

Review Article

Regenerative endodontic therapy: a systematic review of clinical protocols

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Abstract: Aim: The aim of this research was to describe the clinical protocols used for regenerative endodontic therapy through a systematic review of animal and clinical studies. Materials and methods: A systematic review was performed using the MEDLINE, Scopus, Cochrane Library, SciELO, Google Scholar, ScienceDirect and EMBASE databases. Study search and selection was performed by two independent researchers. Animal and clinical studies in which regenerative endodontic therapy was performed on immature necrotic teeth were included. Only prospective studies (with comparative design or case series), with a sample size of 10 or more teeth were included. Results: Twenty-three articles were included in this review. All clinical studies used sodium hypochlorite (NaOCl) as a root canal irrigant, although concentrations varied from 1% to 6%. Chlorhexidine, saline, sodium thiosulfate and hydrogen peroxide were also used in some studies. For intracanal dressing, nine out of eleven studies used a triple antibiotic paste (TAP), although time of application varied from 2 to 6 weeks. Use of EDTA was reported by two clinical studies only. Animal studies used mostly NaOCl for irrigation, at concentrations of 1.25% to 5.25%, and intracanal dressing was mainly a mixture of metronidazole, ciprofloxacin and minocycline, for variable periods of time. Conclusions: Most of the studies did not follow a standard clinical protocol for regenerative endodontic therapy.

Keywords: Regenerative endodontics, immature teeth, clinical protocols

Introduction

Regenerative endodontic therapy (RET) is a new approach for teeth with necrotic pulp and immature roots. RET is defined as a biologically-based procedure designed to replace damaged structures, including dentin and cells of the pulp-dentin complex [1]. RET is possible due to the presence of stem cells in the apical papilla with odontogenic differentiation potential [2]. Moreover, dentin walls provide the scaffolding that supports new tissue formation, as well as growth factors [3], which induce cell proliferation and differentiation.

Before regenerative endodontic therapy came into use, immature necrotic teeth were treated with apexification, which allowed only limited and often negligible root development in terms

of the width and length of the dentin walls [4]. Once the pulp undergoes necrosis, the deposition of dentin ceases and so does the root development. The open apex and the low thickness of the dentin walls make endodontic treatment of the tooth difficult and imprecise, thus compromising its prognosis.

Although RET has the three basic requirements for achieving regeneration (the presence of stem cells, scaffolding and growth factors), true pulp and dentin regeneration have not yet been reported. In animal and clinical studies, radiographically observed root development is due to the ingrowth of cementum-like and bone-like tissue rather than dentin, and intracanal tissue consists of connective tissue without an odontoblast layer, rather than pulp tissue [5]. The most encouraging results have been reported

with the use of dental pulp stem cells seeded into scaffolds and transplanted subcutaneously in animals; the results are regenerated dentin [6] and odontoblast-like cells with cellular processes extending into the dentin tubules [7].

Most studies do not follow a standard protocol for RET. In general, studies report that the necrotic pulp is removed with minimal or no mechanical instrumentation. Disinfection is achieved with different irrigants and intracanal dressings at variable concentrations left in the canal for varying periods of time. Sodium hypochlorite, chlorhexidine and/or EDTA are commonly used as irrigants, as well as calcium hydroxide and antibiotic pastes as intracanal dressings. When the tooth is asymptomatic, the blood clot is induced through over-instrumentation. The blood invades the root canal carrying stem cells and growth factors. However, the absence of clear clinical protocols could be partly responsible for the lack of true tissue regeneration. It has been shown that some irrigants and intracanal dressings, such as sodium hypochlorite, chlorhexidine and antibiotic pastes, have a detrimental effect on stem cell survival and on the release of growth factors from the dentin walls [3, 8-10]. In contrast, other dressings such as calcium hydroxide or EDTA significantly increase SCAP (stem cells from the apical papilla) survival and proliferation [9-11]. The materials used (i.e. irrigants and intracanal medications), and their toxic concentrations, as well as the lack of use of other materials (i.e. EDTA) could eventually be one of the causes of the absence of true pulp and dentin regeneration and for cases with no root development.

