

## REVIEW ARTICLE

# Effectiveness of endodontic tissue engineering in treatment of apical periodontitis: A systematic review

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## Abstract

**Background:** Regenerative endodontics has evolved in recent years with tissue engineering concepts in particular appearing promising. Endodontic tissue engineering (ETE) describes the various approaches based on the orthograde introduction of scaffolds or biomaterials (with or without cells) into the root canal to achieve pulp tissue regeneration. There are currently no systematic reviews investigating whether ETE is a suitable method for the treatment of endodontic disease in both mature and immature permanent teeth.

**Objectives:** The purpose of this systematic review was to determine the effectiveness of ETE in permanent teeth with pulp necrosis in comparison with conventional endodontic treatment.

**Methods:** We searched MEDLINE, Embase and the Cochrane Library for published reports as well as Google Scholar for grey literature up to November 2021. Included were studies of patients with permanent immature or mature teeth and pulp necrosis with or without signs of apical periodontitis (P) comparing ETE (I) with calcium hydroxide apexification, apical plug and root canal treatment (C) in terms of tooth survival, pain, tenderness, swelling, need for medication (analgesics and antibiotics), radiographic evidence of reduction in apical lesion size, radiographic evidence of normal periodontal ligament space, function (fracture and restoration longevity), the need for further intervention, adverse effects (including exacerbation, restoration integrity, allergy and discolouration), oral health-related quality of life (OHRQoL), presence of sinus tract and response to sensibility testing (O). An observation period of at least 12 months was mandatory (T) and the number of patients in human experimental studies or longitudinal observational studies had to be at least 20 (10 in each arm) at the end (S). Risk of bias was appraised using the Cochrane risk-of-bias (RoB 2) tool. Two authors independently screened the records, assessed full texts for eligibility and evaluated risk of bias. Heterogeneity of outcomes and limited body of evidence did not allow for meta-analysis.

**Results:** Two randomized clinical trials investigating cell transplantation approaches with a total of 76 participants (40 treated immature teeth and 36 treated mature teeth) were included for qualitative analysis. Both studies had moderate

concerns in terms of risk of bias. Due to the lack of homogeneity a meta-analysis was not possible. Tooth survival for ETE, root canal treatment and apexification was 100% after 12 months. Teeth treated with ETE showed a higher number of cases with positive pulpal responses to sensitivity tests and with blood perfusion compared with root canal treatment or apexification.

**Discussion:** This systematic review highlights that there is limited evidence for ETE approaches. Even though the results of this review suggest a high survival with ETE in mature and immature teeth, there is a moderate risk of bias due to methodological limitations in the included studies, so the overall results should be interpreted with caution. Lack of a robust control group was a common problem during literature screening, and outcomes besides dental survival were reported inconsistently. Future clinical trials need to address methodical as well as assessment concerns and report long-term results.

**Conclusion:** The benefits and high survival rates reported for ETE techniques suggest that this procedure might be an alternative to conventional procedures for permanent teeth with pulpal necrosis. However, more appropriate studies are needed to derive clinical recommendations.

**Registration:** PROSPERO (CRD42021266350).

#### KEYWORDS

cell transplantation, dental pulp necrosis, periapical periodontitis, regenerative endodontics, tissue engineering

## INTRODUCTION

Caries, trauma or dental anomalies can lead to necrosis of the dental pulp. Hereby, associated bacterial infection of the root canal can initiate an apical periodontitis, which may be asymptomatic (asymptomatic apical periodontitis) or accompanied by pain and other clinical symptoms (symptomatic apical periodontitis). The overall goal of treating apical periodontitis is the resolution of clinical symptoms and radiological healing.

In this context, the treatment of immature teeth presents a particular challenge for clinicians as these teeth have short roots with thin walls and wide apices. Therefore, different treatment modalities are available such as apexification with calcium hydroxide, apical plug with a hydraulic calcium silicate cement (HCSC) or revitalization. The traditional apexification treatment comprises a long-term intracanal dressing with calcium hydroxide to induce the formation of a calcified barrier at the root apex (Mohammadi & Dummer, 2011). Once this barrier is formed, the canal can be obturated with Gutta-percha and restored definitely. However, this treatment has numerous disadvantages rendering the treatment less popular. These include the need for multiple appointments, the long treatment period and the long contact of the tooth with calcium hydroxide, which increases the risk of

root fractures (Andreasen et al., 2002). Given these drawbacks, the current recommendation is to place an apical plug with HCSC and to obturate the rest of the canal with Gutta-percha. In contrast to root canal filling, revitalization offers a regenerative endodontic treatment option for immature teeth (Galler et al., 2016). Here, bleeding is induced into the root canal resulting in a blood coagulum that is covered with collagen and sealed with a HCSC. Thereupon, the coagulum transforms into a repair tissue and enables the regaining of vitality and cold sensitivity (Diogenes & Ruparel, 2017; Widbiller & Schmalz, 2021). Furthermore, an advantage of the revitalization of immature teeth can be seen in the chance of increasing root length and dentine wall thickness (Lin et al., 2017; Nagy et al., 2014; Wikström et al., 2021; Xie et al., 2021), which can improve the tooth's mechanical strength and thus long-term survival (Bucchi et al., 2019; Cvek, 1992). Considering all three treatment methods, it can be said that no significant difference in terms of periapical healing and survival has been observed between apexification, apical plug and revitalization (Lin et al., 2017; Nicoloso et al., 2019; Pereira et al., 2021; Wikström et al., 2021; Xie et al., 2021).

For mature teeth with fully developed roots, root canal treatment (RCT) is the treatment of choice after pulp necrosis, however, even in mature teeth revitalization

therapy is becoming increasingly popular, and the initial clinical data are promising (Arslan et al., 2019). A systematic review showed that the survival probability of root canal-treated teeth ranged from 86 to 93% (Ng et al., 2010). According to another systematic review by Ng et al. (Ng et al., 2007), complete healing after RCT occurred in 68 to 85% of cases. The comparison of the results of revitalization and RCT with respect to tooth survival and periapical health showed no significant differences between the two options (Arslan et al., 2019; Brizuela et al., 2020; Glynis et al., 2021).

