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Regenerative Endodontic Treatment in Necrotic Teeth with the Use of Autologous Platelet Concentrates

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ABBREVIATIONS

AAE – American association of endodontics

AC – Apical closure

APC – Autologous platelet concentrates

BC – Blood clot

CH – Calcium hydroxide

CHX – Chlorhexidine

CRCT – Conventional root canal treatment

CT – Cold testing

DAP – Double antibiotic paste

DWT – Dential wall thickness

EDTA – Ethylenediamine tetraacetic acid

EPT – Electric pulp testing

ICM – Intracanal medication

MTA – Mineral trioxide aggregate

NSET – Non-surgical endodontic treatment

PARL – Periapical radiolucency

PRF – Platelet-rich fibrin

PRP – Platelet-rich plasma

REP – Regenerative endodontic procedure

RCT - Root canal treatment

RRA – Radiographic root area

RL – Root length

RPM – Rounds per minute

TAP – Triple antibiotic paste

SUMMARY

This systematic review aimed to evaluate the effectiveness of autologous platelet concentrates (APCs) compared to traditional blood-clot regeneration for the management of permanent necrotic teeth. The literature published between 2000 and 2024 was searched in the digital databases of PubMed, Web of Science, Science Direct, and Google Scholar. The search revealed 23 eligible articles, which were included in the study. In total, seven Randomized control trials, one prospective randomized trial, one retrospective controlled cohort study, and fourteen clinical studies were analyzed.

The outcomes at postoperative follow-up, such as dentinal wall thickness (DWT), increase in root length (RL), apical closure (AC), vitality response, and success rate, were included in the analysis. The results showed that autologous platelet concentrates significantly improved apical closure and response to vitality pulp tests, whereas no significant effect was observed on root lengthening, dentin wall thickness, or success rate of permanent necrotic teeth treated with regenerative endodontics. Autologous platelet concentrates could be beneficial when treating permanent necrotic teeth for better apical closure and may improve response to vitality tests.

Regenerative endodontic procedures (REPs) can be used to treat immature necrotic teeth with stem cell-containing scaffolds. However, challenges like scaffold formation uncertainty and crown discoloration persist. Autologous platelet concentrates show promise in addressing these challenges, with slightly improved success rates. Further clinical trials and standardization of the research are crucial to give evidence-based recommendations for clinicians and to advance the field of regenerative endodontics.

KEYWORDS

Autologous Platelet Concentrate; Necrotic Teeth; Permanent Teeth; Platelet-Rich Fibrin; Platelet-Rich Plasma; Regenerative Endodontics.

INTRODUCTION

The preservation of natural dentition has long been the main objective of root canal treatment. Achieving this objective is more challenging in young patients presenting with immature necrotic teeth. (1) Trauma or caries-induced pulp necrosis in the younger population can hinder permanent tooth root growth, resulting in distinctive characteristics, such as thin dentinal walls, wide-open apices, and an unfavorable crown-to-root ratio. (2) Challenging root canal debridement and elevating the risk of future root fractures under occlusal forces. (3) Rendering them unsuitable for complete cleaning and obturation with traditional techniques and materials. (4)

Traditionally, apexification has been a common approach for addressing immature necrotic permanent teeth. This method involves inducing an apical barrier using either calcium hydroxide ($Ca(OH)_2$) or hydraulic calcium silicate-based cement, such as mineral trioxide aggregate (MTA) or newer improved specifications biocompatible and bioactive materials, to close the open apex and allow the subsequent filling of the middle and coronal root canal thirds with traditional root canal filling methodologies. (5)

$Ca(OH)_2$ apexification procedures involve long-term application of the dressing material (3 to 24 months) to achieve complete closure of the root apex. (6) The clinical protocol may include one or multiple monthly appointments to replace $Ca(OH)_2$ inside the root canal, eliminating intracanal infection, stimulating calcification, and promoting apical closure. (7)

Sheehy and Roberts reported that the use of calcium hydroxide for apical barrier formation was successful in 74-100% of cases, and the average time for apical barrier formation ranged from 5 months to 20 months. (8) However, Andreasen et al. (9) found that long-term calcium hydroxide dressing in the root canal weakens the root structure. Furthermore, while widely used and inexpensive, long-term $Ca(OH)_2$ therapy has drawbacks such as variability in treatment time, challenges in patient follow-up, and unpredictability of apical seal formation, which can cause reinfection. (10)

Of the aforementioned drawbacks, this traditional approach has been gradually replaced by hydraulic calcium silicate-based cement apexification, which offers a one-step technique, requiring fewer appointments, providing more predictable apical barrier formation, and reducing the need for follow-up visits. (11,12) MTA apexification exhibited clinical successes ranging from 80.77% to 100%. (13)

While both $Ca(OH)_2$ and hydraulic calcium silicate-based cement apexification techniques induce the formation of an apical hard tissue barrier, promoting periapical healing and symptom disappearance, it is crucial to note that these methods do not strengthen the root or encourage further root development in terms of increasing length or width. (14,15) This limitation has led to the recognition of the need for alternative treatment approaches.

Unlike traditional apexification, RET focuses on regenerating the pulp–dentin complex, potentially providing a more comprehensive and sustainable solution for immature necrotic permanent teeth. Regenerative endodontic treatment (RET) presents a promising avenue for addressing challenges associated with immature necrotic permanent teeth, particularly those with incomplete root development and wide-open apices. (13) According to the American Association of Endodontics (AAE), "pulp revascularization" stands out as the primary treatment option in such cases. (16) The goal is not only to treat the existing condition but also to encourage additional root development, aiming to reduce the incidence of future root fractures over time. (13)

Regenerative endodontic procedures (REPs), including RET, are biologically based interventions designed to replace damaged structures like roots and dentin, along with pulp–dentin complex cells. The primary aim of REPS is to create a biomimetic microenvironment within the root canal, supporting the regeneration of mesenchymal stem cells, including osteo/odontoprogenitor stem cells, and promoting continued root development. (17) These procedures are rooted in tissue engineering principles, utilizing scaffolds consisting of stem cells and essential growth factors to facilitate cell proliferation and differentiation. (11) Ideal natural scaffolds should possess suitable porosity, transport nutrients effectively, exhibit proper physical and mechanical strength, elicit a minimal inflammatory response, and biodegrade similarly to the tissue undergoing regeneration. (18)

Most reported *in vivo* studies are based on the concept of cell homing by recruitment of the patient's endogenous stem cells located around the periapical region. (19,20) These include stem cells from the apical papilla, inflammatory periapical progenitor cells, periodontal ligament stem cells, bone marrow stem cells, and dental pulp stem cells. (21)

This process involves the recruitment and migration of the aforementioned stem cells into the root canal space. Cell homing fosters tissue regeneration within the pulp-dentin complex without exogenous cell transplantation. Through chemotaxis, signalling molecules guide the migration of stem cells, facilitating tissue repair and the formation of new dentin. (22)

Cell homing for dentin-pulp tissue regeneration relies mainly on the delivery of growth factors into the canal space. In REPs, two common sources of growth factors exist: internal (i.e., dentin) and the scaffold. Growth factors are found embedded in the extracellular matrix of dentin and can be utilized by conditioning the dentin surface. (23)

The scaffold in regenerative endodontics plays a crucial role in fixing, proliferating, migrating, and organizing cells for structural and functional tissue replacement. (24) The principal objective of scaffold design is to mimic the physical and biochemical microenvironment of the root canal. Furthermore, it should be able to simulate the native extracellular matrix until cells seeded within the scaffold and/or derived from the host tissue can synthesize a new, natural matrix. (25)