The aim of this research was to describe the clinical protocols used for regenerative endodontic therapy of immature necrotic teeth through a systematic review of animal and clinical studies.

Materials and methods

Search strategy

The electronic search was carried out by two independent researchers. The search was made in the MEDLINE, Scopus, Cochrane Library, SciELO, Google Scholar, ScienceDirect and EMBASE databases and the terms used were ("regenerative endodontic" OR revitalization OR

revascularization) AND (tooth OR teeth). The last electronic search was performed on May 9, 2016.

Inclusion and exclusion criteria

We included studies in which regenerative endodontic therapy was performed on immature necrotic teeth. Only prospective animal and clinical studies (with comparative design or case series) with a sample size of 10 or more teeth were included. The studies had to provide clear and adequate information on the clinical procedure and the irrigants/dressings used in regenerative therapy. The search included studies in English, Spanish, French, Portuguese and Italian. No restriction criteria were applied regarding date of publication.

Study selection and data extraction

Selection of the studies was carried out by two independent researchers. In case of disagreement a third reviewer was consulted. The first screening was performed by reading the title and abstract, and articles not complying with inclusion criteria were excluded. The full text of eligible articles was then examined to assess whether they complied with inclusion criteria.

The following information was extracted from clinical studies: patient/tooth characteristics and clinical protocol (age of the patient, teeth, pulp and periapical diagnosis, anesthetic used, mechanical instrumentation, irrigant and its concentration, intracanal dressing and period of time inside the canal, use of any other adjunct, and sealing material), clinical outcomes (pain, response to pulp vitality/sensitivity test, tooth discoloration) and radiographic outcomes (resolution of periapical lesion, root development). Information from animal studies was extracted separately and included: animal species and clinical protocol (teeth, anesthetic used, mechanical instrumentation, irrigant and its concentration, intracanal dressing and period of time inside the canal, use of any other adjunct, and sealing material), information regarding radiographic outcomes (resolution of periapical lesion and root development) and histological outcomes (type of newly formed tissue).

Results

Nine hundred and eighty-four studies were screened in the electronic search, and three

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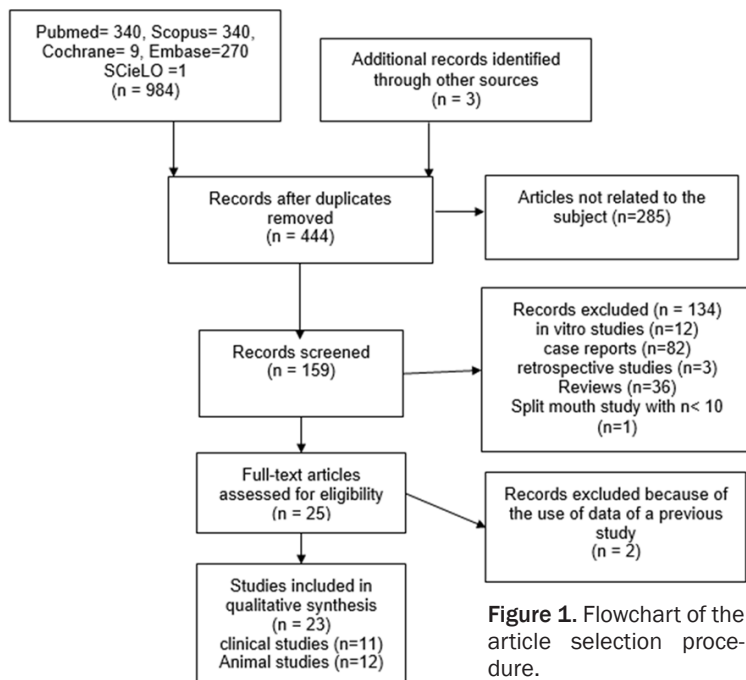


Figure 1. Flowchart of the article selection procedure.

more studies were found through hand searching. Unrelated studies, duplicate studies and articles not complying with inclusion criteria were discarded. Finally, twenty-three articles were included in this review (**Figure 1**), of which eleven were clinical studies and twelve were animal studies.

