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REVIEW ARTICLE

The effectiveness of pulp revascularization in root formation of necrotic immature permanent teeth: A systematic review

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Abstract

Objective. The objective of the present study was to carry out a systematic review to analyse the effectiveness of pulp revascularization in the root formation of necrotic immature permanent teeth, as well as the level of scientific evidence regarding this theme. ***Materials and methods.*** The methodology was based on searching electronic databases such as Web of Science, Pubmed, BVS (Medline, Scielo, Lilacs and BBO), Scopus and Cochrane, including manual searches for the references listed in the studies found. The terms used for the literature search were *pulp revascularization* and *endodontics.* ***Results.*** Initially, 277 articles were identified from the electronic databases; 17 studies remained after analysis and exclusion of duplicates; exclusion criteria also eliminated six articles; 11 remained for evaluation. ***Conclusions.*** Although the results found in the present systematic review are relevant, the scientific evidence should be interpreted with caution as the articles report different methods and evaluation parameters. Despite the capacity of the pulp revascularization technique to stimulate the development of the apical closure and thickening of radicular dentin, several aspects still remain unknown, like the key factors of this repair, the type of tissue formed and the long-term prognosis.

Key Words: *Endodontics, pulp revascularization, incomplete root formation, systematic review*

Introduction

Endodontic management of immature permanent teeth with necrotic pulps and open apices is a significant challenge to endodontists. In this situation, it is not uncommon to see chronic apical periodontitis or even an acute dentoalveolar abscess. Furthermore, the presence of thin dentin walls and the lack of a natural apical constriction for an obturation material to be placed against increases the problem [1,2].

Such teeth have been treated by the apexification procedure for decades, which involves placement of intracanal calcium hydroxide ($Ca(OH)_2$) to induce formation of a calcific barrier at the apex. Despite the widespread use of the $Ca(OH)_2$-based apexification technique, the lengthy treatment period, which might require multiple visits and renewal of the intracanal dressing [3,4], the unpredictability of apical closure [5] and the susceptibility of cervical root fractures after prolonged exposure to $Ca(OH)_2$ [6] have raised serious concern about the merit of this treatment approach.

More recently, the traditional apexification procedure has been modified by the introduction of artificial apical barrier methods with mineral trioxide aggregate (MTA) [2,7,8]. Although this approach might considerably shorten the treatment period, improve patient compliance and result in favorable healing of the periapical tissues [2,7,9,10], it still cannot stimulate the development of the apical closure and thickening of radicular dentin [5,6].

On the basis of these considerations, the fate of apexification in future treatment protocols for necrotic immature permanent teeth appears to be questionable [11,12]. Regenerative endodontic methods have the potential to allow for continuation of root development and might, therefore, offer an alternative therapeutic approach in the management of necrotic

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immature permanent teeth with compromised structural integrity [13].

In 2001, Iwaya et al. [14] showed that continued root development and apical closure in a necrotic immature tooth were possible when successful disinfection of the root canal was achieved. In 2004, Banchs and Trope [15], through a case report, described a new treatment protocol for the management of immature permanent teeth with apical periodontitis, termed revascularization. The first step of this regenerative technique involves disinfection of the root canal with copious sodium hypochlorite (NaOCl) irrigation and a combination of ciprofloxacin, metronidazole and minocycline. After successful disinfection, the antibiotic paste is removed and apical bleeding is induced to produce a blood clot in the canal. As a final step, the canal orifice is sealed with MTA and a permanent coronal restoration is placed. Since the description of the revascularization technique, several case reports and treatment outcome studies have demonstrated the regenerative potential of this treatment, as evidenced by increased root length, thickening of the root wall and apical closure [16,17].

We critically examined the available literature to identify and assess the level of scientific evidence regarding this theme, which were then systematically reviewed to determine the effectiveness of pulp revascularization in root formation of necrotic immature permanent teeth.

Materials and methods

Research question

Is the use of the pulp revascularization technique efficacious in the root formation of immature permanent teeth with pulp necrosis?

Strategy for identification and selection of the studies

An ample search for the studies was performed by two independent researchers. Only those articles published up to July 2014 were considered for review, and the selection process is described in Figure 1. The following electronic databases were used: Web of Science, Pubmed, BVS (Medline, Lilacs and BBO), Scopus and Cochrane for searching potentially relevant studies.

The keywords used for the bibliographic search were *pulp revascularization* and *endodontics*. Both terms were combined in order to refine the search results.