Meanwhile, induced apical bleeding and subsequent blood clot formation act as biological scaffolds for REPs. (16) Challenges arise when there is a failure to induce apical bleeding or achieve sufficient blood volume within the canal space. (26–28) Recent research explores the application of platelet concentrates, such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), introduced into the root canal. These concentrates, derived through blood centrifugation, are rich in growth factors that stimulate collagen production, recruit cells, and enhance soft and hard tissue wound healing potential. (29,30)

The regeneration of teeth with a non-vital necrotic pulp requires the removal of the necrotic pulp tissue to give an empty root canal, which can then be filled with a BC (Blood clot), PRP, or PRF scaffold, populated with viable cells to regenerate replacement pulp tissues to revitalize the tooth. The successful outcome of the REP can be measured as the ability to accomplish apical closure of the tooth root, a periapical lesion healing response, root lengthening, and dentinal wall thickening because these indicate the regeneration of tissues. (30–32)

This study aimed to systematically review and appraise contemporary literature on the clinical and radiographic outcomes and success rate of REPs for the treatment of immature and mature permanent teeth with pre-existing pulp necrosis with APCs as a scaffold and to compare it to the success rate of the conventional REP without a scaffold and non-surgical endodontic treatment (NSET).

AIM

To systematically review and appraise contemporary literature on the clinical and radiographic outcomes and success rate of REPs for the treatment of immature and mature permanent teeth with pre-existing pulp necrosis with APCs as a scaffold and to compare it to the success rate of the conventional REP without a scaffold and NSET.

OBJECTIVES

1. To assess the effectiveness of regenerative endodontic treatment (RET) using autologous platelet concentrates (APCs) as a scaffold in promoting successful outcomes *in vivo*.
2. To compare the success rate of RET using APCs or conventional RET (BC) to apexification with subsequent root canal obturation in immature permanent teeth.
3. To compare the success rate of RET using APCs to conventional RET (BC) in immature permanent teeth.
4. To analyze the reported clinical signs, symptoms, and radiographic findings associated with successful RET outcomes.
5. To identify any potential adverse events or complications related to the use of APCs as a scaffold in RET.
6. To compare the success rate of RET using APCs to conventional treatment modalities in mature permanent teeth with pulp necrosis and/or apical periodontitis.

LITERATURE SEARCH STRATEGY

A systematic literature review was conducted using electronic databases PubMed and Google Scholar. The search was carried out by two independent examiners adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The following keywords were used: ‘immature teeth’ OR ‘immature tooth’ OR ‘immature dentition’ AND ‘pulp revascularization’ OR ‘pulpal regeneration’ OR ‘pulp revitalization’ OR ‘root canal revascularization’ OR ‘root maturation’ OR ‘regenerative endodontic’ OR ‘regenerative endodontic therapy’ OR ‘regenerative endodontic treatment’ OR ‘regenerative endodontic procedure’ AND ‘blood clot’ OR ‘platelet-rich fibrin’ OR ‘platelet-rich plasma’ AND ‘calcified barrier’ OR ‘apical closure’ OR ‘root end formation’ OR ‘root apex closure’.

PICO criteria were applied: Problem/Population: Pulp necrosis and/or apical periodontitis in mature and immature teeth with no age or sex restriction. Intervention: Regenerative endodontic treatment using Autologous platelet concentrates (APCs) as a scaffold for immature and mature teeth. Comparison: NSET in mature teeth and Apexification with subsequent root canal filling in immature teeth. Outcome: overall clinical and/or radiographic success rate.

The inclusion criteria for this review were *in vivo* studies conducted in humans reporting regenerative endodontic treatment (RET) using APCs as a scaffold in cross-sectional studies, cohort studies, or randomized clinical trials. Case studies or case series were excluded. Detailed inclusion and exclusion of studies are shown in Figure 1. The success of RET was defined as the absence of clinical signs and symptoms of periapical inflammation and continued root maturation in immature teeth.

The null hypothesis stated that there was no difference in the clinical success rate of RET when compared to conventional treatment modalities in mature permanent teeth with pulp necrosis and apical periodontitis.

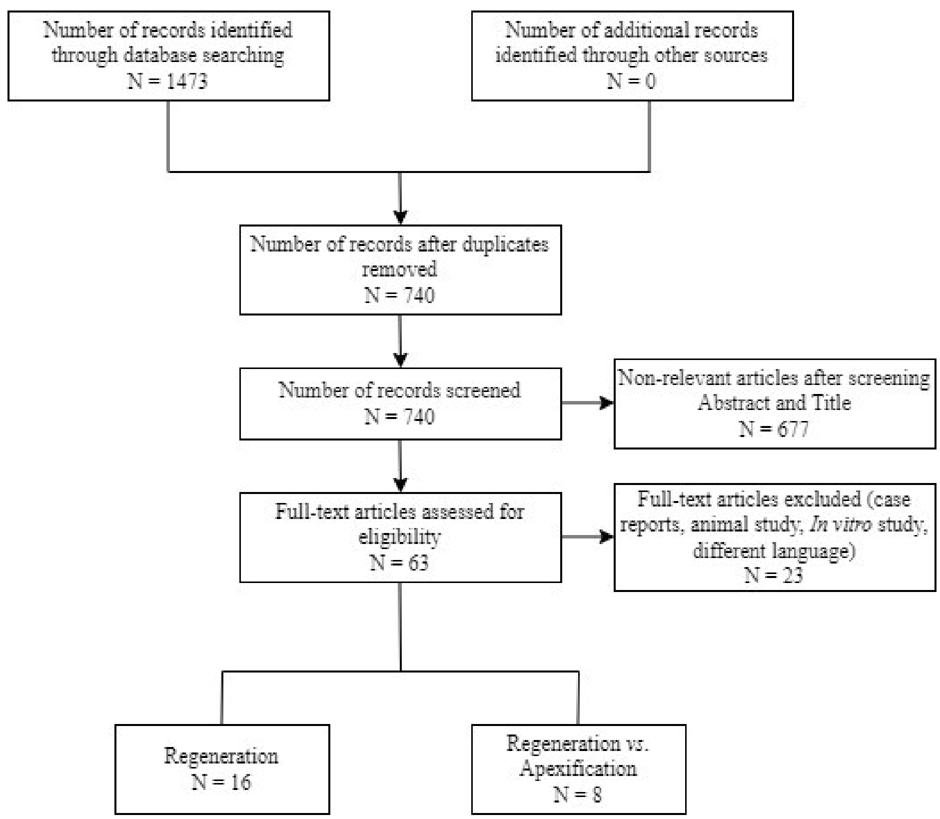

Figure 1. PRISMA 2020 flow diagram for a systematic review that includes searches of databases.

RESULTS

1. Regenerative endodontic treatment with APCs *in vivo*

All studies were performed in the universities of Ankara University (33), All India Institute of Medical Sciences (30), University of Dammam (31), Cairo University (20,34), Suez Canal University (35,36), Banaras Hindu University (37,38), University of Calicut (32), Ondokuz Mayis University (19) and Fujian Medical University (39). According to the inclusion criteria, 5 clinical studies (30,31,33,37,38), 5 randomized control trials (20,32,34–36), 1 prospective randomized trial (19), and 1 retrospective controlled cohort study (39) were included.

The total number of patients reported in the studies was from 8 to 56 (116 patients in the PRP group and 74 patients in the PRF group). With 195 teeth included (116 teeth in the PRP group and 62 teeth in the PRF group). Where the age of the patients ranged from 5-28 years, the age range of patients specific to each group was not mentioned.