Characteristics of clinical studies

Six studies were case series [12-17] and five were pilot clinical studies [18-22]. In all, 222 patients were treated with regenerative endodontic procedures on immature necrotic teeth, of which 160 were associated with periapical lesions. The age of patients was from 6 to 28. All patients were diagnosed with pulp necrosis, except 8 in one study [12], who reported pain when files were introduced into root canals, which could be interpreted as partial pulp necrosis.

Table 1 shows the clinical protocol used for RET. All studies used sodium hypochlorite (NaOCl) as a root canal irrigant, although concentrations varied from 1% to 6%. Chlorhexidine, saline, sodium thiosulfate and hydrogen peroxide were also used in some studies. For intracanal dressing, nine studies used a triple antibiotic paste (TAP), of which the majority used a mixture of metronidazole, ciprofloxacin and

minocycline, and the rest used cefaclor, doxycycline, amoxicillin or clindamycin instead of minocycline (**Table 1**). The time of application varied from 2 to 6 weeks (**Table 1**). At the second appointment, two studies [13, 19] reported using a local anesthetic with a vasoconstrictor. One of these two studies reported difficulties achieving blood clot formation [13]. At the second appointment NaOCl was the most used irrigant, in variable concentrations. The use of EDTA was reported by two studies only (**Table 1**). The follow-up period was from 6 months to 3 1/2 years.

Table 2 includes the radiographic outcomes (root development and resolution of periapical lesions) and the clinical outcomes (response to pulp vitality test and discoloration for TAP, MTA or Ca(OH)₂) of the clinical studies included in this review. Only one patient out of 222 reported post-operative pain in the treated tooth [19].

Characteristics of animal studies

Twelve animal studies were included in this review. **Table 3** shows the protocol for the regenerative endodontic therapy in the selected animal studies. The animals used were dogs [23-32], ferrets [33] and monkeys [34], with a total of 275 teeth and 32 canals treated (**Table 3**). In most studies, the pulp was removed or was disrupted and left in the canal. The pulp chamber was left exposed to the oral cavity, until formation of a periapical lesion. In the rest of the studies, vital teeth were depulped and RET was applied immediately, without the use of intracanal dressing. All teeth with apical lesions were irrigated with NaOCl at concentrations of 1.25% to 5.25%, and intracanal dressing was mainly a mixture of metronidazole, ciprofloxacin and minocycline, for variable periods of time.

Table 4 shows the radiographic outcomes (root development and resolution of the periapical lesion) and histological outcomes (type of newly

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Table 1. Characteristics, clinical protocol, clinical and radiographic outcomes of the clinical studies