These keywords were chosen from the structured and trilingual vocabulary DeCS (Descriptors of Health Science), which was developed based on the Medical Subject Headings (MeSH) of the National

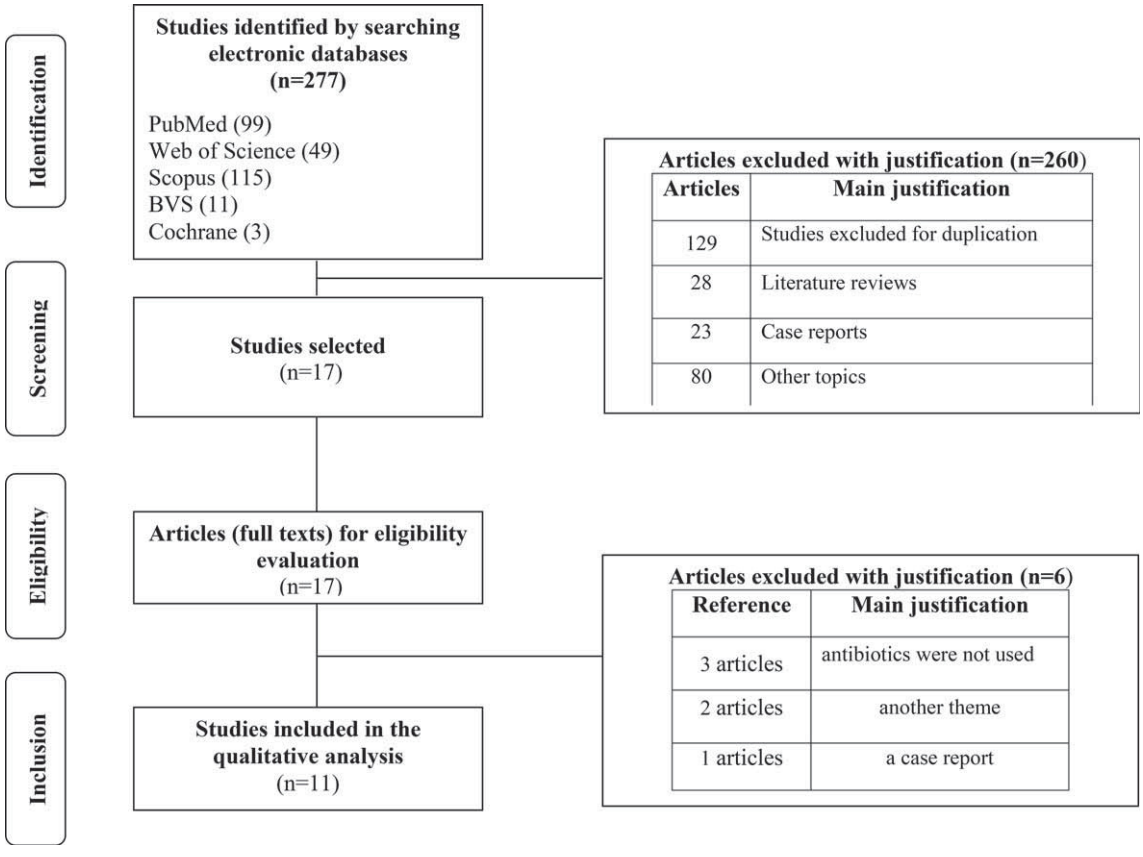

Figure 1. Flowchart of the search process and selection of research studies by using the items recommended for systematic reviews (PRISMA).

Table I. Characteristics of the studies that met the inclusion criteria.