Over the last years, the possibility of regenerating the dental pulp, as mentioned, has stimulated great interest in immature teeth and also in mature teeth, because various biological functions are lost with the dental pulp. In addition to the metabolic processes of the pulp tissue, one of its most important tasks is root formation and secretion of dentine during tooth development and also afterwards. Furthermore, the pulp tissue can counter invading microorganisms by dentine formation as well as specific and unspecific immune actions (Schmalz et al., 2020). In addition, another defence mechanism of the dentine–pulp complex is to increase the outward flow of dentinal fluid (Maita et al., 1991). Thus, the loss of the pulp may also make it easier for bacteria to migrate through the dentinal tubules due to the lack of outward flow of dentine fluid. The protective role of dentine fluid pressure is particularly evident in the considerable bacterial invasion in teeth after RCT (Nagaoka et al., 1995). Ultimately, the dental pulp is also a sensory organ that perceives changes, for example in temperature or pathogenic stimuli. Loss of the pulp inevitably leads to neuronal deafferentation, which may play a role in persistent pain conditions and functional changes in the primary afferent endings of the trigeminal nerve (Lee et al., 2017; Schmalz et al., 2020). Regeneration of the dental pulp would allow recovery of all pulp functions and completion of root formation in juveniles, contributing to mechanical strengthening and thus long-term survival of the tooth.

With the objective of achieving biological regeneration of the dental pulp and providing a predictable and reproducible clinical outcome, researchers have made great efforts in recent years to develop tissue engineering strategies for application in the root canal. In this context, endodontic tissue engineering (ETE) can be subdivided in cell-based (CB-ETE) and primary cell-free procedures (CF-ETE) (Lin et al., 2021; Widbiller & Schmalz, 2021). In the cell-based procedures, which have shown initial clinical success, the cells used must be expanded *ex vivo* and introduced into the root canal by transplantation into prefabricated scaffolds with added growth factors

(Nakashima et al., 2017; Xuan et al., 2018). Although this is an intriguing approach, its clinical implementation poses significant challenges for scientists and clinicians. To obtain cells, a donor tissue or cells must be available or previously stored in a cell bank (Ohkoshi et al., 2018). In contrast, cell-homing procedures use endogenous sources of stem or progenitor cells and bypass *ex vivo* cell manipulation (Galler & Widbiller, 2020; Widbiller & Schmalz, 2021). Here, primarily cell-free scaffold materials are introduced into the root canal together with signalling molecules, where they are supposed to attract cells of the periapical tissue (Galler & Widbiller, 2017, 2020). In addition to the cells, blood vessels and nerve fibres also enter the scaffold material, and a new tissue is supposed to be formed that corresponds to the original pulp.

Besides the blood clot, which forms as a scaffold in the root canal during revitalization treatment, autologous platelet products such as platelet-rich fibrin (PRF), platelet-rich growth factor (PRGF) or platelet-rich plasma (PRP) can also be introduced in an orthograde direction into the root canal. Here, a fibrin matrix encapsulates blood components and platelets as a source of signalling molecules and, according to the concept of CF-ETE, provides the opportunity for cells to populate the root canal and form tissue. However, despite the described success in clinical trials (Bezgin et al., 2015; Rizk et al., 2019; Ulusoy et al., 2019; Xie et al., 2021), the biological outcome of platelet-based procedures is still unclear (Lin et al., 2021).

As a result of an increasing number of studies on approaches based on the introduction of scaffolds or biomaterials (natural or synthetic, allogenic or xenogenic and cell-based or cell-free) into the root canal appearing in recent literature, the question arises as to whether these treatments can be considered alternatives to standard therapies. So far, there are no systematic reviews examining whether ETE is an appropriate treatment choice for both mature and immature permanent teeth with apical periodontitis. The main objective of this review was to determine the effectiveness of cell-based as well as cell-free ETE in comparison with calcium hydroxide apexification, apical plug and root canal treatment.

## METHODS

This systematic review was registered at the international prospective register of systematic reviews PROSPERO (CRD42021266350; 7 July 2021) and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Nagendrababu et al., 2019; Page et al., 2021). The PICOTS (Population, Intervention, Comparison, Outcome, Timing, Study

type) framework was applied to investigate the following clinical question: 'What is the effectiveness of endodontic tissue engineering in treatment of permanent teeth in comparison to conventional techniques?' as follows:

- P = Patients with permanent immature or mature teeth and pulp necrosis with or without signs of apical periodontitis
- I = Clinical approaches based on the introduction of scaffolds or biomaterials (natural or synthetic, allogenic or xenogenic and cell-based or cell-free) into the root canal to facilitate tissue formation
- C = Calcium hydroxide apexification, apical plug or root canal treatment
- O = The main critical outcome was tooth survival and further critical outcomes were pain, tenderness, swelling, need for medication (analgesics and antibiotics), radiographic evidence of reduction of apical lesion size, radiographic evidence of normal periodontal ligament space. Other important outcomes were tooth function (fracture and restoration longevity), the need for further intervention, adverse effects (including exacerbation, restoration integrity, allergy and discolouration), oral health-related quality of life (OHRQoL), presence of sinus tract and response to sensibility testing.
- T = A minimum of 12 months and maximum of as long as possible for all outcome measures
- S = Human experimental studies (randomized controlled trials, comparative clinical trials [CCTs]—non-randomized). The search was supplemented by longitudinal observational studies (retrospective and prospective comparative cohort and case-control studies) to ensure that all relevant clinical information that is often not tested in experimental studies is captured. Number of patients to be at least 20 (10 in each arm) at the end of the study.