All teeth evaluated in the review were permanent necrotic immature teeth with or without periapical lesions. Three studies included incisors and premolars (31,33,39), and one study included all types of teeth. (38) The rest of the studies included only anterior teeth. (19,20,30,32,34–37)

All studies evaluated the success of RET using APCs (PRP or PRF). Follow-up of the patients was reported within 12 and 49 months. Across multiple studies, RET using APCs consistently revealed high clinical success rates. Reported success rates ranging from 85.7% to 100% after an average of 16 months. (19,30–32,34,36–39) Moreover, most teeth treated with RET using APCs demonstrate complete resolution of periapical lesions, with percentages ranging from approximately 60% to 100% across the studies. (19,20,31–33,36–39)

Furthermore, a notable increase in root thickness and length is observed in teeth treated with RET using APCs, with percentage increases ranging from approximately 4.7% to 42.4% for root thickness and 4% to 12.3% for root length. (19,31–34,36,38)

Six out of twelve studies noted positive sensitivity to cold and electric pulp testing, ranging from approximately 15.8% to 100%, suggesting the maintenance or restoration of pulp vitality. (19,31–33,38,39) Additionally, a significant proportion of teeth treated with RET using APCs exhibit complete apical closure, with percentages ranging from approximately 40% to 93.33%. (19,20,30–39) Detailed characteristics of the included studies in RET using APCs *in vivo* are shown in Table 1.

Table 1. Summary of studies using regenerative endodontic treatment with APCs *in vivo*.

Study	Age (years)	Intervention	Number	Irrigation	Final Irrigation	Follow-Up (months)	Primary Goal	Secondary Goal	Vitality	ICM	Radiological Success	RRA	Discoloration	Obliteration
Bezgin 2014	7-13	PRP	10	2.5% NaOCl Saline 5% EDTA	Saline	3-18	100%	70%AC-9.86% RL	50%	TAP (Cip-Met-Cefaclor)	100%	9.86%	60%	40%
Jadhav 2012	15–28	PRP RET	10	2.5% NaOCl	2.5% NaOCl	6-12	100%	70%AC-40% RL(E) 30% RT(E) -50%RT(G)	NR	TAP	100%	NR	NR	NR
Alagl et al., 2017	9 -11	PRP RET	15	2.5% NaOCl Saline 0.12% CHX	17% EDTA	12	100%	93.33% AC	80%	TAP (Cip-Met-Cefaclor)	80%	NR	NR	NR
Elsheshtawy et al., 2020	12.66 (mean)	PRP RET	13	2.5% NaOCl Saline 17% EDTA	17% EDTA	12	85.70%	0.24 RL, 0.03 RT	0%	TAP (Cip-Met-Mino)	85.70%	-1.98	NR	NR
Rizk et al., 2019	8 -14	PRP RET	13	2% NaOCl 17% EDTA	17% EDTA	12	100%	39.27% ± 32.04% IRT 9.88% ± 2.85% IRL	0%	TAP	NR	NR	*(PRP higher than BC)	NR
Ragab et al., 2019	7-12	PRF RET	11	5.25% NaOCl Saline	Saline	12	100%	12.3%RL 63.6%AC	NR	DAP (Met-Cip)	74.2% (Healing)	NR	100%	63.6%
Mittal et al., 2019	NR	PRF RET	4	2.5% NaOCl	2.5% NaOCl	12	100%	100%RT-0%RL 100%AC	NR	DAP	75%	NR	NR	NR
Shivashankar et al., 2017	6-28	PRP	19	5.25% NaOCl	Saline	12	100%	84.2%RT, 73.4% RL	15.80%	TAP (Cip-Met-Mino)	100%	NR	NR	0%
		PRF	20				90%	70%RT, 65% RL	15%					10%
Hazim Rizk et al., 2020	8-14	PRP	13	2.5% NaOCl 17% EDTA Saline	17% EDTA	12	100%	9.88%RL,39.27%RT	0%	TAP (Cip-Met-Mino)	100%	NR	NR	0%
		PRF	12				100%	8.19% RL,42.37% RT			100%			
Narang et al., 2015	<20	PRP	5	2.5% NaOCl	2.5% NaOCl	18	100%	60% RT,99% RL,40% AC	98%	TAP	98%	NR	NR	NR
		PRF	5				100%	20% RT,40% RL,60% AC	80%		80%			
Ulusoy et al., 2019	8–11	PRP	18	1.25% NaOCl 2% CHX, Saline	17% EDTA	49	100%	66.7% AC, 19.01% RT, 4.74% RL	72.20%	TAP (Cip-Met-Clinda)	100%	9.53%	NR	NR
		PRF	17				94.10%	70.6% AC, 9.8% IRT, 6% IRL	88.20%		94.10%	6.93%		
JayLv et al., 2018	9-14	PRF	5	1% NaOCl	17% EDTA	12	100%	80% RT, 80% RL, 80% AC	100%	TAP (Cip-Met-Cefaclor)	100%	NR	NR	NR

ICM – Intracanal medication; RRA – Radiographic root area; PRP – Platelet-rich plasma ;AC – Apical closure ; RL – Increase in root length; RT – Increase in root thickness; RET – Regenerative endodontic treatment; TAP – Triple antibiotic paste; CIP – Ciprofloxacin; Met – Metronidazole; Mino – Minocycline; CHX – Chlorhexidine; Clinda – Clindamycin; NR – Not reported; E – Excellent; G – Good.

2. Success rate of RET with APCs vs apexification in immature permanent teeth

Within the scope of this systematic review, only one clinical study by Narang et al., performed at Banaras Hindu University, addressed the comparison between the success rates of RET with APCs and apexification. 20 patients were included in this study, 5 patients in each of the MTA, BC, PRP, and PRF groups. Each patient had one tooth for treatment. All patients were below 20 years of age. All the 20 cases were evaluated clinically and radiographically at 6 and 18 months after treatment.

In Narang et al.'s study, the bone healing rate in the MTA group was reported to be 58%, significantly lower than the PRP and PRF groups, which showed bone healing rates of 98% and 80%, respectively. The PRP group exhibited a 60% increase in dentinal wall thickness, comparable to the BC group. At the same time, only 20% of the teeth in the PRF group showed increased dentinal wall thickness. A noteworthy 99% of the teeth in the PRP group displayed an increase in root length. The BC and PRF groups demonstrated similar results in root length increase at 40%. Regarding apical closure, the BC and PRF groups showed relatively similar rates at 66.67% and 60%, respectively. In the PRP group, 40% of the teeth exhibited complete apical closure, while none of the teeth in the MTA group showed apical closure. (38)

3. Success rate of RET using PRP vs conventional RET with BC in immature permanent teeth

All studies were performed in universities in different locations: Ankara University (33), University of Dammam (31), University of Leeds (20), Suez Canal University(35), Banaras Hindu University (38), All India Institute of Medical Sciences (30). According to the inclusion criteria, 4 clinical studies (30,31,33,38), 3 randomized control trials (20,35,40) and 1 prospective randomized trial (19) were included.