Authors	Groups	N of teeth/n of periapical lesion	Intrusion	Irrigants 1st session (concentrations)	Intracanal dressing (time)	Irrigants 2nd session (concentration)	EDTA (concentration) x Time	Blood clot
Bezgin et al. 2015 [21]	Control (BC)	10/9	No	NaOCl (2.5%) Chlorhexidine (0.12%), Saline	TAP ² (3 weeks)	Saline S	Yes (17%) x NR	Yes
	Experimental (PRP)	10/7						No
Jadhav et al. 2012 [18]	Control (BC + Collagen sponge)	10/10	Minimal	NaOCl (2.5%)	TAP ¹ (NR)	NR	No	Yes
	Experimental (BC + PRP + Collagen sponge)	10/10						
Nagata et al. 2014 [19]	Control (TAP)	12/6	No	NaOCl (6%) + Sodium thiosulfate + Chlorhexidine (2%) + Saline	TAP ¹ (3 weeks)	Saline	Yes (17%) x 3 minutes	Yes
	Experimental (Calcium hydroxide)	11/5			Calcium hydroxide + Chlorhexidine gel (2%) (3 weeks)			
Nagy et al. 2014 [20]	Control (BC)	12/NR	Minimal	NaOCl (2.6%)	TAP ³ (3 weeks)	NaOCl (2.6%) + Saline	No	Yes
	Experimental (BC + hydrogel + bFGF)	12/NR						
Narang et al., 2015 [22]	BC	5/5	Minimal	NaOCl (2.5%)	TAP ^(NR) (4 weeks)	NaOCl (2.5%)	No	Yes
	PRP + Collagen	5/5						No
	PRF	5/5						No
Chen et al. 2012 [12]	-	20/20	Minimal	NaOCl (5.25%)	Calcium hydroxide (4 weeks)	NaOCl (5.25%)	No	Yes
Dabbagh et al. 2012 [13]	-	18/11	No	NaOCl (5%), Saline	TAP ¹ (in cases of discoloration minocyclin was replaced with ceclafor) (2-6 weeks)	NaOCl (NR)	No	Yes
Kahler et al. 2014 [14]	-	16/14	No	NaOCl (1%)	TAP ⁴ (4 weeks)	NaOCl (1%)	No	Yes
Mctigue et al. 2013 [15]	-	32/22	Minimal	NaOCl (3%) or Chlorhexidine (NR)	TAP ¹ (3-4 weeks) (n=10) TAP ⁵ (3-4 weeks) (n=22)	NaOCl (NR)	No	Yes
Saoud et al. 2014 [16]	-	20/17	No	NaOCl (2.5%), Saline	TAP ¹ (2 weeks)	Saline	No	Yes
Shah et al. 2008 [17]	-	14/14	Minimal	NaOCl (2.5%), Hydrogen peroxide	Formocresol (NR)	N/A	No	Yes

BC: Blood clot. PRP: platelet-rich plasma. PRF: platelet-rich fibrin. bFGF: Basic fibroblast growth factor. TAP¹: Metronidazole, ciprofloxacin and minocycline. TAP²: Metronidazole, ciprofloxacin and cefaclor. TAP³: Metronidazole, ciprofloxacin and doxycycline. TAP⁴: Metronidazole, ciprofloxacin and amoxicillin. TAP⁵: Metronidazole, ciprofloxacin and clindamycin. NR: Not reported. NA: not applicable.

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Table 2. Clinical and radiographic outcomes of regenerative endodontic therapy in clinical studies

Authors	Groups	N of teeth/n of periapical lesion	Apical closure	Root lengthening	Dentin walls thickness	Healing of the periapical lesion	Discoloration for			Pulp vitality
							Minocycline	MTA	Ca (OH) ₂	
Bezgin et al. 2015 [21]	Control (BC)	10/9	6	NR	9	8	NA	12	NA	2
	Experimental (PRP)	10/7	7	NR	9	7				5
Jadhav et al. 2012 [18]	Control (BC + Collagen sponge)	10/10	10	10	10	10	NR	NA	NA	NR
	Experimental (BC + PRP + Collagen sponge)	10/10	10	10	10	10				
Nagata et al. 2014 [19]	Control (TAP)	12/6	8	5	5	6	10	NR	NA	0
	Experimental (Calcium hydroxide)	11/5	6	3	5	4	NA	NR	3	0
Nagy et al. 2014 [20]	Control (BC)	12/NR	NR	NR	NR	NR	NA	NR	NA	NR
	Experimental (BC + hydrogel + bFGF)	12/NR	NR	NR	NR	NR				NR
Narang et al., 2015 [22]	BC	5/5	5	5	5	5	NR	NA	NA	NR
	PRP + Collagen	5/5	5	5	5	5				
	PRF	5/5	5	5	5	5				
Chen et al. 2012 [12]	-	20/20	NR	15	20	20	NA	2	NA	NR
Dabbagh et al. 2012 [13]	-	18/11	NR	NR	NR	9	2	NR	NA	NR
Kahler et al. 2014 [14]	-	16/14	2 (out of 16)	4 (out of 9)	8 (out of 9)	12	NA	12	NA	5
Mctigue et al. 2013 [15]	-	32/22	23	21	22	21	NA	7	NA	NR
Saoud et al. 2014 [16]	-						7 (MTA + minocycline)			
Shah et al. 2008 [17]	-	20/17	10	0	9	15	NR	NR	NA	0
Bezgin et al. 2015 [21]	-	14/14	NR	10	8	14	NA	NA	NA	NR