## Information sources and search strategy

On 2021-11-12, we searched the digital databases MEDLINE (Ovid; inception to present), Embase (Ovid; 1974 to present) and Cochrane Library (Wiley; inception to present). A search for grey literature was carried out in Google Scholar (inception to present). An initial, sensitive search strategy was created for MEDLINE employing a broad range of MeSH terms and text words. The structure of search strategy was Population AND Intervention AND Study Types. The MEDLINE strategy was adapted to the other databases regarding controlled vocabulary, syntax

and study filters. For MEDLINE and Embase, we used published, sensitive search filters to select knowledge syntheses and primary studies:

- Filter for Systematic Reviews/Meta-Analysis/Health Technology Assessment OVID Medline, Embase, PsycINFO (CADTH, 2021).
- Cochrane Highly Sensitive Search Strategy for identifying randomized trials in MEDLINE: sensitivity-maximizing version (2008 revision); Ovid format (Higgins & Green, 2011).
- Filter for controlled non-randomized studies with best sensitivity Ovid MEDLINE. Table 6 in Waffenschmidt et al. (2020).
- Filter for clinically sound treatment studies in Embase, strategy minimizing difference between sensitivity and specificity (Wong et al., 2006).

Results from MEDLINE and Embase were restricted to studies published in English language as justified in the protocol. We strived to follow the PRESS checklist (McGowan et al., 2016) when designing the search strategies, but had no opportunity to have them peer reviewed. The full search strategies of all databases in reproducible form and a documentation of the search results (database accession numbers) are publicly available from a research data repository (s. Data availability).

The tables of contents of the last 20 years (i.e. 2001 to 2021) of both the International Endodontic Journal and the Journal of Endodontics were screened manually for additional relevant studies as well as the reference lists of all included papers and previously published reviews.

Records were imported into Endnote (version X9.3.3) reference management software for deduplication following the method by Bramer et al. (Bramer et al., 2016).

## Study selection process

Following inclusion criteria were established for studies to be eligible for this systematic review:

1. Human experimental studies (randomized control trials, comparative clinical trials—non-randomized) or longitudinal observational studies (retrospective and prospective comparative cohort and case-control studies) with more than 20 patients (10 in each arm)
2. Recall sample size at least 10 in each arm
3. Patients with permanent teeth and pulp necrosis (symptomatic and asymptomatic)
4. Introduction of scaffolds or biomaterials

5. Control by a clinical standard treatment, for example apical plug, root canal treatment or calcium hydroxide apexification
6. Documentation of tooth survival
7. Follow-up for more than one year
8. Full text accessible in English

Studies were excluded if they involved procedures, which were not primarily based on scaffolds that were inserted from orthograde but on the blood clot as a matrix. Bleeding only in the apical part of the root canal was considered acceptable; however, the majority of the canal had to be filled with a scaffold with or without cells to comply with the idea of tissue engineering.

Records were screened for eligibility in two successive rounds, first by title and abstract then by full text using the Rayyan web application (<https://www.rayyan.ai/>). Screening was done blinded by two independent reviewers (MW, FD). After unblinding, disparate votes were discussed and resolved by agreement.

Agreement between reviewers in the full-text screening was assessed by the percentage of agreement and Cohen's kappa coefficient ( $\kappa$ ), which was calculated by SPSS statistical package version 28 (SPSS).

## Data collection

Data were extracted by two independent reviewers (MW, FD) into an established spreadsheet. In case of non-agreement between the reviewers, the data were not used until they were discussed with a third reviewer (HK). In case of studies with more than two arms and/or multiple papers reporting on the same study, only the relevant data of interest was extracted.

The following details were included in the spreadsheet for each study included in the final review: name and country of the first author, year published, name of the journal, type of study design, total number of participants, age distribution, number of participants with apical periodontitis, outcome measures, type of radiographic assessment and method of radiographic assessment. All extracted data were stored in tables.

## Risk of bias assessment

The risk of bias was critically appraised for all included randomized trials. The Cochrane risk-of-bias (RoB 2) tool was used (Sterne et al., 2019), which is based on five distinct domains ('randomization process', 'deviations from the intended interventions', 'missing outcome data', 'measurement of the outcome', 'selection of the reported

result'). Within each domain the two evaluators (MW, FD) independently answered at least one signalling question resulting in an overall judgement ('low risk of bias', 'some concerns', 'high risk of bias'). Discrepancies were discussed and solved by a third evaluator (HK). Specifically, a study is rated as 'low risk of bias' when all domains are low risk of bias, as 'some concerns' when at least 1 domain is rated as such, 'high risk of bias' when it is judged as 'high risk of bias' in at least 1 domain or as 'some concerns' for multiple domains leading to a loss of confidence in the results.

Agreement between reviewers regarding the RoB assessment was analysed as described above.

## Data synthesis

All data regarding endodontic tissue engineering were analysed qualitatively, and a narrative synthesis was performed. The success rate for ETE or the comparative treatment was calculated for each study by dividing the number of successful cases by the total number of cases during a specific time period.

## RESULTS

### Included studies

A total of 5137 records were initially identified in the electronic searches (3344 in MEDLINE; 910 in Embase; 783 in Cochrane Library; 100 in Google Scholar). After eliminating 974 duplicate records, 4163 records remained for title and abstract screening by two assessors. Of these, 8 reports were deemed relevant and a full-text evaluation of each was carried out by each assessor independently in accordance with the preferred reporting of systematic reviews (Moher et al., 2015). 6 reports were then excluded for reasons listed in Table 1, and total of 2 studies (2 reports) were included in the final review. Good inter-reviewer reliability was obtained at the full-text analysis ( $\kappa = .59$ ; 75% agreement). The processes of study identification and selection are illustrated as a PRISMA flow diagram in Figure 1.