The total number of patients reported in the studies ranged from 10 to 65, with 2 to 73 teeth inclusion, where the age of the patients ranged from 8 to 28 years old. A specific number of teeth, patients, and age range in each group were not mentioned. A detailed analysis of the characteristics of the analyzed studies in RET using PRP vs RET with BC is shown in Table 2. All teeth evaluated in the review were permanent necrotic immature teeth with or without periapical lesions. The studies by Bezgin et al. (33) focused solely on premolars, while Jadhav et al. (30), Elshewatya et al. (20), and Narang et al. (38) included all teeth types. Alagl's study exclusively involved single-rooted teeth (incisors, canines, and premolars) (31), and Ulusoy et al. specifically examined maxillary incisors. (19)

All studies evaluated the success of RET with BC and RET with PRP based on radiographic and clinical examination. Success was defined as the absence of clinical and radiographic signs and symptoms. Follow-up of the patients was reported within 12 and 49 months. Overall, all studies showed the superiority of PRP in terms of success rate, ranging from 50% to 100%. Across different research, PRP generally showed higher positive vitality signs than BC. In the PRP group, the sensitivity to cold testing and EPT test ranges from 0% to 98%, and in the BC group, it varies between 0% and 95.2%. The PRP group generally exhibited higher complete apical closure rates than the BC. For the BC group, the range of complete apical closure rates varied from 20% to 76.2%. For the PRP group, the range of complete apical closure rates varied from 40% to 93.3%.

BC and PRP groups demonstrated varied effects on root morphology. BC generally showed higher increases in root length, while PRP tended to exhibit higher increases in root thickness. BC exhibited increases in root length ranging from 4.68% to 15.79% and increases in root thickness ranging from 14.91% to 25.56%. PRP displayed increases in root length ranging from 4.74% to 9.88% and increases in root thickness ranging from 19.1% to 39.27%.

In the PRP group across studies, 30% to 60% of teeth showed a good to excellent increase in root length, while 30% to 99% showed a good to excellent increase in root thickness. Conversely, in the BC group, the range was 40% to 60% for root lengthening and 30% to 50% for root thickening. Measurements in millimetres were not provided.

All contributing teeth presented with uncompleted root development. Study groups included RET with induction of bleeding for blood clot formation. All studies reported control groups being treated using RET with PRP. All studies involved assessment of successful treatment based on clinical and radiographic procedures (success defined as the absence of clinical symptoms and complete healing of periapical lesion) as the primary outcome of interest within 12 to 49 months of follow-up examination. Ethylene-diamine-tetra-acetic acid (EDTA) was reported as a final irrigation solution concentration of 17% in all studies except three. Triple antibiotic paste (TAP) was used for intracanal medication, comprising doxycycline, metronidazole, and cefaclor, among others.

Table 2. Summary of studies using RET with PRP vs conventional RET with BC in immature permanent teeth

Study	Age (years)	Intervention	Number	Irrigation	Final Irrigation	Follow-Up (months)	Primary Goal	Secondary Goal	Vitality	ICM	Radiological Success	RRA	Discoloration	Obliteration
Bezgin 2014	7-13	PRP	10	2.5% NaOCl Saline 5% EDTA	Saline	3-18	100%	70%AC-9.86% RL	50%	TAP (Cip-Met-Cefaclor)	100%	9.86%	60%	40%
		BC	10				90%	60 AC-12.6% RL	20%		90%	12.60%		40%
Jadhav 2012	15–28	PRP RET	10	2.5% NaOCl	2.5% NaOCl	6-12	100%	70%AC-40%RL (E), 30%RT (E)-50%RT (G)	NR	TAP	50% E,40%G,10%S	NR	NR	NR
		BC RET	10				100%	20%AC-60%RL(G)-30%RT(G)			70%G,30%S			
Alagl et al., 2017	9 -11	PRP RET	15	2.5% NaOCl Saline 0.12% CHX	17% EDTA	12	100%	93.33% AC	80%	TAP (Cip-Met-Cefaclor)	80%	NR	NR	NR
		BC RET	15				100%	53.3%AC	40%		86.60%			
Elsheshtawy et al., 2020	12.66 (mean)	BC RET	13	2.5% NaOCl Saline 17% EDTA	17% EDTA	12	88%	0.24 IRL, 0.03 IRT	0%	TAP (Cip-Met-Mino)	88%	NR	NR	NR
		PRP RET	13				85.70%		0%		85.70%			
Rizk et al., 2019	8 -14	BC RET	13	2% NaOCl 17% EDTA	17% EDTA	12	100%	25.56% ± 26.5% IRT 4.68% ± 3.45% IRL	0%	TAP	NR	NR	*(PRP higher than BC)	NR
		PRP RET	13				100%	39.27% ± 32.04% IRT 9.88% ± 2.85% IRL	0%					
Shivashankar et al., 2017	6 -28	BC	15	5.25% NaOCl	Saline	12	100%	93.3%RT,86.7%RL	13.30%	TAP (Cip-Met-Mino)	100%	NR	NR	6.6%
		PRP	19				100%	84.2%RT, 73.4% RL	15.80%					0
Narang et al., 2015	<20	BC	5	2.5% NaOCl	2.5% NaOCl	18	100%	50%RT,40%RL,66.6%AC	60%	TAP	60%	NR	NR	NR
		PRP	5				100%	60%RT,99%RL,40%AC	98%		98%			
Ulusoy et al., 2019	8–11	BC	21	1.25% NaOCl 2% CHX Saline	17% EDTA	49	95.20%	76.2% AC, 14.91% RT, 15.79% RL	95.20%	TAP (Cip-Met-Clinda)	95.2	15.79%	NR	NR
		PRP	18				100%	66.7% AC, 19.01% RT, 4.74% RL	72.20%		100%	9.53%		

PRP – Platelet-rich plasma; BC – Blood clot; ICM – Intracanal medication; RRA – Radiographic root area; PRP – Platelet-rich plasma ;AC – Apical closure; RL – Increase in root length; RT – Increase in root thickness; RET – Regenerative endodontic treatment; CHX – Chlorhexidine; TAP – Triple antibiotic paste; Cip – Ciprofloxacin; Met – Metronidazole; Mino – Minocycline; Clinda – Clindamycin; NR – Not reported; E – Excellent; G – Good; S – Satisfactory.

4. Success rate of RET using PRF vs conventional RET with BC in immature permanent teeth

All studies were performed in universities of different locations: Cairo University (34), Banaras Hindu University (37,38), Ondokuz Mayis University (19), and Fujian Medical University (39).

According to the inclusion criteria, 2 randomized control trials (34,40), 2 clinical studies (37,38), a prospective randomized trial (8), and 1 retrospective controlled cohort study (39) were included.

The total number of patients reported in the studies ranged from 4 to 65, with 4 to 73 teeth inclusion, where the age of the patients ranged from 7 to 20 years old. A specific number of teeth, patients, and age range in each group were not mentioned. A detailed analysis of the characteristics of the included studies is shown in Table 3. All teeth evaluated in the review were permanent single-rooted teeth except one study. (38) All studies evaluated the success of RET with BC and RET with PRF based on radiographic and clinical examination. Follow-up of the patients was reported within 12 and 49 months.

Overall, RET with PRF resulted in high clinical success rates ranging from 75% to 100%, and BC group success rates ranging from 60% to 100%. The vitality of the treated teeth was tested using either with the cold test, electric pulp testing (EPT) or both BC generally exhibited a range of positive vitality signs between 60% and 95.2%. PRF showed a range of positive vitality signs between 0% and 88.2%. One study reported 100% positivity for both BC and PRF groups. (39)

Complete apical closure was noted in all studies; BC demonstrated a range of complete apical closure rates between 25% and 80%. PRF showed a range between 60% and 100%. One study showed comparable results. (39) RET with BC consistently demonstrated higher increases in both root length and root thickness compared to PRF. The range of increase in root length for PRF was approximately 6% to 12.3%, while for BC, it ranged from 14.8% to 15.79%. One study calculated the increase of root thickness in the BC group of 14.91% and 9.8% in the PRP group. Across studies, the BC group demonstrated a range of 50% to 80% of teeth showing an increase in root thickness, and 40% to 80% of teeth showed an increase in root length. Conversely, in the PRF group, the range was also 20% to 80% of cases for root thickness and 40% to 80% of cases for root length without mentioning the root dimensional changes in millimetres.