BC: Blood clot. PRP: platelet-rich plasma. PRF: platelet-rich fibrin. bFGF: Basic fibroblast growth factor. NR: Not reported. Clx: Chlorhexidine.

formed tissue and presence of inflammatory cells) of the animals studies included in this review. In most studies [23, 24, 27-29, 31, 32] the follow-up period was just 3 months. The others lasted 6 to 7 months [23, 25, 26, 34] and one lasted 12 months [30].

Discussion

In general, the regenerative endodontic treatments reviewed could be considered clinically successful, since 84.14% of the treated teeth (or canals) had some degree of root development, and 79.8% of teeth showed healing of periapical lesions. However, the histological analysis of the newly formed tissue in the animal studies showed that the tissues described as responsible for root development are either cementum-like and/or bone-like, and connective tissue, rather than dentin and pulp. Moreover, one animal study showed that, despite having used dental pulp stem cells in immature necrotic teeth, there was no true dentin or pulp regeneration [32]. On the other hand, other studies reported the most encouraging results with the use of dental pulp stem cells seeded into scaffolds and transplanted subcutaneously in animals; the results are odontoblast-like cells with the cell process extending into the dentine tubules [7], pulp-like tissue and regenerated dentin [6].

The materials used (i.e. irrigants and intracanal medications), and their toxic concentrations as well as the lack of use of other materials (i.e. EDTA) could be partially responsible for the absence of true pulp and dentin regeneration and for cases with no root development. It has been proven that irrigation with 2% chlorhexidine is highly cytotoxic for stem cells [8]. In the same way, survival of stem cells of the apical papilla after irrigation with hypochlorite 6% is greatly reduced. However, hypochlorite at 0.5%, 1.5% and 3% induced the lowest decrease in survival of these cells (37% approx.) and final irrigation with EDTA seemed to revert the negative effect of hypochlorite [11]. Moreover, an *in vitro* study showed that irrigation with 1.5% hypochlorite and EDTA gave the best results in stem cell survival [11]. Similarly, expression of odontoblast-like cell markers is completely abolished by irrigation with hypochlorite 6% + EDTA, whereas hypochlorite 1.5% + EDTA has no effects on odontoblast-like cell markers

[11]. Additionally, hypochlorite 6% + EDTA reduced the adhesion of stem cells to dentin walls [35]. All but one of the clinical studies included in this systematic review used a hypochlorite concentration greater than 1.5%, and three used concentrations of 5% or more. Clinical studies that used a concentration of sodium hypochlorite of 5% or more presented mixed results, as did the only two clinical studies that used EDTA (**Tables 1** and **2**).

The survival of SCAP is also conditioned by intracanal medication. An *in vivo* study proved that calcium hydroxide was the only medication tested that was associated with SCAP survival at all concentrations, particularly at a concentration of 1 mg/ml [9]. By contrast, antibiotics at concentrations from 1 to 6 mg/ml led to the death of 50% of the cells [9]. Additionally, an *in vitro* study showed that the release of some growth factors from dentin decreased after the use of TAP or chlorhexidine gel, but increased with the use of calcium hydroxide water-based paste [3]. All but three articles used TAP as intracanal medicament. Only one clinical study in this review compared the use of two different intracanal dressing materials: TAP and calcium hydroxide + chlorhexidine gel (2%). Unfortunately, as calcium hydroxide was not used alone, and chlorhexidine gel (2%) is cytotoxic for stem cells [8], a comparison of clinical outcomes after TAP or calcium hydroxide was not possible.