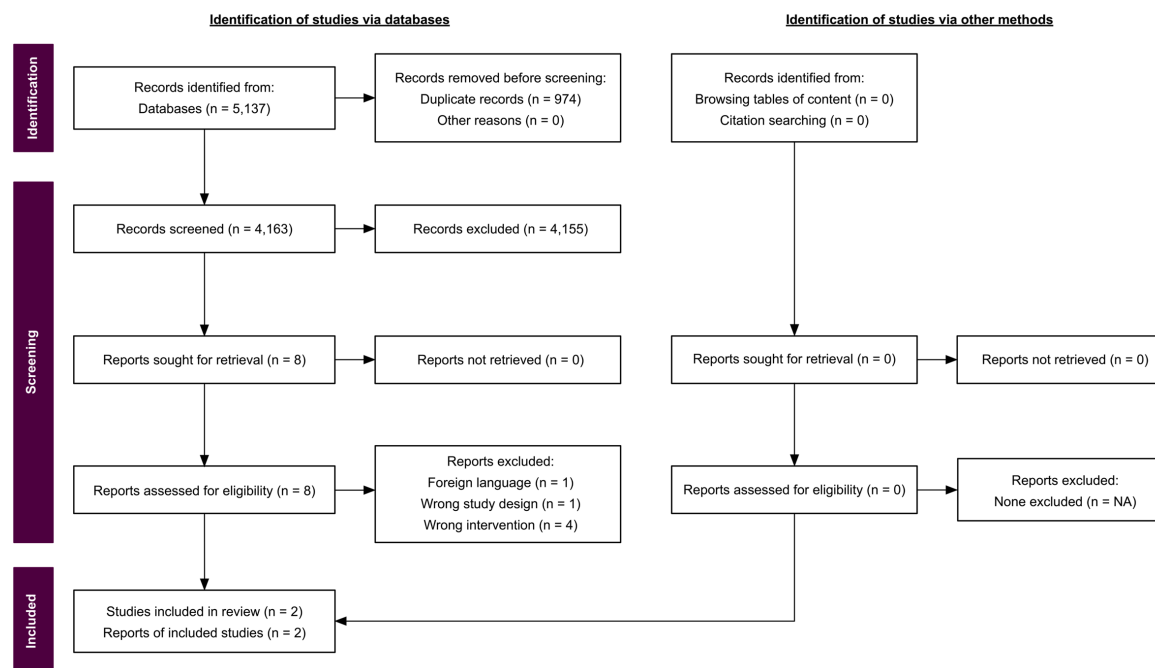
### Included study and population characteristics

The characteristics of all included studies and the particular population are shown in Tables 2 and 3. Both studies were randomized clinical trials and published in the year 2018 and 2020 respectively. The funding statements



**TABLE 1** Exclusion criteria and excluded articles

Reason	Number	Author and year	PMID
Foreign language	1	Rakhmanova & Korolenkova, 2020	33267545
No human experimental study (randomized control trials [RCT], comparative clinical trials [CCTs]—non-randomized) or longitudinal observational studies (retrospective and prospective comparative cohort and case-control studies) with less than 20 patients (10 in each arm)	1	Narang et al., 2015	25684914
No introduction of scaffolds or biomaterials	4	Lin et al., 2017, Pereira et al., 2021, Arslan et al., 2019, Nagy et al., 2014	28864219, 33094527, 31155298, 24461403

**FIGURE 1** Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) flow diagram of the study screening procedure and selection

of both studies list only non-commercial institutions (Brizuela et al., 2020; Xuan et al., 2018). The study by Xuan et al. (2018) included a total of 40 permanent immature incisors and Brizuela et al. (2020) 36 permanent necrotic mature teeth, however, only single-rooted teeth and no molars were treated. Pulp necrosis and apical periodontitis were diagnosed clinically and radiographically, but only Brizuela et al. (2020) reported radiographic lesions in all cases.

Regarding the interventions, Brizuela et al. (2020) compared a cell-based approach based on the introduction of encapsulated umbilical cord mesenchymal stem cells (UCMSC) in a plasma-derived biomaterial with conventional root canal treatment in permanent mature teeth. In contrast, Xuan et al. (2018) evaluated a cell-based approach,

in which aggregates of autologous human deciduous pulp stem cells without scaffold were transplanted into root canals, with apexification in permanent immature teeth.

Follow-up time for both studies was 12 months, and both articles analysed tooth survival, response to sensibility testing and response to vitality testing using cone beam computed tomography (CBCT), laser Doppler flow (LDF) measurement and electric pulp testing (EPT). Brizuela et al. (2020) also evaluated response to sensibility testing with cold and hot tests unlike Xuan et al. (2018) who only used EPT. The article published by Brizuela et al. (2020) also evaluated change in apical lesion size and cortical compromise using CBCT, pain to percussion and postoperative events such as tooth fracture, severe-or-moderate pain and extra-intraoral inflammation. Xuan et al. (2018)

TABLE 2 Study characteristics of the included articles

Author	Design	Location	Journal	Follow-up	Intervention	Control	Outcome	Radiographic assessment
Xuan et al., 2018	Randomized clinical trial	China	Science Translational Medicine	1, 3, 6, 9 and 12 months	Transplantation of human deciduous pulp stem cell (hDPSC) from maxillary deciduous canines	Apexification	Primary: pulpal response, blood perfusion Secondary: root lengthening, root wall thickening, apical closure	CBCT
Brizuela et al., 2020	Randomized clinical trial	Chile	Journal of Dental Research	6 and 12 months	Transplantation of human umbilical cord-derived mesenchymal stem cells (UC-MSC) in a plasma-derived biomaterial	Root canal treatment	Primary: safety <sup>a</sup> , efficacy <sup>b</sup> Secondary: pulpal response, blood perfusion, radiographic healing	CBCT and periapical X-rays

<sup>a</sup>Safety encompassed immediate adverse events as defined by root fracture, severe or moderate pain and extra-intraoral inflammation.  
<sup>b</sup>Efficacy means tooth survival, that is tooth retention in the mouth for a period of 12 months without percussion pain and an apical bone lesion of equal size in the 3 dimensions of space, a decrease in some of them or no more than a 0.1 mm increase in one of them.