All assessed teeth were permanent necrotic immature single-rooted maxillary teeth with or without periapical lesions except in one study in which any type of teeth were included. (38) All contributing teeth presented with uncompleted root development. Interventions included REPs with induction of bleeding for blood clot formation and stimulation of stem cells of the apical region and REPs with PRF. All studies involved the assessment of successful treatment based on clinical and radiographic procedures as the primary outcome of interest within 12 to 49 months of follow-up examination. Success was defined as the absence of clinical and radiographic signs/symptoms. Two studies reported ethylene-diamine-tetra-acetic acid (EDTA) as the final irrigation solution in 17% concentrations. (19,39) In other studies, other irrigants, such as sterile saline or 2.5% sodium hypochlorite, were used as the final irrigants. (34,37,38)

Sodium hypochlorite concentrations ranged from 1% to 5.25%. Studies using DAP (double antibiotic paste) as intracanal medication include Mittal et al. 2019 and Ragab et al. 2019. DAP (double antibiotic paste) or TAP (triple antibiotic paste) of different mixtures were used as intracanal medication.

Table 3. Summary of studies using RET with PRF vs conventional RET with BC in immature permanent teeth.

Study	Age (years)	Intervention	Number	Irrigation	Final Irrigation	Follow-Up (months)	Primary Goal	Secondary Goal	Vitality	ICM	Radiological Success	RRA	Discoloration	Obliteration
Ragab et al., 2019	7-12	BC RET	11	5.25% NaOCl Saline	Saline	12	100%	14.8%RL- 45.4%AC 45.4%AC	NR	DAP (Met-Cip)	80.5% (Healing)	NR	100%	45.4%
		PRF RET	11				100%	12.3%RL 63.6%AC			74.2% (Healing)			63.6%
Mittal et al., 2019	NR	BC RET	4	2.5% NaOCl	2.5% NaOCl	12	100%	100%RT-25%RL 25%AC	NR	DAP	75%	NR	NR	NR
		PRF RET	4				100%	100%RT-0%RL 100%AC			75%			
Shivashankar et al., 2017	6 -28	BC	15	5.25% NaOCl	Saline	12	100%	93.3%RT,86.7%RL	13.30%	TAP	100%	NR	NR	6.6%
		PRF	20				90%	70%RT, 65% RL	15%	(Cip-Met-Mino)				10%
Narang et al., 2015	<20	BC	5	2.5% NaOCl	2.5% NaOCl	18	100%	50%RT,40%RL,66.6%AC	60%	TAP	60%	NR	NR	NR
		PRF	5				100%	20%RT,405RL,60%AC	80%		80%			
Ulusoy et al., 2019	8–11	BC	21	1.25% NaOCl 2% CHX Saline	17% EDTA	49	95.20%	76.2% AC, 14.91% RT, 15.79% RL	95.20%	TAP (Cip-Met-Clinda)	95.2	15.79%	NR	NR
		PRF	17				94.10%	70.6% AC, 9.8% IRT, 6% IRL	88.20%		94.10%	6.93%		
JayLv et al., 2018	10-12	BC	5	1% NaOCl	17% EDTA	12	100%	80% RT, 80% RL, 80% AC	100%	TAP (Cip-Met-Cefaclor)	100%	NR	NR	NR
	9-14	PRF	5				100%	80% RT, 80% RL, 80% AC	100%		100%			

PRF – Platelet-rich fibrin; BC – Blood clot; ICM – Intracanal medication; RRA – Radiographic root area; PRP – Platelet-rich plasma; AC – Apical closure; RL – Increase in root length; RT – Increase in root thickness; RET – Regenerative endodontic treatment; TAP – Triple antibiotic paste; DAP – Double antibiotic paste; Cip – Ciprofloxacin; Met – Metronidazole; Mino – Minocycline; Clinda – Clindamycin; NR – Not reported; CHX – Chlorhexidine.

5. The success rate of RET with BC vs Apexification in immature permanent teeth

All studies were performed at the universities of Ain Shams University (41), Mahidol University (14), Chulalongkorn University (42), Sun Yat-sen University (43), Banaras Hindu University (38), New York University (44), University of Seville (45), State University of Campinas. (46) According to the inclusion criteria 7 clinical studies, (14,38,41,42,44–46), 1 randomized control trial (43) were included.

The total number of patients reported in the studies was from 18 to 187 patients, with 18 to 211 teeth inclusion, where the age of the patients ranged from 6 to 20 years old. A specific number of teeth, patients, and age range in each group were not mentioned. A detailed analysis of the characteristics of the included studies is shown in Table 4. All teeth evaluated in the review were permanent necrotic immature teeth of any type with periapical lesions, except the study performed by Nagy et al. (41), which only included maxillary anterior teeth and the studies by Jeeruphan et al. (14) and Lin et al. (43) which only included incisors and premolars. All studies evaluated the success of RET and Apexification using various materials. Follow-up of the patients was reported within 12 and 66 months.

Across multiple studies, success rates of RET with BC and MTA apexification vary. Notably, BC RET in Jeeruphan's study had a higher success rate (80%) compared to MTA (68%). (14) In Nagy's study, MTA had a higher success rate (100%) compared to BC RET (90%). (41) Conversely, Alobaid (44), Lin (43), and Caleza (45) reported comparable success rates (100%) for both BC RET and MTA techniques. For the MTA group, vitality rates ranged from 0% to 100% (38). For the BC group, vitality rates ranged from 60% to 100% (14,38,44)

Complete apical closure was noted in some studies, where it was higher in the BC group, reaching 66.6% to 78% (38)(38,44)while no complete apical closure was observed in the MTA group. Only one study showed a higher apical closure rate of $Ca(OH)_2$ apexification than RET with BC (82.35% and 62.21%, respectively). (43)

An increase in root thickness was reported in various studies, ranging from 10.2%% to 34.57% in the BC group and from -3.36% to 1.4% in the apexification group. An increase in root length was also reported, ranging from 6.7%% to 20%% in the BC group and from 0.29% to 12.5% in the MTA group. (14,41,42,44–46), Other studies report that 40 % to 81.16% of cases in the BC group showed increased root length, and 50% to 82.6% showed increased root. In the MTA group, 0% to 26.47% showed an increase in root length without mentioning the root dimensional changes in mm. (38,43)

Sodium hypochlorite (NaOCl) irrigation ranged from 1.5% to 6%, and final irrigation included solutions such as saline, EDTA (17%), or 2.5% NaOCl, depending on the study and intervention. Follow-up periods ranged from 6 to 66 months, with most studies having a 12-month follow-up. Intracanal medication commonly included Triple Antibiotic Paste (TAP) consisting of ciprofloxacin, metronidazole, and minocycline or variations thereof, with chlorhexidine sometimes added in the mix. Depending on the study, sample sizes ranged from 5 to 118 teeth per group.

Table 4. Summary of studies using RET with BC vs Apexification in immature permanent teeth.