Disinfection is imperative since apical repair will not occur in the presence of infected tissues. In RET, disinfection depends almost exclusively on irrigants and intracanal medication, since instrumentation should be avoided or minimal in order to preserve the dentin walls. It is necessary to use irrigants at concentrations that are effective as well as non-cytotoxic for stem cells.

This systematic review differs from others reviews of clinical protocols for RET in that it includes animal studies and therefore histological outcomes. However, due to the heterogeneity of the analyzed studies, it was not possible to analyze quantitatively the influence of agents (irrigants and intracanal medications), their concentrations and time for application on the clinical, radiographic and histological outcomes after RET. It is essential to use biocompatible agents in the treatment of immature teeth to

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Table 3. Characteristics, protocol, histological and radiographic outcomes of animal studies

Authors/Year	N/periapical lesion	Instrumentation	Irrigant 1st session (concentration) (n)	Intracanal dressing (n) (time)	Irrigant 2nd session (concentration) (n)	EDTA (concentration) × time	Blood clot formation	Additive
da Silva et al. 2010 [23]	56 (c)/Yes	Minimal	NaOCl (2.5%) + saline (56)	None (28) TAP (2 weeks) (28)	NA (28) NaOCl (2.5%) + saline (28)	No	Yes	None
Khademi et al. 2014 [24]	29 (d)/Yes (20), No (9)	No	NaOCl (5.25%) (20). NR (9)	TAP ² (20) (4 weeks) None (9)	NaOCl (5.25%) + saline (20) NA (9)	No	Yes	None
Londero et al. 2015 [25]	32 (c)/NR	No	NaOCl (2.5%) (32)	TAP (32) (2 weeks)	NaOCl (2.5%) (32)	Yes (17%) × 3 minutes	Yes	None (14) Gelatin-based sponge (18)
Petrovic et al. 2013 [34]	15 (t)/No	NR	NR	None	NA	NA	No	Guttapercha + HA + PRP (7) Guttapercha + HA (8)
Rodríguez-Benitez et al. 2015 [26]	64 (c)/Yes	NR	NaOCl (1.25%) + saline (64)	None (32), TAP ¹ (32) (15 days)	NA (32) NR (32)	Yes (17%) × 1 minute	No (32), Yes (32)	None (32) + PRP (32)
Saoud et al. 2015 [27]	16 (t)/Yes	NR	NaOCl (2.5%) + saline (16)	TAP (16) (3 weeks)	NaOCl (2.5%) + saline (16)	No	Yes	None
Thibodeau et al. 2007 [28]	41 (c)/Yes	No	NaOCl (1.25%) (41)	TAP (10*) (stay) TAP (31*) (4 weeks)	NA (10*) NaOCl (1.25%) + saline (31*)	No	No (10*), Yes (31*)	None (21*). Collagen type I solution (20)
Torabinejad et al. 2014 [33]	21 (t)/No	No	Saline (21)	None	NA	Yes (17%) × NR	Yes (12) No (9)	None (12) PRP (9)
Yamauchi et al. 2011 [29]	96 (c)/Yes	No	NaOCl (2.5%) (96)	TAP (96) (2 weeks)	NaOCl (2.5%) + saline (96)	No (48)- Yes (17%) × 2 minutes (48)	Yes	None (48), Collagen sponge (48)
Yoo et al. 2014 [30]	40 (c)/Yes	No	NaOCl (3.5%) (40)	TAP (40) (2 weeks)	NaOCl (3.5%) + saline (40)	No	Yes	Sponge with conditioned medium from preameloblast (20), Sponge with PBS (20)
Zhang et al. 2014 [31]	36 (c)/Yes	NR	NaOCl (3%) + saline (36)	TAP (36) (4 weeks)	NR	No	Yes (18) No (18)	None (18) PRP (18)
Zhu et al. 2013 [32]	40 (c)/Yes	NR	NaOCl (1.25%) + saline (40)	TAP (40) (2 weeks)	NaOCl (1.25%) y saline (40)	No	Yes	None (18) DPSC (10) PRP (10), DPSC + PRP (19)