TABLE 3 Population characteristics of the included articles

Author	Number of treated patients (treated teeth)	Tooth type	Gender	Age	Maturity	Aetiology	Periapical lesion	Follow-up	Lost to follow-up
Xuan et al., 2018	40 (40) Experimental group: 30 (30) Control group: 10 (10)	Incisors	7 female 33 male	7 to 12 years	Immature	Trauma	Unknown <sup>a</sup>	12 months	Experimental group: 4 (3 lost to follow-up; 1 additional trauma)
Brizuela et al., 2020	36 (36) Experimental group: 18 (18) Control group: 18 (18)	Incisors, canines, mandibular premolars	25 female 11 male	16 to 58 years	Mature	Unknown	36 <sup>b</sup>	12 months	0

<sup>a</sup>Most of the patients were described to have periapical lesions.  
<sup>b</sup>PAI ≥2 and CBCTPAI ≥1.

**TABLE 4** Summary of risk of bias assessment using the risk-of-bias tool for randomized trials per main outcome (survival)

Author	Randomization process	Deviations from the intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall
Xuan et al., 2018	Low risk	Some concerns	Some concerns	Low risk	Low risk	Some concerns
Brizuela et al., 2020	Low risk	Some concerns	Low risk	Low risk	Low risk	Some concerns

quantified the increase in root length, the degree of apical closure and the change in root canal wall thickness using CBCT.

## Quality assessment

The two included studies were randomized clinical trials and evaluated by the RoB 2 tool (Table 4). Both studies raised some concerns in terms of risk of bias. Despite controversial statements in the papers, the blinding of patients, practitioners and examiners appeared to be problematic in both studies. In addition, there were unexplained patient dropouts during follow-up in the study by Xuan et al. (2018) that could influence the results as missing outcomes. The qualitative assessments were performed with good inter-reviewer reliability ( $\kappa = .78$ ; 80% agreement). Further information on the specific domains is provided in Table S1.

## Heterogeneity assessment

The two studies revealed debatable differences with regard to the population characteristics, specifically immature teeth were treated in young patients by Xuan et al. (2018) whereas Brizuela et al. (2020) included mature teeth of older aged patients. Furthermore, Xuan et al. (2018) did not confirm the presence of a periapical lesions in all cases. Despite both trials reporting tooth survival, pulpal response and blood perfusion were documented differently. Whereas in Xuan et al. (2018) reported the decrease in EPT threshold, Brizuela et al. (2020) described the proportion of positive responses amongst the patients. Similarly, blood perfusion was documented as an increase in perfusion units (PU) by Xuan et al. (2018), however, Brizuela et al. (2020) provided PU in comparison with a healthy control tooth. Any other results were inconsistent in both trials and thus not comparable. Considering the heterogeneity of the results and the circumstance that in both studies all teeth survived the maximum observation period of 12 months, pooling of the data did not seem meaningful and a meta-analysis was not possible or reasonable.

## Tooth survival

Tables 5 summarizes results reported by all included studies. Both studies provide the data basis to calculate tooth survival after ETE. Brizuela et al. (2020) showed a 100% survival rate after 12 months when UC-MSC were implanted in mature permanent teeth. Similarly, Xuan et al. (2018) demonstrated a 100% survival rate when stem



**TABLE 5** Success rates and core outcomes of endodontic tissue engineering at a follow-up of 12 months

Author	Intervention	Number of treated teeth	Observation period	Number available at follow up	Survival	Pulpal response	Blood perfusion (LDF)
Xuan et al., 2018	Cell transplantation	30	12 months	26	100%	EPT: Decrease of $43.43 \pm 0.86$ in sensation EPT: 50% positive (16.7% baseline) Cold test: 55.6% positive (5.6% baseline) Warm test: 27.8% (0% baseline)	Increase of $7.19 \pm 0.77$ PU (2.81 $\pm$ 0.41 PU baseline) 78.1% (60.6% baseline)
Brizuela et al., 2020	Cell transplantation	18	12 months	18	100%		

Abbreviation: PU, Perfusion units.

**TABLE 6** Other inconsistently reported results at a follow-up of 12 months

Xuan et al., 2018	Brizuela et al., 2020
Increase in root length: $5.24 \pm 0.92$ mm ( $10.69 \pm 1.32$ mm baseline)	Reduction in apical lesion size: 2.1 mm anterior–posterior 2.0 mm mesial–distal 1.8 mm in height
Decrease of foramen width: $2.64 \pm 0.73$ mm ( $3.17 \pm 0.69$ mm baseline)	Cortical compromise after 12 months: 11% (83% baseline) Safety <sup>a</sup> : 100% Efficacy <sup>b</sup> : 100%

<sup>a</sup>Safety encompassed immediate adverse events as defined by root fracture, severe or moderate pain and extra-intraoral inflammation.<sup>b</sup>Efficacy means tooth survival, that is tooth retention in the mouth for a period of 12 months without percussion pain and an apical bone lesion of equal size in the 3 dimensions of space, a decrease in some of them, or no more than a 0.1 mm increase in one of them.

cells from the pulp of deciduous teeth were placed in immature permanent teeth, with secondary trauma being the only documented loss at 12 months. With regard to the control groups, tooth survival rates were 100% for both RCT and apexification.

## Pulpal response

Brizuela et al. (2020) assessed pulp response using cold, hot as well as electrical pulp tests and observed significant differences between the teeth treated with UC-MSC and those treated with RCT (Table 5). Teeth treated with UC-MSC showed a higher number of cases with positive responses (50%) than the teeth treated with RCT (17%) at 12-month follow-up. However, Xuan et al. (2018) only used the electrical pulp testing to evaluate the pulp's response. The teeth that received stem cells showed a significantly lower stimulation threshold (a mean decrease in sensation of  $43.43 \pm 0.86$ ) than those treated with apexification (a mean decrease in sensation of  $0.17 \pm 0.16$ ).

## Blood perfusion

In addition, both studies used LDF to evaluate blood perfusion (Table 5). The PU reading in the study by Xuan et al. (2018) increased in the cell transplantation group ( $7.19 \pm 0.77$  PU) compared with the apexification group (decrease in  $0.05 \pm 0.48$  PU) after 12 months. Statistically significant differences were demonstrated between the two groups, with tissue engineering allowing vessel formation compared with control.