Study	Age (years)	Intervention	Number	Irrigation	Final Irrigation	Follow-Up (months)	Primary Goal	Secondary Goal	Vitality	ICM	Radiological Success	RRA	Discoloration	Obliteration
Nagy 2014	9–13	MTA	12	2.6% NaOCl	Saline	18	100%	0%RL-0%RT-0%AC	NR	TAP (Cip-Met-Doxy)	NR	NR	NR	NR
		BC	12				100%	4.9%RL-4.7%RT-18.9%AC						
		CH	12				90%	4.7%IRL -3.6%RT-9.8%AC						
Jeeruphan 2012	10.5 (mean)	CH	22	NR	NR	29	77%	1.5%RT-0.4%RL	NR	NA	77.27% healed	NR	NR	NR
	14.6 (mean)	MTA	19	NR	NR		68%	1%RT,6.1%RL		NA	68.42% healed			
	12.9 (mean)	BC	20	2.5% NaOCl	2.5% NaOCl		80%	28.2%RT-14.9%RL		TAP	80% healed			
Silujjai linsuwanont 2017	6-18	BC	17	1.5-2.5% NaOCl 17% EDTA	17% EDTA	35	76.47%	9.51% RL 13.75% RT	NR	TAP (Cip-Met-Mino)	76.47%	NR	NR	NR
		MTA	26	2.5% NaOCl	2.5% NaOCl	49	80.77%	8.55% RL -3.3% RT		CH	80.77%			
J lin 2017	6 -18	BC	69	1.5% NaOCl 0.9% Saline 17% EDTA	17% EDTA	12	100%	81.16% RL 82.6% RT 65.21% AC	NR	TAP (Cip-Met-Clinda)	89.90%	NR	43.47%	37.6%
		CH	34	1.5% NaOCl 0.9% Saline 17% EDTA	17% EDTA			26.47%RL 0% RT 82.35% AC		CH	97%			
Narang et al., 2015	<20 years	MTA	5	2.5% NaOCl	2.5% NaOCl	18	100%	0%RT.0%RL,0%AC	42%	TAP	58%	NR	NR	NR
		BC	5				100%	50%RT,40%RL,66.6%AC	60%		60%			
Alobaid et al., 2014	8.8	BC	15	NaOCl, CHX, EDTA	NR	15-22	78%	10.2 +/- 4.0% IRT 20% IRL	NR	TAP (Cip-Met-Mino) DAP (Cip-Met)	NR	27%	NR	NR
	9.8	MTA CH	15				100%	1.4 +/- 3.2% IRT 12.5% IRL		CH		6.60%		
Caleza-Jimeney et al., 2020	7–10	BC	9	1.5–2.5% NaOCl 17% EDTA	17% EDTA	6–66	100%	12.75%IRL-34.57% IRT	NR	TAP (Cip-Met-Mino)	100%	NR	NR	NR
		MTA	9	2.5% NAOCL	2.5% NAOCL		100%	0.29% IRL, − 3.36 IRT		CH	100%			
Pereira et al., 2021	6-18	BC	22	6% NaOCl 2% CHX 17%EDTA	17% EDTA Saline	12–30	95.45%	12.55% (mean = 1.42 mm) 0.21 mm/6.7% IRT	0%	TAP (Cip-Met-Mino) CH, 2%CHX Gel	95.45%	NR	40.90% *(18.1% TAP,22.7%CH)	13.63%
		MTA	22	2% CHX gel Saline	Saline		86.36%	mean= 0.03 mm/0.99% IRT 6.66% (mean = 0.88 mm) IRL	0%	CH 2%CHX gel ZnO	86.36%		0%	NR

MTA – Mineral Trioxide Aggregate; CH – Calcium Hydroxide; CHX – Chlorhexidine; ICM – Intracanal medication; RRA – Radiographic root area; PRP – Platelet-rich plasma; AC – Apical closure; RL – Increase in root length; RT – Increase in root thickness; RET – Regenerative endodontic treatment; TAP – Triple antibiotic paste; DAP – Double antibiotic paste; Zn0 – Zinc Oxide; Cip – Ciprofloxacin; Met – Metronidazole; Mino – Minocycline; Clinda – Clindamycin; Doxy – Doxycycline; NR – Not reported; NA – Not applicable.

6. Clinical signs, symptoms, and radiographic findings associated with successful RET outcomes

6.1. Clinical Signs and Symptoms

Successful RET outcomes often manifest clinically by resolving symptoms associated with pulpal necrosis and periapical pathology. Patients typically experience alleviation or absence of symptoms such as spontaneous pain, swelling, and sensitivity to percussion or palpation. Additionally, restoration of tooth function and absence of clinical signs of infection, such as sinus tract or purulent discharge, indicate favorable treatment outcomes. (19,20,30,31,33–40)

6.2. Radiographic Findings

Radiographic assessment plays a crucial role in evaluating the success of RET by visualizing changes in periapical tissues, root morphology, and apical closure. Successful outcomes are characterized by the resolution or reduction in size of periapical radiolucencies, indicating healing of periapical tissues and regression of apical pathology. Furthermore, evidence of continued root development, including thickening of dentinal walls, lengthening of the root, and apical closure, reflects the regenerative potential of RET. Overall, radiographic evidence of periapical healing and progressive root maturation signifies successful RET outcomes. (19,20,31,34–40)

6.3. Association with Treatment Success

The correlation between clinical signs, symptoms, and radiographic findings is pivotal in determining the overall success of RET. Patients with complete resolution of clinical symptoms and the absence of periapical pathosis on radiographic imaging typically exhibit favorable treatment outcomes. However, most of the studies evaluating cases that showed ongoing healing of periapical lesions (reduction in the lesion size during the follow-up) were considered successful. Conversely, persistent symptoms, increased or unchanged radiolucency size, or lack of apical closure indicate treatment failure and necessitate further intervention. Therefore, a comprehensive evaluation of clinical and radiographic parameters is essential for assessing the success of RET and guiding subsequent treatment decisions. (19,20,31,35–38,40)

7. Complications related to the RET with APCs

7.1. Crown discoloration

As highlighted by various studies, the prevalence of postoperative coronal discoloration in RET is a significant concern. Caleza et al. (2022) identified a high incidence of coronal discoloration, affecting 82.4% and 78.6% of teeth in the BC and PRP groups, respectively. (45) Discoloration predominantly resulted from MTA placement, affecting 60% of cases, as reported by Bezgin et al. (2014) and Kahler et al. (2014), with an exceptionally high occurrence rate in the BC group. (33,47)

Lin et al. (2017) emphasized discoloration and calcification as primary complications in RET cases, with 43% of BC cases exhibiting discoloration within the initial three months post-treatment. (43) Ragab et al. (2019) further attributed crown discoloration to MTA's composition despite minocycline exclusion. (34) Hazim Rizk et al. (2020) reported a contrasting observation of no discoloration in the PRP and PRF groups. (36) Notably, Rizk (2019) demonstrated a significantly higher incidence of discoloration in the BC group compared to the PRP group, underscoring material-specific influences on discoloration outcomes. (35)

7.2. Root canal obliteration

The radiographic evaluation of various endodontic treatments reveals significant findings. Alobaid et al. observed a higher incidence of apical calcification in revascularization cases (40%) compared to apexification cases (6.6%). (44) Furthermore, intracanal calcific barrier formation and canal obliteration were exclusive to revascularization cases, with rates of 20% and 13.3%, respectively. Pereira et al. found that 13.6% of regenerative endodontic procedures exhibited pulp canal obliteration in the apical third, impacting the analysis of apical angles. (46) Partial pulp canal obliteration was observed in 40% of the PRP group and 40% of the BC group. (33)

Ragab et al. noted a cervical calcific bridge in 36.4% of both PRF and BC groups at the 6-month follow-up. (34) Additionally, Saoud et al. reported hard tissue bridge formation in 15% of BC-treated teeth in the coronal third and 10% in the middle third. (48) Silujjai et al. observed calcified root canals in 23.52% of the BC RET group, emphasizing diverse radiographic manifestations across different endodontic treatments. (42)

8. Success rate of RET using APCs vs conventional treatment modalities in mature permanent teeth

All studies were performed in universities of different locations: University of Health Sciences (49), Universidad de los Andes (50), PDM Dental College (51), and Fayoum University (52). Studies included 3 clinical studies (49,51,52) and 1 randomized control trial (50).