(t): teeth. (c): canals. NR: Not reported. TAP: Metronidazole, ciprofloxacin and minocycline. TAP¹: Ciprofloxacin, metronidazole and cefixime. TAP²: Ciprofloxacin, metronidazole and cefixime. HA: hydroxyapatite. PRP: platelet-rich plasma. DPSC: dental pulp stem cells. EDTA: Ethylenediaminetetraacetic acid. *Information provided by the author. NA: Not applicable.

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Table 4. Characteristics, protocol, histological and radiographic outcomes of animal studies

Authors/Year	N/n periapical lesion	Apical closure	Root lengthening	Dentin walls thickness	Healing of periapical lesion	Connective intracanal tissue	Inflammation tissue	Mineralized tissue	Cementum-like	Bone-like tissue
da Silva et al. 2010 [23]	56 (c)/Yes	NR	NR	NR	26	28	10	24	NR	NR
		NR	NR	NR	20	28	23	20	NR	NR
Khademi et al. 2014 [24]	29 (d)/Yes (20), No (9)	13	NR	8	13	14	NR	16	NR	NR
		7	NR	4	NA	9	NR	7	NR	NR
Londero et al. 2015 [25]	32 (c)/NR	NR	24	NR	NA	28	25	26	26	0
Petrovic et al. 2013 [34]	15 (t)/No	NR	NR	NR	NA	2	NR	NR	NR	NR
Rodriguez-Benitez et al. 2015 [26]	64 (c)/Yes	8	NR	12	14	NR	NR	NR	NR	NR
		20	NR	22	26	NR	NR	NR	NR	NR
Saoud et al. 2015 [27]	16 (t)/Yes	NR	NR	16	16	16	2	16	16	4
Thibodeau et al. 2007 [28]	41 (c)/Yes	5	NR	3	4	2	NR	3	NR	NR
		18	NR	17	12	10	NR	19	NR	NR
Torabinejad et al. 2015 [33]	21 (t)/No	0	NR	0	NA	NR	3	8	NR	8
		0	NR	0	NA	NR	9	9	NR	9
Yamauchi et al. 2011 [29]	96 (c)/Yes	NR	NR	37	33	NR	NR	NR	NR	NR
		NR	NR	33	35	NR	NR	NR	NR	NR
Yoo et al. 2014 [30]	40 (c)/Yes	34	NR	37	32	15	11	NR	NR	NR
Zhang et al. 2014 [31]	36 (c)/Yes	16	NR	12	18	15 (out of 17)	14 (out of 17)	13 (out of 17)	12 (out of 17)	0 (out of 17)
		11	NR	13	18	11 (out of 12)	10 (out of 12)	11 (out of 12)	9 (out of 12)	0 (out of 12)
Zhu et al. 2013 [32]	40 (c)/Yes	NR	NR	28	3	NR	NR	NR	31	24

(t): teeth. (c): canals. NR: Not reported. NA: Not applicable.

maximize root development as well as to contribute to the regeneration of true dentin and pulp. Recently, a clinical protocol for revitalization procedures was published [36]. According to that clinical protocol and according to *in vitro* studies [4, 8-11], the medications that seem to be most adequate for therapy are: irrigation with 1.5%-3% sodium hypochlorite, intracanal dressing with calcium hydroxide water-based paste, and 17% EDTA as a chelating agent, used at the first and second appointment [36]. However, the majority of the studies analyzed in this review did not follow this clinical protocol; several studies used sodium hypochlorite in concentrations higher than recommended, and only two studies used calcium hydroxide as intracanal dressing. Finally, EDTA was used in only six studies. Consequently, it is necessary to conduct clinical and animal studies to establish whether the protocol described above is indeed related to better clinical, histological and radiographic outcomes.

Disclosure of conflict of interest

None.

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