Brizuela et al. (2020) only analysed the PU readings of the UC-MSC group and used as a control a healthy tooth

with similar anatomical characteristics to the tooth of the experimental group. They also reported an increase in PU readings at 12-month follow-up (from 60.6% at baseline to 78.1% at the end of follow-up with respect to the healthy tooth).

## Radiographic evidence of reduction in apical lesion size

Only Brizuela et al. (2020) assessed the change in apical lesion size and compromise of cortical bone by CBCT (Table 6). Fewer teeth were found to have cortical compromise after 12 months (78%) than at baseline (14%). No significant differences were observed regarding cortical involvement between the experimental (UC-MS) (11% of the cases) and the control (RCT) group (17% of the cases). Both groups revealed a reduction in apical lesion size without significant differences.

## Root lengthening and thickening

Root lengthening and thickening was only determined in the study of Xuan et al. (2018) (Table 6). The teeth that underwent cell transplantation showed a root lengthening of  $5.24 \pm 0.92$  mm at after 12 months compared with its baseline situation, where the root length after apexification only increased by  $0.88 \pm 0.67$  mm. Differences amongst the two groups were statistically significant. Regarding the thickening of the root wall, the teeth treated by cell transplantation showed a greater increase in root thickness than the teeth treated with apexification.

## Apical closure

Xuan et al. (2018) furthermore evaluated apical closure of teeth after ETE and apexification. The teeth that underwent cell transplantation showed a decrease in apical foramen width of  $2.64 \pm 0.73$  mm at after 12 months compared with a decrease in  $0.62 \pm 0.22$  mm in the apexification group. Despite groups showed a decrease in apical foramen size, the effect was significantly greater in the teeth that received stem cells from deciduous teeth.

## Adverse events

Brizuela et al. (2020) did not observe any adverse events such as root fracture, severe-or-moderate pain or inflammation in either the UC-MS group or the RCT group

during the 12-month follow-up period. Thus, the 'safety' used to summarize the aspects described was 100%.

## Certainty assessment

According to the PROSPERO protocol, the body of evidence should be assessed qualitatively in the course of data synthesis for each of the main outcomes using the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) concept. Since no meta-analysis was performed, only a narrative synthesis, and no recommendations for health care can be derived, this did not seem reasonable and appropriate (Guyatt et al., 2011).

## DISCUSSION

Regeneration of the dental pulp as a biological treatment alternative to classical endodontic approaches would offer many advantages for patients in both immature and mature permanent teeth. A regenerated pulp could, on the one hand, fulfil immunological and sensory tasks and, on the other hand, contribute to root growth and thus mechanical strength, especially in immature teeth (Bucchi et al., 2019; Cvek, 1992). A large number of high-quality studies on revitalization therapy outline the biological potential (Lin et al., 2017; Shetty et al., 2021; Torabinejad et al., 2017). Furthermore, innovative tissue engineering concepts are increasingly used to regenerate the dental pulp with the help of stem cell transplantation (CB-ETE) as well as the introduction platelet products or growth factor-laden hydrogels into root canals (CF-ETE). For both approaches, promising reports are accumulating and success has been documented in clinical trials and case reports (Nakashima et al., 2017; Rizk et al., 2019; Ulusoy et al., 2019; Zhujiang & Kim, 2016). However, in order to fully evaluate ETE approaches and provide a clinical recommendation for general dental practice, a systematic evidence synthesis comparing these clinical procedures with well-established and standardly applied techniques is imperative.

The different regenerative endodontic approaches, revitalization as well as tissue engineering-based techniques, offer a variety of advantages and disadvantages from both a clinical and biological perspective. In a clinical setting, the induction of bleeding during revitalization is often hesitant, challenging patient compliance and the use of blood products involves venepuncture, which is particularly unsettling for young patients. Furthermore, it is known that the newly formed tissue does not fully correspond to the dental pulp in terms of form and function and thus cannot be said to be biologically regenerated

(Kim et al., 2018; Stambolsky et al., 2016; Widbiller & Schmalz, 2021). In contrast, tissue engineering approaches come with completely new challenges, especially the cell-based techniques. Of course, they are highly complex from a technical perspective, as a donor tissue is required and the cells have to be prepared under laboratory conditions. Strict regulations apply and it takes considerable time and manpower, which makes it a presumably expensive undertaking. Cell-free techniques based on the implantation of platelet products or customized hydrogels, however, offer advantages, because an *ex vivo* manipulation of cells is not necessary, as there is no donor organ. Another advantage of ETE approaches that are not based on blood products is the painless and uncomplicated application by injection into the root canal.

Although there were only two studies that could be included from the field of endodontic tissue engineering, a major strength of the current systematic review is the implementation of strict inclusion and exclusion criteria. The intention was to achieve a very high standardization for included studies (follow-up time) in order to create the best possible comparability and the precondition for pooling the results. Thus, only randomized or comparative clinical trials or longitudinal observational studies with more than 20 patients were considered. Furthermore, a follow-up of at least one year had to be provided for at least 10 patients in each arm. However, what proved to be the greatest difficulty in the course of the title and abstract screening was less the study design but, in general, the selection of the control or comparative group. A requirement for inclusion was that the endodontic tissue engineering procedures were compared with conventional methods such as apical plug, apexification or root canal filling. This is the only way to compare the potential of the innovative procedures with that of standard, non-regenerative methods and derive a pertinent clinical recommendation. In many cases, ETE approaches, mostly techniques using platelet products, have been compared with revitalization (ElSheshtawy et al., 2020; Mittal & Parashar, 2019; Ramachandran et al., 2020; Rizk et al., 2020). In this regard, it should be recognized that this is, of course, a legitimate thematic set-up, as both cell-based and cell-free tissue engineering applications compete with revitalization in the clinic. Nonetheless, revitalization must also be seen as an innovative procedure, which is currently used mainly in specialized facilities as it requires appropriate training and specific equipment. Consequently, in order to be able to deduce clinical recommendations for general practitioners, comparisons must be made with techniques that can be applied in all dental practices and are used there on a regular basis.