		Methodology							
			First visit			Second visit			
Reference, year	Objective	Patients/ Kind of teeth	Mode of disinfection	Medications	Drug time	Techniques	Sealing	Follow-up	Outcome
Jung *et al.* [34], 2008	Reports the outcomes of immature permanent teeth with pulpal necrosis and apical periodontitis, divided into two groups with the difference of stimulation of bleeding to form a clot or not	8/ 9 premolars	5–5.25% NaOCL 10–30 min	Cipro, Metro, Mino	1 week	Group I: Ca $(OH)_2$ and/or MTA Group II: blood clot + MTA	IRM and later Composite Resin	10 months–5 years	In both groups of patients, there was evidence of satisfactory postoperative clinical outcomes; the patients were asymptomatic, no sinus tracts were evident, apical periodontitis was resolved and there was radiographic evidence of continuing thickness of dentinal walls, apical closure or increased root length
Ding *et al.* [31], 2009	Examine the effect of a pulpal revascularization procedure for immature necrotic teeth with apical periodontitis	12/ 5 incisors + 7 premolars	20 ml 5.25% NaOCL	Cipro, Metro, Mino	1 week	Blood clot + MTA	Composite Resin	15 months	Six patients dropped from the study (as a result of pain or failure to induce bleeding after canal disinfection) and instead received a standard apexification procedure. Another three patients did not attend any recall appointments. The remaining teeth (n = 3) were found to exhibit complete root development, with a positive response to pulp testing
Petrino *et al.* [35], 2010	Report three cases of immature permanent teeth with pulpal necrosis and apical periodontitis that used revascularization protocol	3/ 4 incisors + 2 premolars	10–20 ml 5.25% NaOCL	Cipro, Metro, Mino	14–34 days	Blood clot + MTA	Composite Resin	6 months–1 year	For follow-up, all six teeth showed resolution of periapical radiolucencies, whereas three of six teeth showed continued root development. Two teeth displayed a positive response to vitality testing. One tooth showed color change
Nosrat *et al.* [26]., 2011	Describes successful revascularization treatment of necrotic immature first mandibular molars	2/ 2 molars	20 ml 5.25% NaOCL 20 min	Cipro, Metro, Mino	21 days	Blood clot + CEM	Glass Ionomer + amalgam or stainless steel crown	15–18 months	In radiographic and clinical follow-ups both cases were asymptomatic and functional, periapical radiolucencies were healed and roots continued to develop. In addition, placing CEM as a new endodontic biomaterial over the blood clot formed inside the canals provided good seal and favorable outcomes

Table I. (*Continued*).

Reference, year	Objective	Methodology: Patients/ Kind of teeth	First visit: Mode of disinfection	First visit: Medications	First visit: Drug time	Second visit: Techniques	Second visit: Sealing	Follow-up	Outcome
Kim *et al.* [29]., 2012	The present case reports evaluations of the long-term prognosis of revascularization in necrotic immature teeth, with the aim of providing reliable evidences for the revascularization technique	3/ 3 premolars	10–20 ml 5.25% NaOCL 2–5 min	Cipro, Metro, Cefa	2 weeks	Blood clot + MTA	Gutta-percha and Composite Resin	24–48 months	All three cases showed resolution of the periapical radiolucency and thickening of the dentin walls
Jeeruphan *et al.* [23]., 2012	Evaluate radiographic and clinical outcomes of immature teeth treated with Ca $(OH)_2$ apexification, MTA apexification and revascularization	61/ 36 incisors + 25 premolars	2.5% NaOCL	Ca $(OH)_2$	Ca $(OH)_2$ 17 ± 12.6 months	Gutta-percha	Glass Ionomer and Composite Resin	Ca$(OH)_2$ 27.32 ± 30.47	The percentage of change in the width of the root was significantly higher in the revascularization group; the percentage of increase in root length was significantly higher in the revascularization group; the survival rate of the teeth treated with revascularization were higher
				Cipro, Metro, Mino	Revasc - 28.85 ± 13.08 days	Blood clot + Collaplug		Revasc - 21.15 ± 11.70	
				MTA	-	-		MTA- 14.21 ± 7.84	
Sönmez *et al.* [27]., 2013	Reports the outcome of revascularization treatment in three necrotic immature molars	3/ 3 molars	10 ml 5.25% NaOCl	Cipro, Metro, Mino	2 weeks	Blood clot + MTA	Composite Resin	24 months	All patients continued to be asymptomatic and thickening of the dentinal walls continued with apical closure. The radiographs showed complete resolution of periapical radiolucencies
Jadhav *et al.* [28]., 2013	Evaluate and compare revascularization, with and without PRP in non-vital, immature anterior teeth	3/ 6 incisors	20 ml 2.5% NaOCL	Cipro, Metro, Mino	4 weeks	Group I: no PRP; Group II: Blood clot + PRP	Resin modified glass ionomer cement or porcelain fused to metal crowns	12 months	All cases showed resolution of clinical signs and symptoms and also apical closure, thickening of the dentin walls and increased root length. The group that used PRP reported better results

Table I. (*Continued*).

