figure 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 (Figure 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

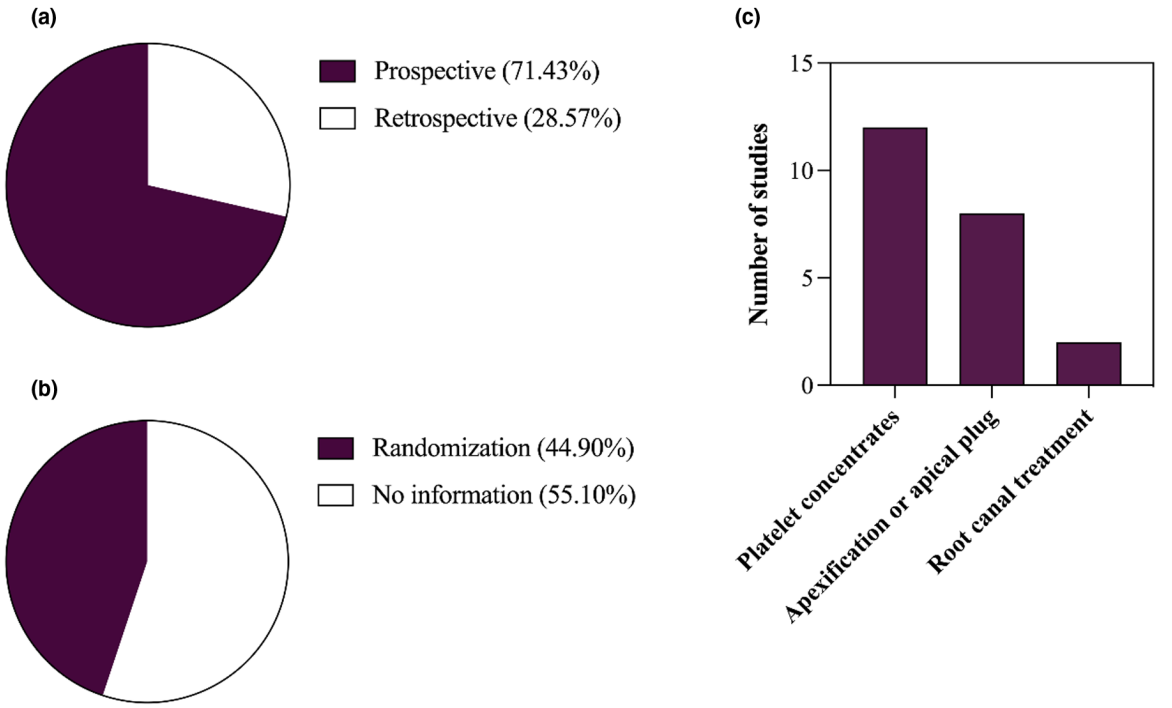

FIGURE 1 Study parameters. (a) Proportion of data collected prospectively or retrospectively. (b) Use of randomisation procedures. (c) Selection of control/comparative groups for revitalisations in the studies