Since the field of ETE is very broad and it became clear in pilot searches that the number of eligible studies might

be low, primarily cell-free techniques, such as platelet-based applications, and cell-based methods were investigated together. Both concepts are based on the basic principle of tissue engineering (Langer & Vacanti, 1993), although the distinction between subgroups (CB-ETE vs CF-ETE) is certainly of great interest if a sufficient number of studies are performed in the context of a meta-analysis. A meta-analysis was not possible in this work, but the review provides information about in which work the greatest necessity can be seen in the future, and guidance on what to pay particular attention to. In addition to the standardization of follow-up and outcome data, it is necessary to take into account the adequate control or comparison groups in the study design. To allow pooling of data, at least generally accepted outcomes such as survival and radiographic healing must be reported. Both will increasingly enhance the evidence of ETE procedures, which is urgently needed at present.

## Limitations of the studies

However, the included studies had several limitations, which are elaborated as follows. According to the consolidated standards of reporting trials (CONSORT), the registration of the study protocol in a corresponding database before the start of a clinical trial is a very important methodological aspect. This ensures the transparency of the research project and guarantees that the study design cannot be changed arbitrarily and adapted subsequently. It also prevents the data obtained from being selected and creating a one-sided picture, because readers can compare the published article with the protocol at any time. Usually, this is assured as part of the quality management during publication process (Schulz et al., 2010; Sponchiado-Júnior et al., 2021). Both studies were registered accordingly, but minor discrepancies were present that were not discussed in the publication. In the study by Xuan et al. (2018) the information on [ClinicalTrials.gov](https://www.clinicaltrials.gov) was not updated adequately and still results are missing despite the study was published in 2018. Furthermore, the changes in the methodology were not reported and therefore there are discrepancies between the study protocol and the published manuscript. For example, the sample size defined in the protocol was 80 patients, however, only 40 patients were finally included the published study. In addition, the registered protocol stated that the reduction in periapical lesion at 12 months should be assessed, which again was not included in the publication. In contrast, Brizuela et al. (2020) updated changes to the study protocol accordingly in the [ClinicalTrials.gov](https://www.clinicaltrials.gov) archive as the study proceeded, which is clearly documented in the history of changes. Thus, there were no discrepancies

between the study protocol and the published article. However, it should be noted that originally (first protocol version) a 24-month follow-up of patients was planned, but this was eventually reduced to 12 months. Since failures are known to often appear late, this is considered limiting and long-term observations are required in studies of regenerative endodontic procedures (Almutairi et al., 2019). In addition, in the last update of the registered protocol, the assessment of tooth discoloration was eliminated.

A particular challenge in a topic that provides only few comparative studies is certainly the preliminary calculation of sample sizes. Brizuela et al. (2020) chose the number of participants arbitrarily, and Xuan et al. (2018) also did not compute the number of samples for each outcome. The lack of an accurate sample calculation has a number of drawbacks, because the sample size affects the validity of the results, as an insufficient sample size can lead to low statistical power (Sponchiado-Júnior et al., 2021; Tong et al., 2017).

Blinding is a difficulty not only in the included papers but also in principle in all studies on regenerative endodontics. In the context of randomized clinical trials, blinding of the patient (and their guardian), the practitioner and the outcome assessor is a methodological quality criterion. It is important to reduce performance as well as detection bias and to add credibility to the validation of data, especially when results are based on subjective parameters. Nevertheless, not every aspect of this is implementable in the course of the treatments examined.

On the one hand, the procedures are often associated with particular steps on the basis of which the patient receives information on the treatment. In the case of autologous cell-based ETE, the cells must be obtained in advance and prepared in the laboratory, which in the case of Xuan et al. (2018) was done by removing a deciduous canine, for example (Xuan et al., 2018). Consequently, blinding of the patient is hardly possible here. Blinding would be thinkable in the case of allogeneic cell transplantation, as described by Brizuela et al. (2020). However, in this particular case, a special medical briefing is required and the patient's consent to receive stem cells from a foreign donor depending on randomization. In contrast, blinding of the patients would be feasible at primarily cell-free approaches, even though in the case of platelet products venepuncture can provide information. In contrast to the patients, blinding of the operator is by no means possible, because the procedures are fundamentally different. This would only be conceivable in the case of minor modifications of a process, for example the alteration of signalling molecules in a comparable scaffold, which can be kept secret from the operator. In the case of outcome assessor, blinding is essential and possible under the condition that the person only collects clinical parameters

and has no further radiological information. The identification of completely different procedures, for example cell transplantation and a root canal filling, is possible on two- or three-dimensional radiographs at any time, which is why the examiner cannot evaluate in a blinded manner here. As described above, blinding would be feasible if the compared procedures are similar and only differ in radiologically not detectable aspects, for example signalling molecules, cells or scaffold materials.

Both the study by Brizuela et al. (2020) and that of Xuan et al. (2018) reported the blinding of participants, study personnel and outcome assessors, that is triple blinding, which is not possible based on the previous considerations and the fact that RCT or apexification was used as a control. Poor description of the blinding steps was also criticized by other authors (Loguercio et al., 2017; Pandis et al., 2010; Reis et al., 2018). In principle, it should be noted that the blinding steps must be described in detail and precise information should be provided about who was blinded and which blinding strategy was used (Schulz & Grimes, 2002).

Another point of discussion is the inclusion of patients in regenerative endodontic trials. Basically, the treatment of a selected subpopulation leads to a potential selection bias and thus to a limitation of external validity. For example results from subjects in specialized facilities may not reflect those in a general dental practice. In addition, other variables may be reflected in the inclusion criteria that could impact the results. For instance, for revitalization, the aetiology of pulpal necrosis, the patient age as well as the apical diameter are debated controversially (Almutairi et al., 2019; Estefan et al., 2016; Koç & Fabbro, 2020; Scelza et al., 2021). If a dental trauma was the cause of pulpal necrosis, there is a considerable risk of collateral resorptive lesions due to luxation (Galler et al., 2021), and especially in young patients, damage to the Hertwig epithelial root sheath (HERS) is conceivable. Furthermore, the stage of root development and the width of the apical foramen may also be relevant, since a wider foramen provides a better connection to the periapical tissue. These effects cannot be excluded in the work of Xuan et al. (2018) in which only traumatized incisors of young patients, mainly males, were treated, whereas Brizuela et al. (2020) included primarily female patients of a wide age range and diverse aetiology.

## Outcomes

In addition to the survival of a tooth, which is based on absence of clinical symptoms, the resolution of an apical radiolucency is a decisive criterion for success of endodontic treatment in general. In analogy, the evaluation



of radiographic healing assumes an important role in the course of regenerative endodontic procedures (Chugal et al., 2017; Diogenes & Ruparel, 2017).

It is all the more surprising that this outcome was not taken into account in the study by Xuan et al. (2018) when comparing the experimental and control groups after 12 months. For this reason, it is not possible in this study to compare the efficacy CB-ETE with apexification in terms of resolution of radiological signs of inflammation, although the data basis would be available with the CBCTs. In contrast, the study by Brizuela et al. (2020) only reported data for cortical involvement and quantification of lesion reduction in three planes of the CBCT. However, based on the data provided, it is not possible to determine the probability for reduction or resolution in apical radiolucency. Thus, the information content is seriously limited, since an evaluation of the radiological treatment success is not possible in this way. In addition, neither of the studies included in the qualitative synthesis examined outcomes that were significant from a clinical perspective, such as canal obliteration, discoloration or oral health-related quality of life (OHRQoL).

The evaluation of the pulp after regenerative endodontic treatment is of great importance to the practitioner as it is the only specific clinical parameter for success besides absence of inflammation. However, this proves difficult for several reasons. First, the patient's compliance is required to verify the sensitivity, but this is often limited especially in young patients. Usually, tests based on thermal or electrical stimulation are used for this purpose. Here, thermal tests provoke intratubular fluid movement and thus indirectly excite A $\delta$ -fibres, whilst electric pulp tests stimulate these fibres directly (Jafarzadeh & Abbott, 2010). Both tests are used as surrogates for pulp vitality and require a functional vasculature as prerequisite for innervation. Since regeneration of neuronal structures is difficult to predict, as many revitalization studies show, positive sensitivity can only be expected in about 50% of cases (Diogenes & Ruparel, 2017). A lack of cold sensitivity alone does not in the least indicate pulpal necrosis or failure, as it is still possible that non-sensitive, but vital tissue is present at the time of testing (Widbiller & Schmalz, 2021). Another problem, especially with thermal tests, is coronal restoration. Since the endodontic space for regeneration usually does not extend further than the root canal entrance, the insulating layer of composite and bioactive cements significantly attenuates the stimulus.

Another way to objectively check pulp vitality is to use methods that directly assess the blood supply to the pulp, such as laser Doppler flow measurement, ultrasound Doppler flow measurement or pulse oximetry. These vitality tests provide higher diagnostic accuracy compared with sensitivity tests (Balevi, 2019; Ghouth et al., 2018),

but problems often occur and their application in particular is clinically complex (Alghaithy & Qualtrough, 2017; Ghouth et al., 2019). In addition to the clinical parameters described, radiographs of course also offer a way to infer the status of a newly formed tissue. In particular, the growth in length and thickness of roots of immature teeth or ectopic mineralization allow conclusions. All these factors must be taken into account and combined when assessing pulp vitality.

For both thermal and electrical testing, it is advisable to exclude false positives by comparison with healthy teeth or, especially in the case of EPT, by a baseline measurement prior to regenerative treatment. Although the assessment of pulp sensitivity in the control group with conventional treatment, for example RCT or apexification, might also give an idea of false-positive responses, the bias must be regarded as very critical. Whilst possibly unblinded patients hope for cold sensitivity after regenerative treatments, the opposite is expected after conventional treatment.

Furthermore, it should be mentioned at this point that even a vitality or sensitivity response is no proof of biological regeneration of the pulp as repair tissues can also show those features (Austah et al., 2018).

## Limitations of this review

Study registries were not searched and therefore studies not reported in journal articles may have been missed. Searching study registries was not specified in the protocol common to a whole set of systematic reviews aiming to provide the basis of an ESE guideline. Also as specified in the protocol one study was excluded during full-text review due to not being published in English language (Rakhmanova & Korolenkova, 2020). We did not provide quantitative syntheses for individual outcomes due to the small number of studies and their high heterogeneity. This also precluded derived analyses.

## CONCLUSIONS

In summary, based on this systematic literature review, the clinical evidence for effectiveness of ETE approaches is very limited, a lack of studies controlled with conventional procedures such as calcium hydroxide apexification, apical plug and root canal treatment was particularly evident. Within the limitations of this review, the benefits and high success rates reported for ETE techniques suggest that these procedures may represent an alternative to conventional procedures for permanent teeth with pulpal necrosis. Although the existing work suggests high



survival rates, the observation period is short and there is a moderate risk of bias due to methodological limitations in the included studies, so a recommendation cannot be derived at this stage.

## AUTHOR CONTRIBUTIONS

MW and FDST contributed to conceptualization, methodology, investigation and writing—original draft; HK contributed to methodology, formal analysis, writing—review and editing; NM contributed to methodology and writing—review and editing.

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## CONFLICT OF INTEREST

The authors declare to have no potential conflict of interest.

## DATA AVAILABILITY STATEMENT

PRISMA-2020 checklist, PRISMA-S checklist, full search documentation including database accession numbers of records found and details of RoB-assessment are publicly available from a research data repository (DOI: [10.5283/epub.52428](https://doi.org/10.5283/epub.52428)): <https://doi.org/10.5283/epub.52428>

## ETHICAL STATEMENT

The ethical approval was not required for the systematic review.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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