		Methodology							
			First visit			Second visit			
Reference, year	Objective	Patients/ Kind of teeth	Mode of disinfection	Medications	Drug time	Techniques	Sealing	Follow-up	Outcome
Nagy *et al.* [24]., 2014	Assess the effectiveness of three techniques of revascularization for immature necrotic teeth (MTA apical plug, regenerative endodontic protocol [blood clot] and regenerative endodontic protocol [blood clot + injectable scaffold])	36/ 36 incisors	10 ml 2.6% NaOCL	Cipro, Metro, Doxy	3 weeks	MTA apical plug Blood clot Blood clot + bFGF	18 months	Composite Resin	The percentages of recall for the MTA, REG and FGF groups were 75%, 83% and 83% respectively. Clinical and radiographic examination during the follow-up period showed signs and symptoms of failure in three of the 29 recalled cases. Two cases belonged to the FGF group and one case belonged to the REG group. The success rates for the MTA, REG and FGF groups were 100%, 90% and 80%, respectively. The use of artificial hydrogel scaffold and basic fibroblast growth factor was not essential for repair
Kahler *et al.* [30]., 2014	Present a preliminary result of 16 cases of immature permanent teeth with pulp necrosis treated with a pulp revascularization technique	12/ 13 incisors + 3 premolars	1% NaOCL	Cipro, Metro, Amox	4 weeks	Blood clot + MTA	18 months–3 years	Glass Ionomer	Qualitative assessment showed 90.3% resolution of the periapical radiolucency. Apical closure was assessed as incomplete in 47.2% and complete apical closure in 19.4% of cases. Quantitative assessment showed change in root length varying from 2.7–25.3% and change for root dentin thickness of 1.9–72.6%. Discoloration of the crown was a common consequence, with unesthetic results in 10 of the 16 cases
Nagata *et al.* [25]., 2014	Evaluate traumatized immature teeth treated with two protocols of pulp revascularization (TAP and CHP)	23/ 23 incisors	20 ml 6% NaOCL and 10 ml 2% CH	G1 = Cipro, Metro, Mino G2 = $Ca(OH)_2$ with 2% chlorhexidine gel	21 days	Blood clot + CollaCote + MTA	Coltosol + Composite resin	9–19 months	Revascularization outcomes for traumatized patients treated with the tested protocols presented similar clinical and radiographic data. The success rate of periapical lesion repair was considered close to 100% TAP caused esthetic problem leading to tooth discoloration, which can be considered a disadvantage when compared with CHP

Cipro, Ciprofloxacin; Metro, Metronidazole; Mino, Minocycline; Metro, Metronidazole; Doxy, Doxycycline; Amox, Amoxicillin; Cefa, Cefaclor; PRP, Platelet rich plasma; CEM, Calcium enriched mixture cement; Ca (OH)2, Calcium hydroxide; MTA, Mineral trioxide aggregate; TAP, Triple antibiotic paste; CH, Chlorhexidine; CHP, Ca(OH)2 with 2% chlorhexidine gel; NaOCl, Sodium hypochlorite.

Library of Medicine of the US to allow terms to be used in Portuguese, Spanish or English. This method provided a unique and consistent way to retrieve information, regardless of the language used. The above-mentioned keywords yielded the best search results during the pre-test phase.

After an initial electronic search, both abstracts and titles were evaluated. In those cases in which the abstract and title were found to not be clear, the article was fully read in order to minimize the possibility of disregarding important studies. In addition, a manual search for the references of the studies was also performed.

Criteria for study selection

Inclusion criteria

- Clinical studies (clinical research studies and serial case reports);
- Studies that evaluated the performance of pulp revascularization in incomplete root formation with pulp necrosis
- Studies using NaOCl for disinfection of the root canal;
- Studies using medication based on antibiotics;
- Studies inducing apical bleeding to produce a blood clot; and
- Studies with no language restriction.

Exclusion criteria

- Literature reviews;
- Dissertations;
- Theses;
- Qualitative studies;
- Case reports;
- Chapters of textbooks or textbook; and
- Annals of congress.

Critical evaluation of the studies

Full copies from the potentially relevant studies were obtained and analyzed by two investigators independently (AS and MD). Next, the selected studies were compared and any disparity found was discussed so that a consensus could be met by a third investigator (LA).

After assessing the articles, studies not meeting the inclusion criteria were excluded.

Data extraction

Data were retrieved by two investigators (AS and MD) on an independent basis by fully reading the articles and considering the following variables: objective, methodology (number of patients/kind of teeth, first visit [mode of disinfection and medications], second visit techniques, follow-up time and sealing) and outcome.