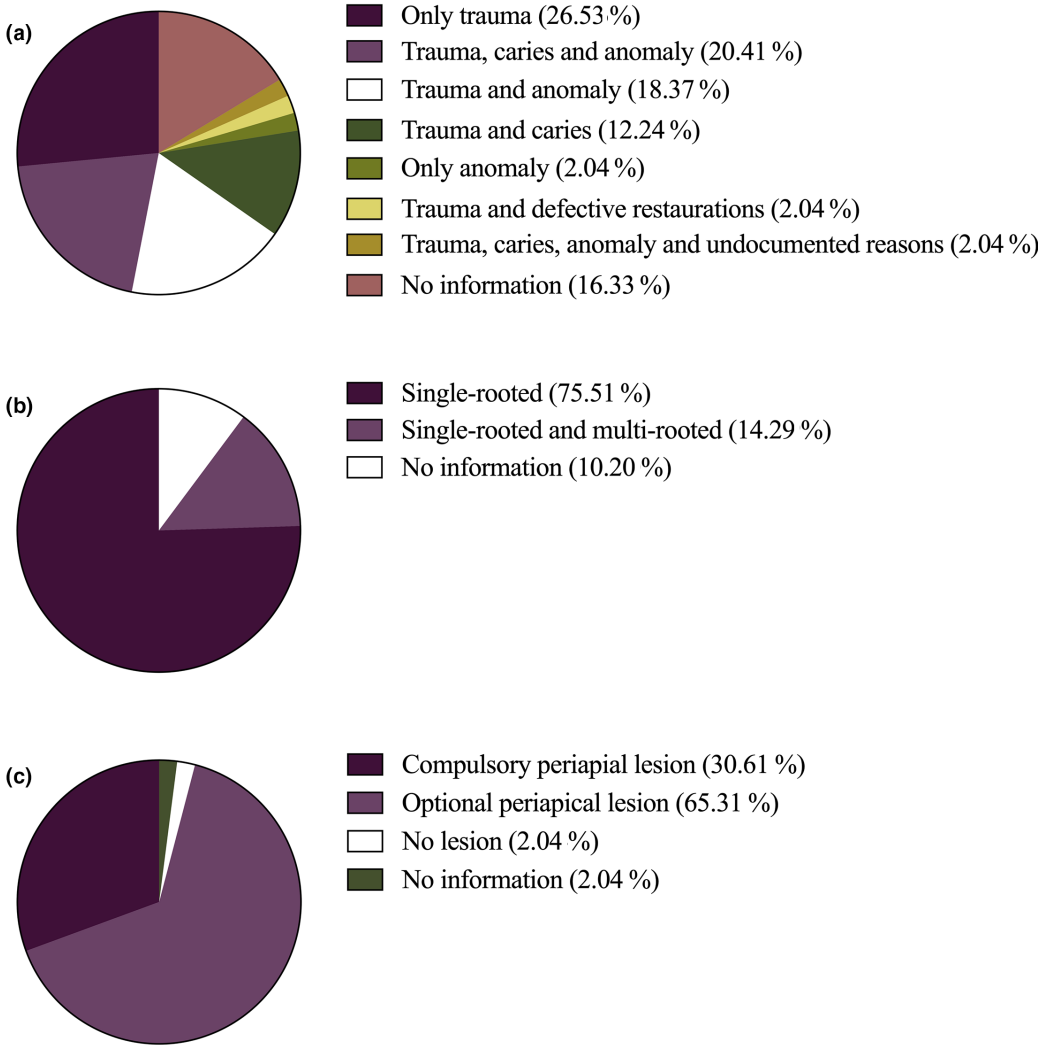

FIGURE 2 Case-specific details. (a) Documented aetiology. (b) Proportion of single-rooted teeth or inclusion of multi-rooted teeth. (c) Presence of periapical lesions prior to revitalisation

1 study (Figure 2c). Another study exclusively included only 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 (Figure 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, 1 study reported only using cold tests and 7 studies only electric pulp tests. Five studies did not specify sensibility 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 (Figure 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 (Figure 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,