There were a total of 15 to 36 patients with 18 to 46 teeth included, whose ages ranged from 9 to 58. Table 5 shows a detailed analysis of the characteristics of the included studies. All teeth evaluated in the review were single-rooted necrotic mature teeth with or without periapical lesions except in two studies in which all types of teeth were included (51,53). All contributing teeth presented with completed root development.

All studies evaluated the success of RET with BC or PRF or compared it with the success rate of NSRCT based on radiographic and clinical examination. Follow-up of the patients was reported within 12 and 18 months. Overall, RET resulted in high clinical success rates ranging from 86.6%% to 100% (49–52). The sensitivity of the treated teeth was tested using cold testing methods and was reported to range from 50% to 60% in RET.

In three studies, ethylene-diamine-tetra-acetic acid (EDTA) was reported as the final irrigation solution in 17% concentrations. (49,51,52), except for one study in which the irrigation protocol was not mentioned. (50) Sodium hypochlorite concentrations ranged from 1% to 2.5%. Each study used either TAP, DAP, or calcium hydroxide as intracanal medication.

Table 5. Summary of studies RET with APCs vs conventional treatment modalities in mature permanent teeth

Study	Age (Years)	Intervention	Number	Irrigation	Final irrigation	Follow-up (months)	Success Rate	Vitality	Intracanal Medication	Radiological Success
Arslan et al 2019	18-30	BC RET	26	1% NaOCl	5% EDTA	12	92.30%	EPT: 50%	TAP (Dox, Met, Cipro)	Healed 46.2% Healing 46.2%
		NSRCT	20				80%	EPT: 5%	CH	Healed 60% Healing 25%
Brizuela et al 2020	16 -58	RET	18	NR	NR	6-12	100%	CT: 56% EPT: 50%	CH	NR
		NSRCT	18				100%	EPT: 16.6%	CH	
Nageh et al. 2018	18-40	PRF RET	18	1.5% NaOCl	17% EDTA Saline	12	100%	CT 60% EPT 60%	DAP (Met, Cipro)	100%
Jha et al 2019	9-15	BC RET	15	2.5% NaOCl	17% EDTA	18	86.60%	NR	TAP	Healed: 86.6% Healing:13.3%
		NSRCT	15	2.5% NaOCl			80%		-	Healed:80% Healing:20%

BC – Blood clot; ICM – Intracanal medication; PRP – Platelet-rich plasma; RET – Regenerative endodontic treatment; NSRCT – Non-surgical root canal treatment; TAP – Triple antibiotic paste; DAP – Double antibiotic paste; Cip – Ciprofloxacin; Met – Metronidazole; Mino – Minocycline; Doxy – Doxycycline; NR – Not reported; CT – Cold testing; EPT – Electric pulp testing.

DISCUSSION

Regenerative endodontic procedures (REPs) harness tissue engineering principles, relying on a scaffold comprising stem cells and essential growth factors to nurture the proliferation and differentiation of stem cells. The ideal scaffold should possess specific attributes: adequate porosity for cell seeding, efficient transport of nutrients, oxygen, and waste, as well as sufficient physical and mechanical strength, minimal inflammatory response, and biodegradability akin to the tissue regeneration process. Blood clots (BC) and autologous platelet concentrates (APCs) serve as commonplace scaffolds in REP. (11)

Clinically, the revitalization protocol involves meticulous debridement and disinfection of the tooth, employing low concentrations of sodium hypochlorite (2–2.5%) during the initial appointment, often with minimal or no instrumentation. Subsequently, intracanal medication with calcium hydroxide or triple antibiotic paste (TAP) is administered. Most TAPs used included Ciprofloxacin, Metronidazole, and Minocycline. Some studies have replaced Minocycline either with Cefaclor or Clindamycin. The subsequent appointment entails canal irrigation with EDTA, followed by the induction of bleeding to recruit stem cells from periapical tissues, facilitating the formation of a scaffold for new tissue ingrowth. (54) This approach offers several advantages over alternative methods, including the absence of allergic reactions, reduced cost and visitation time, and enhanced convenience and comfort for patients. The clotting process involves a multitude of blood cells and clotting factors. (11)

While this approach simplifies the therapy's widespread adoption, it also presents potential drawbacks. The predictability of blood clot formation within the canal is not assured, and inadvertent blood contamination of the tooth crown may result in discoloration. (55) In response, autologous platelet concentrates emerge as an intriguing and cost-effective solution for regenerative endodontics. Their high concentration of growth factors stimulates migration, proliferation, and differentiation of stem cells, supported by a dense fibrin matrix acting as a stable scaffold, along with bacteriostatic properties. (56)

The success of regenerative endodontic therapy extends beyond primary outcomes, encompassing desirable secondary outcomes associated with continued root development. Primary outcomes include the alleviation of apical periodontitis symptoms and evidence of bony healing, often indicated by periapical radiolucency (PARL) resolution. Secondary outcomes gauge root development through increased wall thickness, length, and apical closure. (16) These facets of ongoing root development are intricately linked to vitality preservation and the interaction between Hertwig's epithelial root sheath and mesenchymal stem cells of the apical papilla. (57,58) This physiological process leverages vital cells like fibroblasts, odontoblasts, and cementoblasts, facilitating continued root growth and narrowing. (59)

Several methodologies and patterns emerge when reviewing regenerative endodontic treatment (RET) studies with autologous platelet concentrates (PRP and PRF). Firstly, while RET appears to have a generally high clinical success rate, there is a need for studies with more extended follow-up periods to assess the sustainability of outcomes. Secondly, universities and regions that have been active in RET research include those with strong dental programs, such as those in Asia, Europe, North America, South America, and Africa. Specific institutions may include universities with renowned dental schools, such as Columbia University, University of Seville, New York University, and Chulalongkorn University, among others.

There is variability across studies regarding group sizes and teeth included in the analyzed objectives. Some trials had larger sample sizes (ex. 56), while others may focus on specific subgroups or clinical scenarios. Studies mainly included single-rooted teeth (incisors and premolars), and only a few studies included all types of teeth. However, there was a general trend towards randomized controlled trials and clinical studies with well-defined inclusion criteria and comparable group sizes to ensure robust data analysis. Nonetheless, more standardized approaches to study design and reporting could enhance the comparability of findings across studies and contribute to the advancement of RET research.