Results

Initially, 277 studies were identified through their abstracts in the following electronic databases: 115 articles from Scopus, 99 from Pubmed, 49 from Web of Science, 11 from BVS and three from Cochrane. After disregarding the studies duplicated and analysing the titles and abstracts, 17 articles were selected.

Of these, three did not use antibiotics [18–20], two were not about the topic [21,22] and one was a case report [15], thus not corresponding to the inclusion criteria of this systematic review. Therefore, 11 articles were analyzed in detail.

Data extraction from the articles selected was described in Table I. In the studies assessed, the number of patients ranged from 2–61, as well as the number of teeth. Only three studies evaluated > 20 patients/teeth [23–25]. The main teeth evaluated were incisors (n = 123), followed by pre-molars (n = 48) and only two studies evaluated molars (n = 5) [26,27]. Three studies evaluated only incisors: Nagy et al. [24], n = 36; Nagata et al. [25], n = 23; and Jadhav et al. [28], n = 6. The others presented incisors and pre-molars together.

The necessary first step for treating infected necrotic teeth is root canal disinfection. To do this, sodium hypochlorite (NaOCl) was used at concentrations ranging from 2.5–6%, with minimum or no instrumentation. This was followed by the application of an antibiotic paste, formed by ciprofloxacin, metronidazole and minocycline, as recommended by Banchs and Trope [15]. In order to prevent discoloration of the tooth, three studies replaced Minocycline with Cefaclor [29], Doxycycline [24] and Amoxicillin [30]. The time the drugs spent in the root canal ranged between 1–4 weeks, until no more exudate, swelling, sinus tract and pain or tenderness to percussion were observed.

Once the infection was quelled, bleeding into the canal was induced, followed by sealing with MTA and restoration with glass ionomer cement or composite resin. In order to facilitate the sealing procedure, some studies modified the technique by introducing a matrix of Collaplug (Zimmer Dental, Carlsbad, CA) [23] or CollaCote (Zimmer Dental) [25] and then sealing the element with MTA and a restoration. In other studies, we saw a blood clot supplemented with an autologous Platelet Rich Plasma (PRP) [28] or a blood clot and an injectable scaffold impregnated with basic fibroblast growth factor. (bFGF) [24].

The most used restorative material was composite resin. There were reports of difficulty in following the progress of cases because of non-attendance and drop-out of treatment [24,31]. In one study, seven of the 36 patients were excluded because of inadequate compliance and failure to recall. Clinical and radiographic examination during the follow-up period

showed signs and symptoms of failure in three of the 29 recalled cases [24]. In another study, only three of the 12 cases were successful. Regarding cases of failure: In four cases there was a failure to induce bleeding, two experienced pain after placement of the antibiotic mixture and three were lost to follow-up [31].

Resolution of apical periodontitis, sinus tract healing, asymptomatic teeth, thick dentin walls and root length with apical closure were reported.

Discussion

The use of the pulp revascularization technique has been identified as an effective method to stimulate the development of the apical closure and thickening of radicular dentin. However, there are few histological and clinical studies assessing its action within root canals, and there is a lack of standardization of the parameters used for clinical treatment in endodontics.

The objective of the present study was to analyze the effectiveness of pulp revascularization in root formation of necrotic immature permanent teeth.

Through the present systematic review, we observed that there are few studies on the theme. Due to the fact that systematic reviews are based on inclusion and exclusion criteria and extremely rigorous methodological criteria, the number of articles tends to decrease, mainly when new criteria are imposed by the authors. This occurred in the present study, as articles that used other disinfection methods instead of a triantibiotic mix were excluded.

With regard to the methodology employed, there were several observed differences between the articles selected.

Although the Banchs and Trope's [15] protocol for pulp revascularization exists, most authors have introduced modifications to it. These modifications started as early as the disinfection step, where the concentration of sodium hypochlorite (NaOCl) used in irrigation ranged from 2.5–6%, which interferes directly with the solution's properties and possibly, in high concentrations, exterminates the surviving cells within the root canal. Nagata et al. [25] used calcium hydroxide and 2% chlorhexidine gel for disinfection, both of them known to be cytotoxic when in direct contact with the cells. Despite the variations of techniques in the disinfection process, all studies reported clinical success with resolution of apical periodontitis and fistulas and obtained totally asymptomatic teeth.

Several studies have reported discoloration on teeth [25,30]. This represents a disadvantage of the procedure. In order to replace the minocycline and prevent color changes, Cefaclor [30], Doxycycline [24] and Amoxicillin [30] were used. Studies also suggest use of Rokitamicina, Fosfomycin and Cephradine [32]. Sealing the dentinal tubules of the crown with the bonding agent composites was also proposed [33].

Most studies applied the revascularization technique on incisors, which are the most affected by trauma, followed by premolars, which are the most affected by carious lesions. Only two studies used two molars. Nosrat et al. [26] highlights the difficulty in achieving bleeding in the mesial canals of molars. At this point, a question arises: is there an influence or limitation of the anatomy in the use of revascularization technique? The solution found by Nosrat et al. [26] was the use of distal channel blood. Is this a valid solution? This difficulty was not unique to molars. It was also found in incisors and premolars. This led to most studies using anesthetic without vasoconstrictor in this step, but larger studies are needed to evaluate this point.

Some authors have proposed to optimize the properties of the blood clot by adding autologous Platelet Rich Plasma (PRP) [28] or basic fibroblast growth factor (bFGF) [24], but this optimization will only be possible, in our point of view, when the key factors of this type of repair are determined. Furthermore, a study reported conditions where blood clot was not necessary for root development [34].

Petrino et al. [35] considered it a challenging technique, as several authors have found difficulty in its execution. It was difficult to seal a root canal with MTA over a blood clot, where it mingled with the material when some pressure was applied. Two studies successfully proposed the use of a matrix with Collaplug (Zimmer Dental) [23] or CollaCote (Zimmer Dental) [25] to overcome this problem.

Composite resin was the choice for restoration of the element in most studies because of its esthetic value.

Patterns of continued root maturogenesis were variable at an 18-month review, although periapical pathology appeared to be resolved completely. Reviews at 36 months showed continued root maturogenesis for some cases [30]. Only two studies followed the cases for 48–60 months [29,34], which leaves doubts on the long-term prognosis. There is also difficulty in maintaining patient monitoring for such long periods, as reported by Ding et al. [31] and Nagy et al. [24].

Ratings during the monitoring period were clinical and radiographic. Clinical results clearly demonstrate the success of the technique and its advantages when compared to the $Ca(OH)_2$ apexification technique and the induction of an apical barrier with MTA. The studies showed positive results in quelling the infection. However, in radiographic analysis, only Kahler et al. [30] bothered to eliminate variations in the images that can distort the qualitative assessment of outcome. To do so, this study used the Turbo Reg Plug-in of the Image J Program (Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, VD, Switzerland).

Two other studies only digitized the images to perform measurements, ignoring the changes caused by different incidences of the central X-ray beam [23,24]. Other studies also presented radiographs without any standardization. This demonstrates the necessity of a protocol for the radiographic evaluations.

Despite these studies demonstrating the clinical success of the technique, there are very few histological studies on human teeth describing the type of tissue formed in this kind of repair [34]. Existing studies were performed with teeth submitted to revascularization and subsequently extracted for some other reason. One animal study showed that three types of tissues were generated after treatment including cementum-like tissue along the dentinal walls responsible for root wall thickening, bone-like tissue and periodontal ligament-like tissue. Also, in one case, partially survived pulp tissue and the presence of odontoblast cells lining the dentinal walls were evident. The study concludes that the tissue formed in the canal space is not pulp and does not function like pulp tissue, which means that revascularization is not pulp regeneration but resembles the wound repair process [26,36]. There are also reports of complete calcification of the blood clot space and ankylosis.

It is important to emphasize that the authors of the selected studies adopted different parameters both for the implementation of the technique of pulp revascularization and for evaluating outcomes. The lack of standardization in the methodology does not allow the results to be compared by meta-analysis. The diversity of parameters involved, as well as the limited amount of articles using this type of approach, makes any comparison difficult and less reliable.

All these questions lead us to believe that it is necessary to establish a standardized protocol for the technique of pulp revascularization, as well as strict criteria for clinical and radiographic evaluation. With the possibility of reproducibility within the criteria and parameters pre-established, randomized controlled prospective clinical studies would be very welcome. Moreover, identification of determinants for this type of repair, the type of tissue formed and the prognosis of the tooth in the long-term also becomes essential.

The results found in the present study are relevant. The scientific evidence should be interpreted with caution as the articles report different methods and parameters. Despite the capacity of the pulp revascularization technique to stimulate the development of apical closure and thickening of radicular dentin, several aspects still remain unknown, like the key factors of this kind of repair, the type of tissue formed and the long-term prognosis.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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