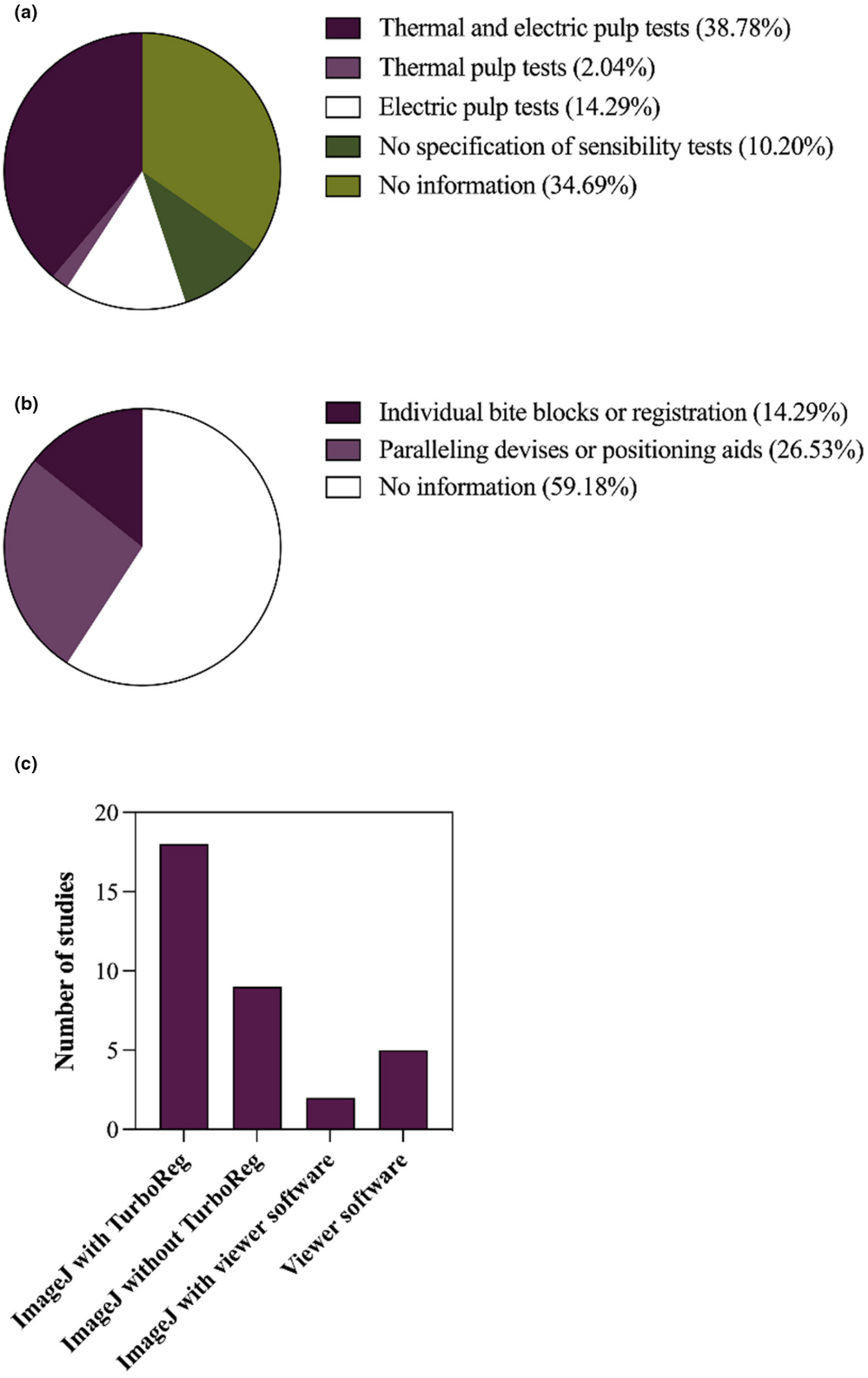

FIGURE 3 Diagnostic landmarks. (a) Use of sensibility tests in the course of postoperative control. (b) Tools used to improve the reproducibility of radiographs. (c) Software used to process and measure radiographs

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.