The results indicate that autologous platelet concentrates (APCs) significantly enhanced apical closure, APCs groups ranged from 40% to 100% while the BC group ranged from 18.9% to 80%, and responsiveness to sensitivity pulp tests, APCs group ranged from 15.8% to 98% and BC group ranged from 13.3% to 95.2%. Only one study has reported comparable results of 100% responsiveness to sensitivity tests (EPT and Cold test) in both APCs and BC groups. (39) However, no notable differences were observed regarding root lengthening, dentin wall thickness, or the success rate of immature, necrotic teeth undergoing regenerative endodontic treatment with BC. This result aligns with the findings of other research conducted by Panda et al. (60)

This discrepancy may arise from the deliberate induction of bleeding in the periapical region during revascularization procedures for immature necrotic teeth, wherein the formation of a blood clot within the root canal serves as a scaffold, facilitating angiogenesis, guiding stem cell migration from the periapical area, and stimulating pulp regeneration and root maturation. (61)

In the subgroup analysis, teeth subjected to platelet-rich plasma (PRP) treatment exhibited superior secondary outcomes compared to those treated with platelet-rich fibrin (PRF). The PRP groups showed a 4.74% to 9.88% increase in root length, a 19.1% to 39.27% increase in root thickness, and 40% to 100% showing complete apical closure. Meanwhile, the PRF group groups showed a 6% to 12.3% increase in root length, a 9.8% to 42.37% increase in root thickness, and 60% to 100% of teeth showing complete apical closure. One potential explanation for PRF occasionally proving less effective than PRP could stem from the distinct bioactivities of these two autologous platelet concentrates (APCs). PRF consists of a dense and stable fibrin network (62), enabling a slower release of growth factors compared to PRP. Consequently, PRP releases a notably higher quantity of growth factors within the initial 15–60 minutes post-clot formation, while PRF maintains a continuous and gradual release of a modest amount of growth factors over ten days (63). The elevated concentrations of bioactive molecules released by PRP could potentially account for its apparent beneficial effects over PRF, particularly in the short term. Notably, two studies (32) evaluated tooth response to vitality testing following treatment with both PRP and PRF, indicating comparable outcomes. (36,40) One study stated the superiority of PRP over PRF on this variable (38), and the other study stated the opposite. (19) Based on these findings, it could be stated that there is a tendency for PRP to yield superior results compared to PRF in regenerative endodontic procedures. Nonetheless, further clinical investigations involving large sample sizes and standardized, comparable methodology are warranted to validate or refute this trend over an extended follow-up period.

The process used to obtain the autologous platelet concentrates was also heterogeneous. Studies used different rotations per minute (RPM), activators and anticoagulants, and numbers of centrifugation steps to obtain the concentrates. In order to make it easier to compare the trial results, future research should employ the published, standardized protocols for APCs together with standardized protocols for REPs.

In each study, the teeth selected for regenerative endodontic treatment (RET) were those affected by trauma (64), secondary caries (65), or developmental anomalies (64). Trauma had the highest rate of pulp necrosis aetiology; *Dens Evaginatus* was the second highest, and caries had the lowest rate out of all three. Several factors influencing the outcome of RET include the irrigation protocol, final canal rinsing, intracanal medicaments (ICM), and apex diameter. A wider apical diameter permits mesenchymal stem cell migration into the root canal space, facilitating host cell homing and new tissue formation within the canal. An apical diameter of 1.1 mm or greater is advantageous, with natural regenerative endodontic treatment observed in approximately 18–34% of teeth with immature roots. (66) Additionally, the patient's age, which correlates with the stage of root formation and apical diameter, likely acts as a modifying factor in regenerative endodontic procedures. (67)

The included clinical trials presented significant differences in protocols regarding treatment performance. Most studies did not follow the standardized protocol for revitalization, as published by the American Association of Endodontists (16) and the European Society of Endodontics. (68) Among variations concerning the standard treatment were the use of sodium hypochlorite in high concentrations (5.25% or 6%) (34,40,69) the lack of use of EDTA (14,30,33,34,37,38,40,41,47,48) and the use of triple antibiotic pastes with high concentrations and/or those containing minocycline All these variations may have affected the clinical outcomes of revitalization. (20,36,42,44–46,69) High sodium hypochlorite concentrations reduce the viability of stem cells and their odontogenic/osteogenic differentiation. (70) EDTA, on the other hand, reduces the deleterious effect of sodium hypochlorite and improves cell survival and differentiation. (70) Moreover, EDTA liberates the growth factors present in dentin that positively affect stem cell adhesion, migration, and differentiation. (71) TAP, especially in high concentrations, has cytotoxic effects on stem cells and reduces mineralization (72), and when minocycline is included, it can cause significant tooth discoloration. (73)

Crown discoloration after RET is multifactorial in origin. It can be caused by the presence of minocycline in the antibiotic intracanal medicaments, the induction of bleeding during RET, and the application of MTA coronal barrier after RET. (74) Various approaches have been suggested to minimize discoloration after RET. These approaches include the use of minocycline-free antibiotic medicament or $Ca(OH)_2$ (75), the application of intracanal medicament below the cementoenamel junction using an injectable syringe (16), sealing the pulp chamber with a dentin bonding agent (76), and the use of hydraulic calcium silicate-based cement with eliminated potential of crown discoloration for the coronal barrier, such as Biodentine. (77)

REPs are also gaining attention as an alternative to conventional root canal treatment (CRCT) for mature teeth with periapical radiolucencies. A preliminary study comparing REPs with CRCT showed favorable outcomes in 92.3% and 80% of cases, respectively, over a 12-month follow-up. The study suggests REPs as a viable treatment option, especially considering their potential to induce vital tissue formation. (49) Another phase I/II clinical trial explored the use of encapsulated human umbilical cord mesenchymal stem cells in REPs, reporting 100% clinical efficacy and increased pulp sensitivity in the REP group. This innovative approach holds promise for dentin-pulp regeneration. (50) Furthermore, a study utilizing platelet-rich fibrin (PRF) in modified REPs demonstrated significant improvement in pulp sensibility, indicating potential vital tissue formation. However, further investigation on a larger scale is warranted. (52) Additionally, the SealBio technique, stimulating periradicular cells for healing and regeneration, showed comparable outcomes to conventional obturation, with the added benefits of reduced chair time and cost-effectiveness. (51) These findings underscore the evolving landscape of endodontic therapies, offering promising alternatives for managing periapical pathology while addressing practical concerns in treatment delivery.

Regenerative endodontic procedures hold promise as a viable treatment option for immature necrotic teeth, offering the potential for tissue regeneration and continued root development. While using autologous platelet concentrates and meticulous protocol adherence can enhance treatment outcomes, challenges such as scaffold formation predictability and crown discoloration remain areas of ongoing research and improvement. Standardization of protocols, further clinical investigations with larger sample sizes, and the exploration of alternative scaffold materials are essential for advancing the efficacy and predictability of regenerative endodontic therapy. With continued advancements in tissue engineering, biomaterials, and clinical protocols, regenerative endodontics stands poised to play an increasingly significant role in modern endodontic practice, offering patients improved outcomes and enhanced oral health.

CONCLUSIONS

In essence, regenerative endodontic procedures present a hopeful method for treating immature necrotic teeth by leveraging tissue engineering principles and scaffolds that contain stem cells and growth factors. Despite the potential advantages of regenerative endodontic procedures, there are challenges, such as the uncertainty surrounding scaffold formation and the occurrence of crown discoloration that require attention. The utilization of autologous platelet concentrates resulted in slightly higher success rates and clinical outcomes of the reviewed studies. Particularly, autologous platelet concentrates significantly improved apical closure and response to vitality pulp tests, whereas no significant effect was observed on root lengthening, dentin wall thickness, or success rate of permanent necrotic teeth treated with regenerative endodontics. Therefore, it appears promising in addressing some of these challenges. Furthermore, variations in treatment protocols highlight the importance of further research and clinical studies aimed at advancing regenerative endodontics. As tissue engineering techniques and protocol standardization continue to progress, regenerative endodontic procedures have the potential to significantly enhance patient outcomes and contribute to the evolution of modern endodontic practices.

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