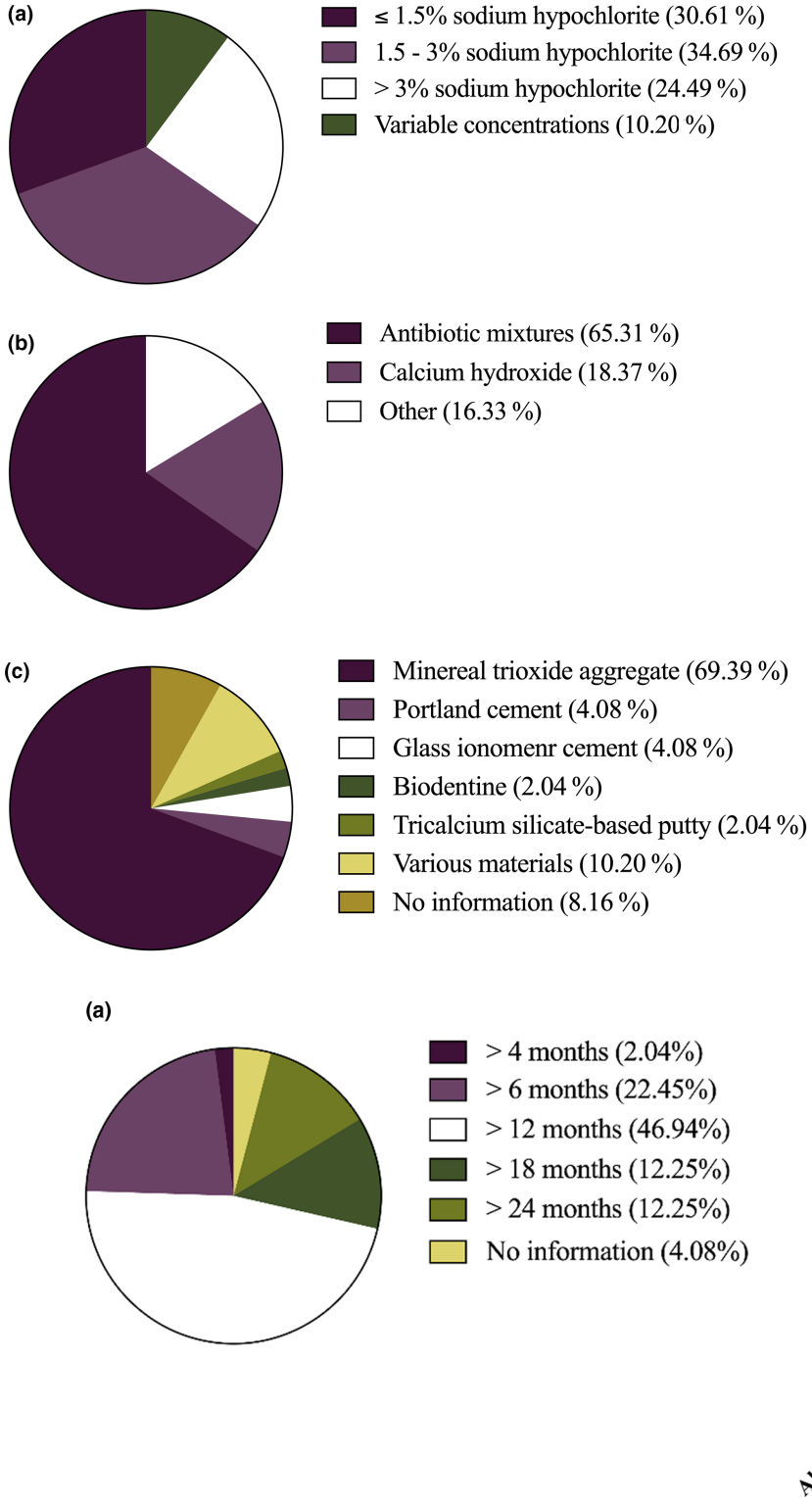

FIGURE 4 Treatment-related features. (a) Concentration of sodium hypochlorite used for canal disinfection. (b) Intracanal dressing applied before revitalisation. (c) Materials described to cover the blood clot

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

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% (Figure 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). Amongst them were two studies that used formocresol as intracanal medication (Figure 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 (Figure 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 4 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 months and annually afterwards. In the evaluated studies, follow-up times ranged from 4 to 96 month. Whilst 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 (Figure 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 were collected at least every 2 months (1 study), every 3 months (19 studies) or every 6 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 (Figure 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, for example 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 with regard 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 ≥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, for example *dens evaginatus*, outcomes have shown to be quite different from those after a dental trauma (Austah et al., 2018; Banchs & Trope, 2004; Chen et al., 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, 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 dosed 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 whilst 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 can have a positive effect on 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 silicate cements 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 cement 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 anterior 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 with regard 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).

Whilst 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 checklist, 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 reviews and the analysis of pooled data. Nevertheless, it must be acknowledged that an innovative field such as regenerative endodontics, in particular, benefits also from reporting relevant observations beyond the proposed outcomes.

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 amongst studies.

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AUTHOR CONTRIBUTIONS

Matthias Widbiller involved in conceptualization. Helge Knüttel and Matthias Widbiller involved in methodology. Tobias Akamp, Helge Knüttel and Matthias Widbiller involved in literature review data curation. Kerstin M. Galler, Tobias Akamp and MW involved in writing original draft. Kerstin M. Galler, Tobias Akamp and Matthias Widbiller involved in writing - review and editing.

CONFLICT OF INTEREST

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

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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 bio-medical 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

	Author:
	PubMed-ID:
Study parameters	Year of publication:
	Study type:
	Randomization procedure:
	Control/comparative groups:
	Patient number:
	Tooth number:
Case-specific details	Age range:
	Age (regarding individual groups):
	Specific tooth type:
	Apical closure before treatment:
	Aetiology of pulp necrosis:
	Presence of periapical lesions:
Diagnostic landmarks	Pulp tests before treatment:
	Pulp tests after treatment:
	Type of radiograph:
	Individualized radiographs:
	Standardized paralleling technique:
	Image processing:

	Author:
	PubMed-ID:
Treatment-related features	Use of NaOCl:
	Use of EDTA:
	Interappointment canal dressing:
	Matrix:
	Restoration material:
	Number of operators:
Outcome measures	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: