Universidad Europea

GRADUATION PROJECT

Degree in Dentistry

PULP REVASCULARIZATION
OF
IMMATURE TEETH.

Madrid, academic year 2022/2023

Identification number: 101

ABSTRACT

Introduction: Immature teeth with necrotic pulp require prompt and appropriate treatment to be maintained and avoid complications. Revascularization, a treatment modality that encourages natural regeneration of pulp-dentin complex, has gained increasing popularity over the past decade; **Objectives**: This systematic review aims to evaluate the current evidence regarding the effectiveness and safety of the protocols of revascularization in immature teeth; **Methodology**: A comprehensive literature research was performed among PubMed, Medline Complete, and Oral and Sciences source databases of the articles published in between 01/2013 and 01/2023. The inclusion criteria were original studies published in English that evaluated revascularization as a treatment modality in immature permanent teeth. Data were extracted and gathered in tables; **Results**: This search identified 19 articles that met the inclusion criteria. The studies involved a total of 276 immature teeth treated with revascularization. The several protocols employed all involved: accessing the root canal, disinfecting the canal, creating a blood clot, and placing a coronal seal. The follow-up periods ranged from 12 to 36 months. The most common complications were crown discoloration and intracanal calcification; **Conclusions**: Revascularization is a promising treatment modality for immature teeth with necrotic pulp, with a high success rate and low incidence of complications when the patients are selected wisely. However, the quality of evidence is limited due to the heterogeneity of study designs and lack of standardization in treatment protocols. Further high-quality randomized controlled trials with standardized protocols and evaluation methods, with longer follow-up periods are needed to establish the efficacy and safety of revascularization in immature teeth.

Keywords: Dentistry; revascularization; immature tooth; pulp necrosis; apex closure.

Resumen

Introducción: Los dientes inmaduros con pulpa necrótica requieren un tratamiento rápido y adecuado para mantenerse y evitar complicaciones. La revascularización, una modalidad de tratamiento que fomenta la regeneración natural del complejo pulpo-dentina, ha ganado popularidad en la última década; **Objetivos**: El objetivo de esta revisión sistemática es de evaluar la evidencia actual sobre la efectividad y seguridad de los protocolos de revascularización en dientes inmaduros; **Metodología**: Se realizó una investigación bibliográfica exhaustiva entre PubMed, Medline Complete, y las bases de datos de fuentes orales y científicas de los artículos publicados entre 01/2013 y 01/2023. Los criterios de inclusión fueron estudios originales publicados en inglés que evaluaron la revascularización como modalidad de tratamiento en dientes permanentes inmaduros. Datos extraídos y recogidos en tablas; **Resultados**: Esta búsqueda identificó 19 artículos que cumplían los criterios de inclusión. Los estudios incluyeron un total de 276 dientes inmaduros tratados con revascularización. Los varios protocolos empleados todos involucraron: acceso al canal radicular, desinfección del canal, la creación de un coágulo de sangre, y la colocación de un sello coronal. Los períodos de seguimiento fueron de 12 a 36 meses. Las complicaciones más frecuentes fueron decoloración de corona y calcificación intracanal; **Conclusiones**: La revascularización es una modalidad de tratamiento prometedora para dientes inmaduros con pulpa necrótica, con una alta tasa de éxito y baja incidencia de complicaciones cuando los pacientes son seleccionados sabiamente. Sin embargo, la calidad de la evidencia es limitada debido a la heterogeneidad de los diseños del estudio y la falta de estandarización en los protocolos de tratamiento. Se necesitan ensayos controlados aleatorios de alta calidad con protocolos y evaluación estandarizados, y períodos de seguimiento más largos para establecer la eficacia y la seguridad de la revascularización en dientes inmaduros.

Palabras claves: Odontología; revascularización; diente inmaduro; necrosis pulpar; cierre apical.

INDEX

1 INTRODUCTION

1.1 The permanent immature tooth (PIT)

1.1.1 Dental growth

Dental growth and development of a tooth is an organized process starting during embryogenesis that takes place in several sequences during pregnancy, childhood, and adolescence. A permanent mature tooth is composed of non-vascularized hard tissue and soft vascularized and innervated tissue, the pulp dental arch are considered immature unless the apical cementum-dentine junction is present. PIT have histological, anatomical, and physiological characteristics that make their treatment unique and individualized (4).

1.1.1.1 Enamel immaturity

Enamel is the outer layer of the tooth, the hardest tissue produced by our body. Its formation is due to ameloblast derived from embryological epithelium that produces a scaffold of proteins where the further fixation of calcium and phosphorus ions can deposit producing the hydroxyapatite crystals. After this scaffold formation, ameloblast goes through apoptosis, and the enamel organ then becomes acellular, shunting off the ability for self-regeneration (2). The full enamel maturation called mineralization occurs after the eruption process, at this time enamel tissue is still porous and very sensible to caries that will expand fast to the dentin (5,6).

1.1.1.2 Dentin immaturity

The dentin is an acellular hard tissue produced by the odontoblasts. Dentin formation is initiated by ameloblast stimulation of the odontoblasts that will produce pre-dentin, a non-calcified protein scaffold for its further calcification of it. After the scaffold formation, odontoblasts remain in the pulpar cavity and are the cells mediator between the surface of the dentin and the nerve inside the pulp through cytoplasmic elongation called Tome's fiber. Odontoblasts keep their ability of tissue formation;

indeed, it can be observed a regular secondary dentin apposition starting at the eruption in response to minor irritants such as biomechanical forces, and thermal changes in the odontoblasts site and inside the tubule of the Tome's fiber reducing the pulp and tubule's wideness. In the event of a dental injury, dentin has little capacity for tissue regeneration: the inflammatory factors and signaling pathway from pulp tissue allows to recruit odontoblasts and stimulate the differentiation of dental pulp stem cells (DPSCs) into new odontoblast-like cells for the formation of localized tertiary dentin as a response to major irritation. As shown in Figure 1 dentin is one of the niches of stem cells (SCs) in the dental organ. This last one is molecularly close to the original dentin tissue. The dentin apposition and mineralization process is a life-long process, and an immature tooth has only a thin porous primary dentin because of wide tubules; meaning that irritants crossing the enamel barrier can easily transit through Tomes fiber and reach the pulp (2,3,7).

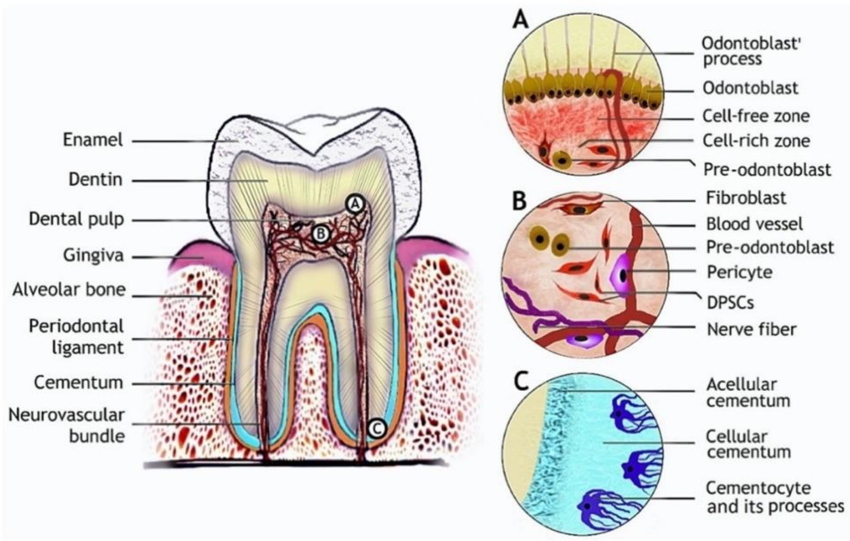

Figure 1: Tooth structure and dental tissues with the respective stem cell populations.
(A) The odontoblast niche (B) Dental pulp cells (C) Cementocytes residing in the lacunae of cellular cementum (2).

1.1.1.3 Cementum immaturity

Cement formation, as dentinal formation, occurs in a corono-apical direction. Its composition is different in function of the position on the root, while a thin, mainly acellular cementum is observed, there is a thick cementum with cementocytes inlaid in it (Figure 1). Hertwig epithelial root sheath (HERS) or epithelial root sheath (epithelial origin) is believed to be at the origin of the initial acellular cementum while the cementoblasts furtherly recruited come from the dental follicle lineage (mesenchymal origin) take a part in the apical root cementum apposition making those last mainly involved in the apical closure (3). Cementum apposition occurs during the whole life, cementoblasts and their precursors remain in the periodontal ligament space and can be recruited for cementum generation (2).

1.1.1.4 Radicular immaturity

The eruption corresponds to the movement of the tooth to reach its functional place, it is to be distinguished from the emergence which corresponds to the appearance in the mouth of the crown by perforation of the gum (8). The beginning of the eruptive sequence corresponds to the beginning of root formation. During emergence, the formation of the root of the immature tooth is only at its 2/3 of complexion (9). The neurovascular bundle is important in a wide radicular canal with the apical part remaining "open" for a period of up to 3 years. During this period the dentine surrounding the apex remains a weak point (9–11). In the following Figure 2, the eruption of the tooth occurs in the 3rd image of the very immature stage.

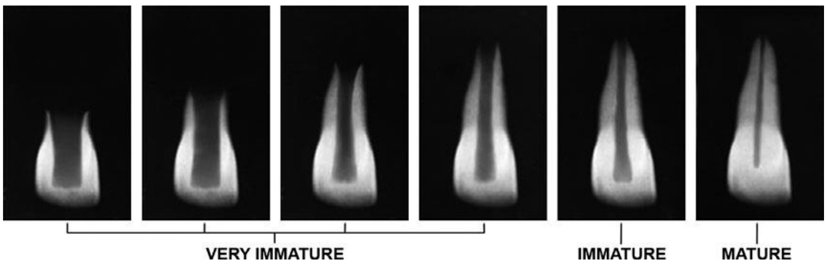

Figure 2: Classification of root development stages: Very immature root development = the tooth has incomplete root formation or complete root formation with a wide-open apex.
Immature root development = the tooth has full root formation and half-closed apex.
Mature root development = the tooth has full root formation and a closed apex (12)

The HERS cells are known to be involved in the root shape, it induces the recruitment of dental papilla cells and their differentiation in root odontoblasts. At one point of root formation, the HERS become fenestrated, and some of the inner layer's cells differentiate into cementoblast while some outer layer's cells take a part in the osteogenesis and periodontal ligament formation. It is believed that resting cells from fenestrated HERS remain sprinkled in the periodontium space as stem cells (SCs) to maintain homeostasis and allow regeneration (3). Some other resting epithelial structure from HERS, specifically the cervical loop, was supposed to be transient structure, however, it is now considered that they could play a role in the regeneration of the root (2).

1.1.1.5 Nerve immaturity:

The innervation is also immature, which helps to make it less sensitive to different external stimuli. The absence of apical constriction means that the nerve pathways are not compressed during any inflammation, which explains a reduction or an absence of pain, even in advanced stages of pulpar disease making the diagnosis difficult (13).

1.1.2 Pulpar pathology

The pulp contains the neural ends that participate in the reception of sensory information of the oral environment and is perfused by blood vessels providing the needs for the resident cellular population and the odontoblasts of the dentin, and guiding immune cells in case of attack (2). It remains very sensitive to irritation, which means that irritants from microbial, physical, or chemical origin involving the pulp can lead to its necrosis (12).

1.1.2.1 Pulpar etiopathology

One of the three main actors of pulp pathology is of bacterial origin: caries. Affecting nearly 100% of the adult world population, it is a multifactorial disease inducing localized demineralization and further destruction of dental tissue (6). Indeed, it was observed a prevalence of extensive caries in the 1st molar of 10% in the 2-3 years after eruption (5).

An irritation of physical or chemical nature can also affect the pulp. It can be from different origins: iatrogenic thermal exposure mainly with burs, iatrogenic chemical irritation due to adhesives, iatrogenic pulp exposure, and direct trauma can all provoke pulpar inflammation. Even if the pain is usually present, the pulp can cure itself in a major part of cases (12,14).

The presence of an anatomical abnormality can produce pulpar or periodontal inflammation and can also lead to pulp necrosis.

1.1.2.2 Pulpar counterbalance

Histologically only the non-affected tooth shows normal tissues, even initial stages of reversible pulpitis show histological abnormality (15,16). The course of pulpar affection begins with pulpitis considered reversible initially and goes through an irreversible process of inflammation followed by necrosis. Necrosis is the cellular death within the tooth at different levels: the canals and the pulp chamber (2,15).

Pulpar inflammation is the response to irritation of odontoblasts, as a matter-of-fact dentin and pulp act as one unit. The information of irritation is transmitted through Tome's fibers causing reversible vasodilatation. It follows the cellular recruitment of defensive cells and as said earlier, the production of tertiary dentin. The initial response is identical regardless of etiology and is proportional to aggression, a greater distress can imply irreversibility and chronicity of inflammation (16).

In chronic inflammation, on top of vascular and cellular reactions, it can be observed tissue destruction caused by an exacerbated inflammatory response by the immune cells leading to progressive tissular death where pulpectomy would be the indicated treatment (17).

The symptoms of pulp pathology can be from nothing to intense pain depending on the stage. In the case of pulp necrosis, it is possible to observe symptoms such as swelling, sinus tract, pain to percussion and palpation, mobility, and self-reported pain but also color changes after some time (12,13).

1.1.2.3 The particularity of immature tooth

In case of necrosis, the apical formation is unable to complete itself. As the young population is the most affected by caries and trauma is therefore possible to encounter PIT. Their preservation on the arch is then a key issue for the dentist (10).

1.1.3 Diagnosis of tooth necrosis and open apex

1.1.3.1 Clinical examination, basic information

Carious progression on a PIT is faster than on a mature permanent tooth, because of tissue immaturity described above. Meticulous pulpar and peri-radicular diagnosis are therefore essential. To do so, a good medical and dental history of all the symptoms and course of the pain should be undertaken, just as much as a description of the main medical conditions of the patient. A visual examination will be carried out to provide the basic information (10,18). Pain to percussion, dullness on palpation, and the presence of mobility are some of the specific components to assess for pulpar diagnosis. Additional periodontal assessment, fluoroscopy, and the presence of occlusal discrepancies may be required (18).

1.1.3.2 Complementary examination (in vivo)

It has been shown that the formation of the root is not necessary for the completion of the eruption, this means that the diagnosis of an incomplete apex can only be made using the complementary examination beginning with the radiological examination (1). Radiological examination is mandatory and essential for the diagnosis, treatment, and follow-up of suspected cases of pulp necrosis. Indeed, if the other tests bring us clues, the latter brings with certainty the status of maturity of the tooth. In addition to this, the x-ray can indicate to the practitioner the presence of periapical lesions and root resorptions before symptoms appear (10).

Regarding vitality testing, the heat test is not a definitive test for the diagnosis of pulpal status even if the sensitivity to heat is an element that suggests pulpal necrosis. On the other hand, the cold test and the electrometric test showed very good results in terms of sensitivity and specificity (19).

The diagnosis of the pulp condition cannot be drawn from the response to a single test nor the history of the patient alone. Even more in the case of young patients where the disparity of the response to tests and the variety of pain do not coincide with the histopathological condition of pulp in PIT, moreover in case of a traumatic injury, the response to a vitality test will be negative even if the tooth's ability to recover is not lost. Specifically, when it comes to PIT the use is limited due to the inconsistency of response associated with open apex, preventing an accurate diagnosis on its own. Nonetheless, it is expected to work as a conclusion of all the tests carried out: the association of several tests increases the specificity of detection, combined with the proper examination and history for the practician to generate an accurate diagnosis in most cases (10,13,19).

1.2 Treatments available

The decision on endodontic treatment is made according to the appropriate diagnosis, taking into consideration the possible complications associated with each patient. The latter will be informed about the procedure beforehand and must provide informed consent (18,20). The practitioner may face two scenarios:

- Either the preservation of pulp vitality is possible, in which case direct pulp capping, indirect pulp capping, partial pulpotomy, or total pulpotomy represent treatment alternatives allowing physiological root closure.
- Either the pulp vitality is compromised and treatment orientation towards endodontic treatment is necessary for non-physiological apical closure or through revascularization (10).

1.2.1 Reparative endodontics:

Among non-surgical reparative techniques, there is the apexification technique which is defined by the American Association of Endodontics (AAE) as "a method to induce a calcified barrier in a root with an open apex or the continued apical development of an incompletely formed root in teeth with necrotic pulps" (21).

1.2.1.1 Traditional apexification

Pulp canals are cleaned and the apex is temporally sealed up with calcium hydroxide ($Ca(OH)_2$) apically and in the conducts, then it is sealed and maintained in a sterile environment before the final endodontic treatment. It was originally used because it showed great biocompatibility, predictable results, and could be mixed with other materials (10,22). Nonetheless, apex formation with $Ca(OH)_2$ has also some cons: the treatment time is ranging from 5 to 20 months, compromising the result outcomes in case of fracture, patients that don't come, etc. At the end of the treatment, membrane rupture is not ruled out because it is thin and porous (22,23). Resorption of the $Ca(OH)_2$ before apex formation, has been observed and the need to replace it doesn't rely on evidence (10).

1.2.1.2 Apical barrier formation

For the reasons previously mentioned, other materials such as Calcium silicate, Biodentine®, and mineral trioxide aggregate (MTA™) are used more often to produce a more resistant, non-porous apical barrier (22,23). The following Figure 3 is showing the procedure. It consists of the placement of 5mm of MTA™ intracanal. It is not necessary to apply humidity during the setting because the moistness of the surrounding tissues would suffice. Consequently, the final obturation can take place on the same day as the opening (10).

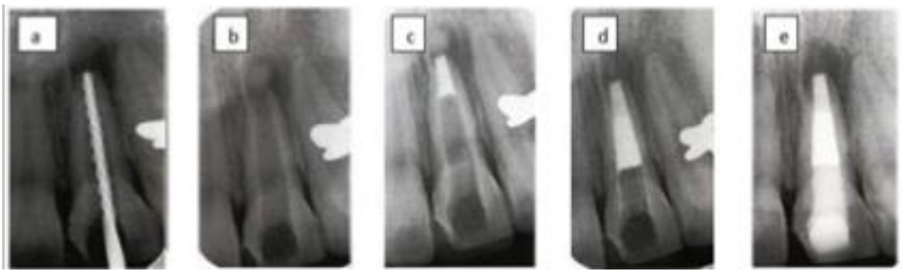

Figure 3: Apexification with MTA as an apical plug. A) The canal is disinfected with copious irrigation B) Calcium phosphate is placed through the apex as a barrier. C) 4mm of MTA is placed at the apex. D) The canal is filled with thermoplastic gutta-percha. E) Bonded resin resorted is placed at a level below CEJ (10).

1.2.2 Pulp regenerations

Pulp regeneration has been studied for a long time, starting in the early 60s with Östby et al. (24), as an alternative to apexification in the case of PIT with necrotic pulp. Based on the cleaning and sterilization of the canals, with the introduction of the Ethylenediaminetetraacetic acid (EDTA) product, the induced bleeding and partial obturation of the canal presented progressively in the periapical region formation of granulation tissue, fibrous tissue, and calcified tissue closing the apex. However, the lack of knowledge about pathogens and SCs conducted to hasty conclusions about this protocol (25). It is only at the beginning of the 20th century that the interest in the procedure is renewed; in particular, the searches of Iwaya et al. (26) which have made it possible to relaunch the search for a fairer protocol, he then noted that the treatment by revascularization of a bacteria-free pulp on a young permanent tooth allowed apical closure after 30 months.

1.3 Pulp regeneration in a necrotic immature tooth

1.3.1 Concept

It is now known that pulp treatment is not limited to simple pulpectomy and root canal treatment. According to the AAE, "regenerative techniques is the concept of tissue engineering to restore the root canals to a healthy state" (27). Revascularization consists of the use of the native properties of the patient's tissues to regenerate the pulp-dentin complex histologically and anatomically, to allow the end of root edification by preventing or curing the associated apical periodontitis as presented in Figure 4 (26,28). In case of a failure of tissue regeneration, it is possible to complete the treatment with a reparative technique (18).

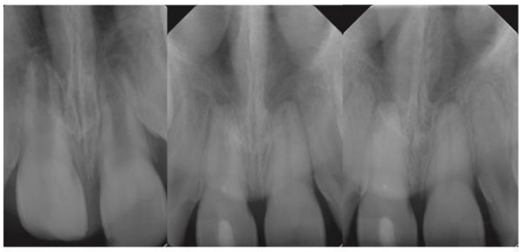

Figure 4: Degrees of apical closure.
1: open apex; 2: partially closed apex; 3: closed apex (12).

1.3.2 Indications according to AAE & European Society of Endodontology (ESE)

The main indications are the presence of a necrotic pulp with an open apex and thin radicular walls; regarding the apical diameter an apex with a diameter as reduced as 0,5mm to 1mm shows good results, however, the best outcomes in terms of increased thickness, length, and apical closure are obtained when the preoperative apex diameter is superior to 1mm. The subject must therefore be young (between 8 and 13 years old). Several studies suggest that the younger the individual, the better the SCs' regenerative potential and healing capacity (4,20).

In addition, the patient should not show allergies to antibiotic content necessary for the procedure nor general pathology (20). Pulp regeneration may be indicated as an alternative to apexification when root space is not necessary for post/core or restoration but when a too-short root and a too-thin wall would make the tooth too fragile and the success of treatment by apexification would be questionable (18,29).

1.3.3 The mechanism of the treatment: the 4 pillars

This part is based on clinical observations as well as clinical trials on animals, histological studies being complicated to perform on humans for ethical reasons. The concept of the four pillars has been described by Brizuela C. et al. (30).

1.3.3.1 Stem cells – progenitor source

The first cornerstone to obtain pulpar regeneration is the need for SCs subsistence nearby the apical region. There are two possible sources of SCs: endogenous and exogenous (30). The generated blood clot provides the blood-derived SCs and together with resilient cells forms the endogenous SCs load needed for pulpar regeneration. The resilient cells can be from pulpar, radicular, and periodontal origin. Among SCs dental pulp stem cells (DPSCs) have shown the best self-replication and differentiation ability once stimulated by growth-factor (GF) (4,31,32).
However, the availability of autologous SCs is limited in empty pulp cavities and exogenic supply can imply an immune reaction. Yet it’s now known that to magnify the quality of apical formation, the practician has in his tools different types of exogenous SCs (2,30,31).

1.3.3.2 Matrices – conductive material

The second pillar for tissular regeneration is revascularization and reinnervation of the cavity, to provide the latter SCs the supply and support they need(30). Neovascularization is highly dependent on the apical diameter and root length (4,33). Proteins provided by the blood clot act as a scaffold for the neoformation of capillaries, despite that, in the case of small apex, some porous biomaterial could be used to ease and strengthen the affluence of blood vessels (4,30,34,35).

1.3.3.3 Growth factors – inductive material

Another crucial element for regeneration would be the GF; they are molecules secreted directly by surrounding cells or from distant cells and reach their location thanks to the blood supply (36). Their origin is often in the area as their lifespan is short, MSCs contained in the blood clot have shown important paracrine secretion of GF to stimulate differentiation (2,30). They are promoting immune defense, tissue formation, and tissue repair through cellular proliferation and cellular differentiation. In the matter of dental regeneration, where hard and soft tissue together with neovascularization are needed, the intervention of growth factor is unquestionable (2,3,30).

Research in regenerative dentistry is oriented towards the use of additional autologous blood derivates because of their high content in GF and other signalization proteins useful for regeneration, they are called platelet-rich plasma (PRP) and platelet-rich fibrin (PRF)(34,37). However, the extraction of plasma derivate makes the procedure high-priced with argumentative results, indeed for some authors blood clot is thought to remain the gold standard when in other studies the use of plasma-derived products has shown better outcomes (37,38).

1.3.3.4 Interaction between progenitor, conductor, and inductor

The synergic effect of those three pillars is the last point to complete the backbone of pulpar regeneration. Indeed, some materials on the market allow delivering progenitor cells, a scaffolding component, and inductive material in addition to the induced blood clot (30,31).

Recent research has shown that several presentations of PRF, such as gel or membrane, allow it to act as matrices for further faster and more effective vascularization (34,37,38).

DPSCs can be cultured over a membrane for further reimplantation. Depending on the GF present, DPSCs can differentiate in odontoblasts-like cells or neuronal cells due to their ectodermic origin. More recent research has considered the potential angiogenic properties of DPSCs (7,31).

1.4 Justification

This therapy is a relatively new technique for the treatment of permanent teeth with an immature apex, it allows to promote self-regeneration of the tissues. While its effect is proven to work over root regeneration after cessation of growth due to inflammation processes, the techniques are still not consolidated. This study seeks to outline the major advances in the regenerative endodontic field and is justified by the need to centralize information on this revolutionary technique for the students and professionals concerned.

1.5 Research question

The research question was: "In immature teeth with pulp necrosis does the revascularization provide a good outcome when compared to apexification respecting the apical closure?"

2 OBJECTIVES

- To evaluate the current evidence regarding the effectiveness and safety of the protocols of revascularization in immature teeth.

- To establish parameters to measure the success rate in pulp revascularization.

- To establish a comparison between pulp revascularization and apexification.

3 MATERIAL & METHOD

3.1 Literature search

3.1.1 Research strategy

To emphasize the main characteristics of dental revascularization, this study systematically searched the following databases: PubMed®, MEDLINE® Complete, and Dentistry & Oral Sciences® Source available with access provided by the Universidad Europea de Madrid.

Based on the PICO strategy this research was made by combining the research keywords with Boolean modulators (AND & OR) to pin down the relevant articles.

This review follows the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 recommendations (39). All the articles included were uploaded to the Mendeley reference manager desktop for bibliographical and data management.

The PICO components were settled as listed below:

- Population (P)— Patients with necrotic, immature permanent tooth OR patient with tooth condition allegedly leading to further pulp necrosis;
- Intervention (I)— regeneration procedure;
- Comparison (C)—apexification procedure;
- Outcomes (O)—clinical and radiographical successful outcome: apical closure, root lengthening, thickening and absence of symptoms.

3.1.2 Research equations

The following combination of keywords was used based on the PICO strategy:

((Pulp necrosis OR necrotic pulp OR necrotic tooth) AND (open apex OR Immature tooth)**) AND (**(Pulp revascularization OR pulp revascularisation OR pulp regeneration OR regenerative endodontics) **AND** (Apexification)**) AND (**Apical closure**)**

3.2 Selections of the articles

3.2.1 Criteria established by automatization tools

The results were limited by automatization tools to the English language with a date range of January 2013 to January 2023, with full text available, nonetheless, to discover the origin, and understanding the evolution of the treatment and its considerations in the literature and by the clinical professional.

3.2.2 Implemented eligibility criteria

On the one hand, were accepted, only the scientific articles fulfilling the following <u>inclusion criteria</u>:

- Scientific articles published in the last 10 years;
- Case or study about revascularization in an immature tooth;
- Presence of pulp necrosis and/or periapical lesion;
- Techniques, protocols, and material of regenerative endodontics;
- Revascularization together with apexification as a comparison of the treatments.

On the other hand, were evicted from the study, the articles fulfilling one of these <u>exclusion criteria</u>:

- Treatment done in permanent mature tooth or temporal dentition;
- Literature review without data to use;
- Apexification alone;
- No blood clot induced;
- Animal research;
- *In vitro* studies.

4 RESULTS

4.1 Flowchart diagram

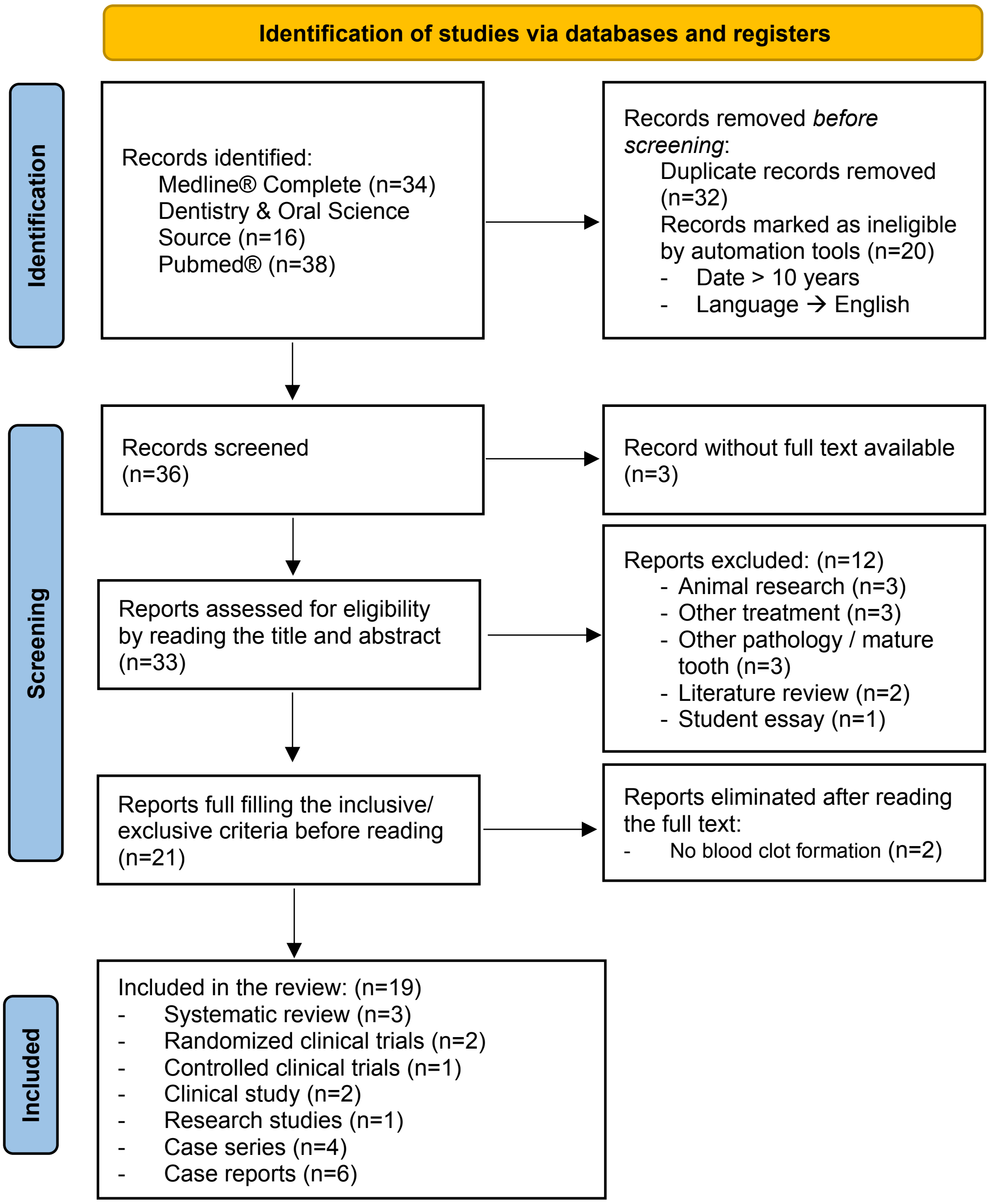

Figure 5: Flowchart of the article selection process. Flow diagram following Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 design (39).

From the elected databases, 88 articles were identified (Figure 5). After deduplication, language selection, and date restriction, 36 records were screened. The pertinent data were acquired automatically for further analysis on an Excel sheet; missing data were included manually. From the initially screened records, 3 articles didn’t present a full text. 12 of the 33 examined articles were excluded after reviewing the title and abstract for non-compliance with the inclusion/exclusion criteria. The full text was read for 21 articles and 2 were eliminated for the reason explained in Figure 5. The overall number of articles included in the study was 19 articles, a total of 3 systematic reviews, 2 randomized clinical trials, 1 controlled clinical trial, 2 clinical studies, 1 research study, 4 case series, and 6 case reports were included in the review.

Abbreviations corresponding to tables of results:

- AC: Apical closure
- CHX: Chlorhexidine
- F/M: Female/Male
- GIC: Glass ionomer cement
- NaOCl: Sodium hypochlorite
- NR: not reported
- PA: periapical
- PA H: periapical healing
- PAI: periapical index
- RL: Root lengthening
- RT: root thickening
- TAP: triple antibiotic paste
- Tooth #: tooth number following FDI
- Y: years old

4.2 Tables of results

Table 1: Data extracted from case reports.

Authors, Year	N° of session follow-up	Protocol							Patients Anamnesis	Outcome			
		Anesthesia	Disinfection / irrigation	Instrumentation	Medication + sealing / duration	Cleaning / final irrigation	Supplement material	Coronal barrier / obturation		PA H	AC	RL	RT
Soares ADJ et al. (40) **Mar 2013**	2 sessions Follow-up: 1, 3, 6, 9, 12, 15, 24 months	Local anesthesia	• Initial sterile saline • 2% CHX gel	YES	$Ca(OH)_2$ and 2% CHX gel [1:1] 21 days Coltosol™	• Sterile saline • 3mL, 17% EDTA 3 min • Sterile saline	NO	• MTA™ • Coltosol™ + Composite	• Sex/age: F,9y • Tooth #: 21 • Pulp status: Pulp necrosis • Etiology: trauma ⇒ Intrusive luxation and enamel-dentin-pulp fracture • Periodontal status: No PA lesion	NR	YES	YES	YES
Makkar S et al. (41) **May 2013**	4 sessions Follow up: 3, 6, 9 months	1st session: NR 2nd session: 2% lignocaine hydrochloride 1:200,000 adrenaline	20mL 5.25% NaOCl	apical opening with 110 K file	TAP 2 weeks Cavit™ (x2)	10mL, 5.25% NaOCl	Gelspon™	• MTA™ ⇒ Cavit™ 6weeks • GIC™ (2nd base) • Composite	• Sex/age: M,17y • Tooth #: 11 • Pulp status: Pulp necrosis, • Etiology: trauma (8y ago) and caries • Periodontal status: Symptomatic chronic apical periodontitis	YES	YES	YES	YES
Kottoor J et al. (42) **Jul 2013**	3 sessions Follow-up: 3, 12, 36 and 60 months	1st session: 2% lidocaine with 1:100,000 epinephrine 2nd session: local anesthesia	20mL 5.25% NaOCl 20min	Coronal third with Gates Glidden drill size #4	TAP 3 weeks Cavit™	10mL, 5.25% NaOCl	NO	• MTA™ ⇒ Cavit™ 1 day • 2mm GIC™ liner • Composite.	• Sex/age: M,11y • Tooth #: 12 • Pulp status: Pulp necrosis, • Etiology: trauma ⇒ enamel-dentin-pulp fracture • Periapical status: Symptomatic apical periodontitis	YES	YES	YES	YES
Becerra P et al. (28) **Jan 2014**	3 sessions Follow-up: 1, 12, 18 and 24 months	1st session: No anesthesia 2nd session: 3% plain mepivacaine	• 20mL, 5.25% NaOCl • 1.2mL 2% CHX	NO	TAP 26 days IRM™	• Sterile saline • 5.25% NaOCl 3 min	NO	• MTA™ ⇒ IRM™ 1 week • Composite	• Sex/age: F,11y • Tooth #: 35 • Pulp status: Pulp necrosis • Etiology: fractured Dens evaginatus • PA status: abscess with sinus tract	NO	NR	YES	NO

Table 1: Data extracted from case reports. (continuation)

Authors, Year	N° of session follow-up	Anesthesia	Disinfection / irrigation	Instrumentation	medication + sealing/ duration	Cleaning / final irrigation	Supplement material	Coronal barrier / obturation	Anamnesis	PA H	AC	RL	RT
Bakhtiar H et al. (43) **Jun 2014**	3 sessions Follow-up: 1, 12, 18 and 24 months	1st session: 2% lidocaine 1:80,000 epinephrine 2nd session: NR	• 8mL, 0.5% NaOCl • Sterile saline	NO	TAP 2 weeks	NR	PRGF	⇒ Cavit™ 2 weeks • MTA™ • Composite	• Sex/age: F,11y • Tooth #: 21 & 11 • Pulp status: Pulp necrosis • Etiology: Trauma • PA status: Acute apical abscess	YES	YES	NR	YES
									• Sex/age: F,8y • Tooth #: 11 • Pulp status: pulp necrosis • Etiology: trauma • PA status: Symptomatic apical periodontitis	YES	YES	NR	YES
									• Sex/age: F,8y • Tooth #: 21 • Pulp status: Pulp necrosis • Etiology: trauma • PA status: Symptomatic apical periodontitis	YES	YES	NR	YES
Rehman, ST et al. (44) **Oct 2015**	3 sessions Follow-up: 3, 6, 12 to 18 months	Local anesthesia	• CHX • Sterile saline • 10mL, 2.5% NaOCl	Cervical and middle third debridement #35k file	TAP 3 weeks Cavit™	NR	CollaPlug™	• MTA™ • GIC™ After 1 week: • Composite	• Sex/age: M,9y • Tooth #: 11&21 • Pulp status: pulp necrosis • Etiology: trauma • PA status: Symptomatic apical periodontitis	YES	YES	YES	YES
	4 sessions Follow-up: 3 to 6 months, 12 and 18 months	1st session: Local anesthesia 2nd session: Local anesthetic without vasocon strictor	• 2.5% NaOCl • Sterile saline		• $Ca(OH)_2$ • 2% CHX gel [1:1] 21 days Cavit™	• Sterile saline • 17% EDTA • Sterile saline		• MTA™ • Cavit™ 1 week Then: • Composite	• Sex/age: M,9y • Tooth #: 11&21 • Pulp status: pulp necrosis • Etiology: trauma • PA status: Symptomatic apical periodontitis	YES	YES	YES	YES

Table 1: Data extracted from case reports. (continuation)

<table>
<tr><th>Authors, Year</th><th>N° of session and follow-up</th><th>Anesthesia</th><th>Disinfection/ irrigation</th><th>Instrumentation</th><th>medication + sealing/duration</th><th>Cleaning/final irrigation</th><th>Supplement material</th><th>Coronal barrier/ obturation</th><th>Anamnesis</th><th>PA H</th><th>AC</th><th>RL</th><th>RT</th></tr>
<tr><td>Bukhari S et al.
(45)

Dec 2016</td><td>2 sessions

Follow-up: 7-31 months</td><td><u>1st session</u>:
Local anesthesia

<u>2nd session</u>:
3% plain mepivacaine</td><td>3% NaOCl</td><td>minimal or no</td><td>TAP

Minocycline replaced with clindamycin

≈ 37days
Cavit™</td><td>• 3% NaOCl
• 17% EDTA</td><td>NR</td><td>• EndoSequence® Bioceramic Putty™ or MTA™

• Composite</td><td colspan="5"><table>
<tr><th>28 teeth</th><th>Complete healing</th><th>Incomplete healing</th><th>Failure</th></tr>
<tr><td>Incisors n=21</td><td>80,9%</td><td>9,4%</td><td>9,5%</td></tr>
<tr><td>Premolar n= 5</td><td>60%</td><td>40%</td><td>-</td></tr>
<tr><td>Molar n = 2</td><td>50%</td><td>-</td><td>50%</td></tr>
<tr><td>Maxilla n = 22</td><td>77%</td><td>14%</td><td>9%</td></tr>
<tr><td>Mandible n= 6</td><td>66,6%</td><td>16,6%</td><td>16,6%</td></tr>
<tr><td>Caries n= 5</td><td>60%</td><td>20%</td><td>20%</td></tr>
<tr><td>Anatomical n=3</td><td>67%</td><td>33%</td><td>-</td></tr>
<tr><td>Trauma n=20</td><td>80%</td><td>10%</td><td>10%</td></tr>
<tr><td>S & S (+) n=18</td><td>83%</td><td>11%</td><td>6%</td></tr>
<tr><td>S & S (-) n=10</td><td>70%</td><td>20%</td><td>10%</td></tr>
<tr><td>PA lesion (+) n=22</td><td>77%</td><td>14%</td><td>9%</td></tr>
<tr><td>PA lesion (-) n=6</td><td>66,6%</td><td>16,6%</td><td>16,6%</td></tr>
</table></td></tr>
<tr><td rowspan="4">Carmen L et al.
(46)

Mar 2017</td><td rowspan="4">Preliminary antibiotic treatment
+
2 sessions

Follow-up: 7-12 months</td><td rowspan="4">NR</td><td rowspan="4">• Crown cleaning: 2% CHX
• 5% NaOCl</td><td rowspan="4">Minimal</td><td rowspan="4">TAP

3weeks</td><td rowspan="4">5% NaOCl</td><td rowspan="4">NO</td><td rowspan="4">• MTA™
• GIC™, Composite
• Metal crown for molars</td><td>• <u>Sex/age</u>: NR,8y
• <u>Tooth #</u>: 46
• <u>Pulp status</u>: Irreversible pulpitis
• <u>Etiology</u>: caries
• <u>PA status</u>: No PA lesion</td><td>NR</td><td>YES</td><td>YES</td><td>YES</td></tr>
<tr><td>• <u>Sex/age</u>: NR,8.5y
• <u>Tooth #</u>: 21
• <u>Pulp status</u>: Pulp necrosis
• <u>Etiology</u>: trauma
• <u>PA status</u>: Acute apical abscess</td><td>YES</td><td>YES</td><td>YES</td><td>YES</td></tr>
<tr><td>• <u>Sex/age</u>: NR,6.5y
• <u>Tooth #</u>: 36
• <u>Pulp status</u>: Irreversible pulpitis
• <u>Etiology</u>: Caries
• <u>PA status</u>: Symptomatic apical periodontitis</td><td>YES</td><td>NO</td><td>YES</td><td>YES</td></tr>
<tr><td>• <u>Sex/age</u>: NR,8y
• <u>Tooth #</u>: 36 // 46
• <u>Pulp status</u>: Pulp necrosis
• <u>Etiology</u>: Caries
• <u>PA status</u>: Symptomatic apical periodontitis // No PA lesion</td><td>YES</td><td>YES</td><td>YES</td><td>YES</td></tr>
</table>

Table 1: Data extracted from case reports. (continuation)

Authors, Year	N° of session and follow-up	Anesthesia	Disinfection/ irrigation	Instrumentation	medication + sealing/duration	Cleaning/final irrigation	Supplement material	Coronal barrier/ obturation	Anamnesis	PA H	AC	RL	RT
Meschi N et al. (47) **Oct 2018.**	2 sessions Follow-up: 3, 6, 12, 24, 36 months	<u>1st session</u>: Local anesthesia, <u>2nd session:</u> Local anesthetic without vasoc onstrictor	30mL, 2.5% NaOCl	NR	DAP (*ciprofloxacin, metronidazole*) 2-4 weeks GIC	1. 20 mL, 2.5% NaOCl 2. EDTA (5 mL, 17%)	CollaPlug™	• MTA™ • GIC™ • Composite.	• <u>Sex/age</u>: F,8yo • <u>Tooth #</u>: 22 • <u>Pulp status</u>: Pulp necrosis • <u>Etiology</u>: Dens invaginatus • <u>PA status</u>: PA lesion	YES	YES	YES	YES
									• <u>Sex/age</u>: F,11y • <u>Tooth #</u>: 21 • <u>Pulp status</u>: Pulp necrosis • <u>Etiology</u>: trauma • <u>PA status</u>: apical periodontitis	YES	NO	YES	YES
									• <u>Sex/age</u>: M,8y • <u>Tooth #</u>: 21 • <u>Pulp status</u>: Irreversible pulpitis • <u>Etiology</u>: trauma (with fracture) • <u>PA status</u>: PA lesion	YES	NO	YES	YES
									• <u>Sex/age</u>: F,6y • <u>Tooth #</u>: 21 • <u>Pulp status</u>: Pulp necrosis • <u>Etiology</u>: trauma • <u>PA status</u>: PA lesion	YES	NO	YES	YES
									• <u>Sex/age</u>: M,8y • <u>Tooth #</u>: 11 • <u>Pulp status</u>: Pulp necrosis • <u>Etiology</u>: trauma • <u>PA status</u>: PA lesion	YES	YES	YES	YES
Kang, K et al. (48) **Oct 2022**	Preliminary drainage and antibiotic treatment + 4 sessions Follow-up: 6, 12, 36 months	<u>1st session</u>: Local anesthesia <u>2nd session</u>: 3% plain mepivacaine	1. 20ml, 1% NaOCl 2. 5ml, 17% EDTA activated (Endoactivat -or®) 1min 3. Sterile saline	NR	TAP [1:3:3] *metronidazole minority*	1. NaOCl with Endoactivator® 2. 5 mL, 17% EDTA Endoactivator® 1min 3. Distilled water	NO	• EndoSequence® Bioceramic Putty™ ⇒ 1week • Composite	• <u>Sex/age</u>: M,11y • <u>Tooth #</u>: 35 • <u>Pulp status</u>: Pulp necrosis • <u>Etiology</u>: dens evaginatus • <u>PA status</u>: Acute apical abscess	YES	YES	YES	YES

Table 2: Data extracted from clinical studies

Authors, Year	N° of session and follow-up	Anesthesia	Disinfection/ irrigation	Instrumentation	medication + sealing/ duration	Cleaning/final irrigation	Supplement material	Coronal barrier/ obturation	Patients	Outcome
Kahler B et al. (49) **Mar 2014**	2sessions follow up at 18 months for all, 2 cases up to 3 years	1st session: local anesthesia 2nd session: No vasoconstrictor	1% NaOCl	NO	TAP *minocycline replaced by amoxicillin* 4weeks Cavit™/Fuji IX	1% NaOCl	NO	• MTA™ • GIC™	Subjects: • 16teeth, 12patients • average age≈10,4y Teeth characteristics: • 3 mandibular second premolar, dens evaginatus • 13 traumatized central incisors 84% of periapical lesions	At 18 months: • Periapical image in 9,7% of cases • Apical closure: ⇒ Incomplete in 47,2% ⇒ Complete in 19,4% • root length ⇒ 2.7% to 25.3% • dentin wall thickening ⇒ 1.9% to 72.6%
Nagata JY et al. (50) **May 2014**	2sessions mean follow-up period, 15 months, between 9 and 19 months	1st session: 2% lidocaine with vasoconstrictor 2nd session: 2% lidocaine with vasoconstrictor	1. 20mL 6% NaOCl 2. 5mL sterile 5% sodium thiosulfate, 1 min 3. 10mL sterile saline solution 4. 10mL 2% chlorhexidine 5. 5% Tween 80 and 0.07% soy lecithin	NO	TAP or $Ca(OH)_2$ and 2% CHX gel [1:1] 21days Colostol™ + composite	1. sterile saline 2. 3mL 17% EDTA 3. Sterile saline	CollaCote™	• White MTA™ • Coltosol™ • Composite	Subjects: • 23 teeth (incisors) • Age ≈ 7–17 years old • Pulp necrosis, trauma Study characteristics: • Randomly divided in 2 groups ⇒ TAP (n=12), ⇒ CHP (n=11).	• Complete PA healing in 95% of all cases • Asymptomatic in 95% of all cases • Tooth discoloration: ⇒ TAP 83,2% ⇒ CHP 27,3% • Apical closure: ⇒ TAP 66,7% ⇒ CHP 54,5% • Root lengthening: ⇒ TAP 41,7% ⇒ CHP 27,3% • Root thickening: ⇒ TAP 41,7% ⇒ CHP 45,4%
Saoud TM et al. (51) **Dec 2014**	2 sessions Follow-up at 1, 3, 6, 9, and 12 months	NR	• Initial 2.5% NaOCl • 2.5% NaOCl, copious amounts • sterile saline solution	Gentle debridation K-file	TAP 2weeks IRM™	Sterile saline	NO	• MTA™ • Composite	Subjects: • 17 patients • 70% males • Average age: 11.3 years • 20 anterior teeth • No drop-out Teeth characteristics: • Periapical lesion (85%) • Active swelling or sinus tract 50% Etiology: • 100% trauma, some coronal fracture	AT 12 MONTHS • Periapical lesion: 90% resolution • Apical closure: 55% → On average 79% reduction of apical foramen size compared to preoperative radiograph (clinical significance) • Root lenght: on average 5% increase • Root width: on average 21% root width increase COMPLICATIONS: • Canal obliteration: ⇒ 3 coronal third ⇒ 2 middle third

Table 2: Data extracted from clinical studies. (continuation)

Authors, Year	N° of session and follow-up	Anesthesia	Disinfection / irrigation	Instrumentation	medication + sealing/duration	Cleaning / final irrigation	Supplement material	Coronal barrier/ obturation	Patients	Outcome
Shivashankar VY et al. (52) **Aug 2017**	NR	NR	5.25% NaOCl	Minimal	TAP 3 weeks IRM™		NR		<u>Subjects</u>: • 60 patients (6-28 years), anterior teeth • Wide apical foramen (>1mm) thin dentinal walls <u>Study characteristics:</u> ⇒ randomly categorized • PRF as scaffolding material Group A: n=20, • Conventional revascularization Group B: n=20, • PRP as the biomaterial Group C: n=20.	• Mean PAI score (preoperative and at 12months): ⇒ A: 4,35→1,85 ⇒ B: 4,4→2,07 ⇒ C: 4,26→1,32 • Root lengtening and thickening: no significant difference ⇒ Good RT ≈25,4 % ⇒ Good RL ≈27,6 % • Apical response ⇒ Majority of type 3 response • Vitality testing ⇒ PRP and PRF ≈ 15% of (+) ⇒ Induced bleeding ≈ 13% of (+)
Lin J et al. (53) **Nov 2017**	3 sessions follow up at 3, 6, 9, and 12 months	<u>1st session</u>: 2% plain lidocaine <u>2nd session</u>: NR	1. 20mL 1.5% NaOCl 2. 0.9% saline, 3. 20mL 17% EDTA	NO	TAP *Minocycline replaced with clindamycin hydrochloride* 3weeks Caviton™	• 0,9% Sterile saline • 20mL 17% EDTA	Resorbable collagen matrice	• MTA™ ⇒ GIC™, 7days *GIC™ removed* • Composite	<u>Subjects</u>: • 118 teeth, 103 completed study • Average age: 10.5y <u>Teeth characteristics:</u> ⇒ Pulp necrosis etiology: • All central incisors (CI): trauma • All premolars (PM): dens evaginatus <u>Study characteristics:</u> • Randomly assigned to REG or Apexification with a 2:1 ratio • Ech group was subdivided regarding etiology of pulp necrosis • REG; n=69; 21CI and 48PM (11 cases lost) • Apexification n=34; 13 CI and 21 PM (4 cases lost)	After 12months: • 100% periapical healing and asymptomatic tooth • REG 89,8% success ⇒ RL in≈81,16% ⇒ RT in≈82,6% ⇒ AC in≈65,21% • Apexification 97% success ⇒ RL in≈26,47 % ⇒ RL in=0 cases ⇒ AC in≈82,35% Better RL in the dens evaginatus subgroup
					$Ca(OH)_2$ paste placed 7days Caviton™	17% EDTA	**Apexification procedure** • Vitapex™ paste injected into the canal ⇒ Temporally sealed with GIC™ • Rx confirmation of apical barrier: ⇒ Obturation with β-gutta percha, and composite resin.			

Table 3: Data extracted from research study

Authors, Year	Objective of the study	Results
St Paul A et al. (54) **Jun 2017**	Survey destined to endodontist practicing REG or apexification, asking their preferences over the treatment of immature permanent teeth with plural necrosis in children.	Apexification preferred when considering: ⇒ evidence base supporting the treatment (60%) ⇒ predictability of treatment outcome (77.8%) ⇒ patient compliance (57.3%) ⇒ number of required patient appointments (51.2%) ⇒ likelihood of tooth discoloration (53.3%) REG preferred when considering: ⇒ continued root development (89%) ⇒ apical closure (66.7%) Age and continuing education courses taken by the respondents were substantially correlated with their preferred course of therapy.

Table 4: Data extracted from systematic review and metanalysis

Authors / Year	Objective of the study	Results
Antunes LS et al. (55) **Jan 2016**	To conduct a systematic review to evaluate the quality of scientific data on this topic as well as the efficacy of pulp revascularization in the root development of necrotic immature permanent teeth.	• Various studies proposed a different protocol, applying modification in the material used to enhance the effect (PRP and PRF) and to prevent the complications coronal sealing). • Most teeth studied are incisors affected by trauma. • Undeniable positive effect of regenerative procedure for AC, RL and RT, however the key factor of revascularization remains unknown.
Xie Y et al. (56) **Sep 2021**	• To evaluate if revascularization produce superior periapical healing than apexification when treating immature necrotic teeth. • To assess chances of treatment failure with revascularization and apexification for the treatment of immature necrotic teeth. • To appraise the superiority of REG in root development when treating immature necrotic teeth as opposed to apexification.	• Both treatment plans are viable solutions for treating periapical periodontitis and closing open apices. • RET should be used cautiously, and the discoloration risk should be considered in the treatment plan for patients with high aesthetic demands. • When treating young necrotic teeth with significant root maldevelopment, RET may be the initial option since it is more effective at root elongation and thickening without posing a greater danger to the validity of the entire treatment.
Panda P et al. (57) **Jul 2022**	To assess each intervention separately and compare how is REP and apexification clinical outcome when treating young immature necrotic teeth.	• Consider REP when the root development is severely deficient, with insufficient dentine, and where the tooth's prognosis is hopeless even with an apexification procedure. • APCs significantly improve vitality and AC compared to blood clot only. The outcome of each procedure was similar. • In the apexification procedure, the apical barrier formation is similar in both MTA and $Ca(OH)_2$. • With a degree of confidence, it can be said that the apexification and regeneration processes both have identical overall effects and are equally comparable therapies.

5 DISCUSSION

5.1 Pulp revascularization protocols

5.1.1 1st appointment:

Tissue regeneration is a slow process that must be carried out in several stages. At first, the practitioner must make sure to clean and sterilize the pulp cavity, this procedure is done under local anesthesia and absolute isolation in all the studies except in Becerra P et al. (28) where they didn't use anesthetic to confirm the absence of vitality. Figure 6 (Annexes) displays the steps to follow during the first appointment.

Preliminary antibiotic treatment was done in a few studies where the patient had a periapical abscess with amoxicillin for a week (46,48). Kang KZ et al. (48) did the opening to drain the abscess through the canal prior to disinfection.

5.1.1.1 Instrumentation

The latest recommendations from the ESE and the AAE do not indicate if mechanical instrumentation is or isn't necessary (20,29). According to most studies, the PIT have thin dentinal walls that risk being weakened by the procedure, along with the removal of some of the SCs that are there (20,29,43,46–48).

Some of the authors did instrument the canal either apically to remove the bacteria and to widen the opening supposedly to enhance the tissue formation in the cavity so as did Makkar S et al. (41); either coronally as Soares ADJ et al. (40) and Kottoor J et al. (42) with Gates Glidden burs to widen the opening to facilitate the medication insertion.

Nonetheless, a recent expert consensus commented that in case of deep infection, the residual presence of bacteria in the dentinal tubules could cause the treatment to fail. They recommend using the largest files available k-files or Hedström to remove this bacterial biofilm up to 1mm from the apex (32).

5.1.1.2 *Irrigation*

The first step required in treating infected and necrotic teeth is root canal disinfection. After removing the pulp, the cavity is irrigated. Irrigation is done using a needle with a closed end and side vents by all the authors.

- Sodium hypochlorite (NaOCl)

The considerations to be taken in the choice of the agent will not be based solely on its antibacterial properties but it must be remembered the future need for SCs, NaOCl was used in concentrations ranging between 1 to 6%. NaOCl is known to have deleterious effects on populations of SCs within the duct. To avoid excessive cytotoxicity among the SCAPs population during irrigation, only a few authors followed AAE recommendation: the irrigation needle placed at 1～2mm from the apex, and the use of a lower NaOCl concentration [1.5-3%] (20mL/canal, 5 min) followed by irrigation with saline water or 17% EDTA (20 mL/canal, 5 min) which helps to recover from the deleterious effects of NaOCl (29,43,44,48,51,53). Kottoor J et al. (42) reported an application of NaOCl for a longer time, 20min. Only a 2022 study reported the use of an Endoactivator® while using the EDTA 17% to enhance its activity (48). The study of Nagata JY et al. (50) study reported the use of sodium thiosulfate as the neutralizing agent of NaOCl.

- Chlorhexidine

Other studies reported the use of 2% chlorhexidine gel as the only irrigant (40,50) or as a complement of the NaOCl (28,40,44,50). Despite that, AAE and ESE agree on the fact that chlorhexidine use is harmful to SCs and doesn't produce tissue solubilization (20,29).

- Other options

AAE recommends being accompanied by a suction system such as EndoVac™ to avoid the extrusion of liquids during irrigation (29). On top of negative pressure irrigation, other methods are available to improve sterilization: passive ultrasonic irrigation and photon-induced photoacoustic streaming that show better smear layer dissolution with less damage to the dentin due to lesser working time (32).

5.1.1.3 Intracanal medication

Thereafter a thorough drying of the conduits with paper points is required.

In all the studies, the authors used antibiotic paste or calcium hydroxide as antibacterial medication. The retention time of the drug in the root canal ranged from 2 to 4 weeks until no exudate, swelling, sinus ducts, and pain or tenderness were observed. Bukhari S et al. (45) did report an average duration of 37 days. The official recommendation on the period during which the drug is left is between 1 to 4 weeks, several clinical trials have extended the period to several months but concluded that the optimal duration would be 2-4 weeks for TAP and $Ca(OH)_2$ (20,29,58).

- Triple antibiotic paste (TAP)

Intraductal infections are unique from one patient to another in terms of bacterial strain and quantity. A large majority of the bacteria involved are anaerobes, so metronidazole is an agent of choice for this fight but must be combined with other antibiotics to make it bactericidal. However, because of the complexity of the infection, it is rare for a single type of antibiotic to meet this demand; a combination of substances significantly increases the efficacy, up to the total sterilization observed *in vitro* studies (55). The most used TAP is composed of minocycline, metronidazole, and ciprofloxacin, and has been developed and tested by Sato I et al. (59) in 1996, it is effective to kill gram (+) and gram (-) anaerobes and spirochetes. Regarding the examined studies, the use of TAP was reported in 13 articles (28,41–46,48–53). Minocycline is known to provoke discoloration of dental tissue; it is therefore possible to change the composition of the paste. It was replaced with clindamycin hydrochloride or amoxicillin in 3 studies to prevent tooth discoloration (45,49,53), another antibiotic recommended is cefaclor (29). It was removed in one study by Meschi N et al.(47)for the same reason. Another recommendation to minimize staining is to apply a dentin bonding agent to seal the dentin tubules in coronal dentin (29,42,49,55).

The mixture must be made by the practician or could be obtained from a pharmacist, and it follows a 1:1:1 ratio and then diluted in propylene glycol or sterile saline solution to make it injectable through a syringe or placed with lentulo spiral by all authors studied. The ratio was changed by Kang KZ et al. (48), where it was [1:3:3] of metronidazole, minocycline, and ciprofloxacin. The final recommended concentration doesn't exceed [1-5]mg/L, higher concentrations have been tested but in a limited number of studies and could be noxious for SCAPs (29,32). In addition to staining and cytotoxicity, other disadvantages are to be considered, notably: sensitization, development of bacterial resistance, and difficulty of removal from the root canal (20).

- Calcium hydroxide ($Ca(OH)_2$)

In the examined articles, $Ca(OH)_2$ was used in 3 studies conjointly with 2% chlorhexidine gel to increase the disinfectant potential against anaerobes; they lead to successful revascularization (40,44,50). Since in the study of Nagata JY et al. (50), chlorhexidine was less effective than TAP to favor apical closure and root lengthening further studies need to be done to confirm of refute its upgraded effect. $Ca(OH)_2$ is simply placed in the conduit making this a simpler technique. Despite a slightly lower antibacterial effect compared to TAP, it remains a material of choice, the only one recommended by the ESE because it does not lack advantages compared to TAP (20). The absence of minocycline makes it an interesting alternative if the risk of dentin discoloration is important at the aesthetic level (50). According to some studies, $Ca(OH)_2$ proves protection of the SCs, by favoring the liberation of GF from the processed dentin. In addition, its consistency makes it an easier material to remove from the canal. $Ca(OH)_2$ does not, however, strengthen the dentinal walls because its application is temporary and for antibiotic purposes (32). Nonetheless, there are some suggestions concerning the possible deleterious effect of $Ca(OH)_2$ on the SCs (40,44,50). To diminish the cytotoxicity of chlorhexidine, Nagata JY et al. (50) study suggested using 5% Tween 80 and 0.07% soy lecithin to neutralize its effect.

5.1.1.4 Temporary obturation

The tooth was temporarily filled with 3-4mm of restorative material such as to prevent bacterial filtration within the medicament (29). In the investigated articles 5 studies used Cavit™ (41–45), 4 used IRM™ (28,48,51,52), and 1 used GIC™ (47). Other materials have been reported such as Colostol™ and Caviton™ which are eugenol-free temporary cement that is known to be easier to remove (40,50,53). Some authors did a double seal using 2 materials, so as did Kahler B et al. (49) using Cavit™ and GIC™ or Nagata JY et al. (50) with Colostol™ and composite resin to obtain better sealing and avoid filtrations.

5.1.2 2nd appointment

Secondarily, the continuity of the treatment consists of the induction of the bleeding through the apex, but it can be carried out only if the pathology is solved as shown in Figure 7, Annexes. If signs and symptoms of active infection persist, intracanal medication was replaced for another period of 2-4 weeks and consider changing the product to be used or administer systemic antibiotics (20,22,29,51).

5.1.2.1 Anesthesia

The main causes of failure during this stage are based on the poor acquisition of bleeding, leading to a weak scaffold, and a lower amount of SCs and GF. The origin may be the inflammation created by the medication or allegedly because of the involvement of a vasoconstrictor during anesthesia which is not recommended. Mepivacaine 3% is therefore the benchmark product since it won't impede blot clot formation (20,28,29,32,45,48). Some authors didn't specify the type of anesthetic used but commented that it shouldn't contain epinephrine. However, Makkar S et al. (41) used 2% lignocaine hydrochloride containing 1:200,000 adrenaline.

5.1.2.2 *Cleaning of the intracanal medication*

After complete isolation, it is time to reopen the cavity and remove the rest of the medication, the same type of needle for the irrigation as during the first appointment was used by all the authors. Some authors used NaOCl as the sole irrigant or together with EDTA (28,41,42,45,46,48,49). At the same time, to avoid cytotoxic irrigants, some studies avoided NaOCl in favor of EDTA 17% (20mL/5min) which will itself be removed using a sterile saline solution or only with saline solution (20,22,29,40,44,50,51). Before continuing the canal is dried using paper points.

5.1.2.3 *Induced bleeding and obturation*

Afterward, ensues the stage of bleeding triggering, the goal is to obtain bleeding from the entire canal up to the CEJ level (20,29). A two-millimeter over-instrumentation of the apex using a manual pre-bent file or hand-spreader is done as a standard. Some authors recommended the stimulation of bleeding up to 5-3 mm below CEJ to avoid coronal placement of the MTA (22,42,44,49,50). If adequate bleeding is not achieved, the file can be soaked in EDTA solution for its anti-coagulant properties. Indeed, the presence of the blood clot is essential, as said earlier, the stem cell concentrations are higher there than in the circulating blood (58).

5.1.2.4 *Additional materials*

To facilitate the sealing procedure, some studies introduced matrices made of CollaPlug™, CollaTape™, or CollaCote™ to facilitate the placement of MTA™, and to avoid any filtration of the restorations; the membrane is placed in a sterile solution to avoid air entrapment (22,44,47,50,55).

Another study by Shivashankar VY et al. (52) looked at clots supplemented with autologous platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) to optimize revascularization. Still, some authors have observed root growth in cases where they were unable to obtain proper bleeding thanks to the use of PRP and PRF. Used combined with a blood clot or as an alternative, they make it possible to supplement the roles of matrix and GF supply of the blood clot; some materials, tested on large animals and present great regenerative potential such as angiogenic hydrogel, as in a 2021 study (34) where blood clot induction wasn't needed. Research into their effectiveness continues to lead to contradictory conclusions, however, all agree that their use can be considered on a case-by-case basis (32,37,38,52,60).

5.1.2.5 Obturation

After 15 minutes, the blood clot should be formed, and the cavity is ready for obturation (20,29). All the authors recommended MTA™ as best used as a pulp space barrier, made of a thin layer of 2-4 mm. According to AAE white MTA™ shows many advantages over other materials, notably its biocompatibility and reduced discoloration compared to common MTA™ (29,50,58). Kang KZ et al. (48) and Bukhari S et al. (45) used EndoSequence® Bioceramic Putty™ because of the reduced setting time, no discoloration, and easier handling, with a similar sealing ability. For cases where aesthetics is the priority, the satisfactory use of glass ionomer has been reported (58).

On top of that, the cavity is obturated temporarily or definitely, mostly with composite; only one study obturated with GIC (49). It could also be filled up with flowable GIC, $Ca(OH)_2$, or Coltosol™ and then covered with adhesive restoration to promote better sealing and avoid filtration (20,40–42,44,47,50).

5.2 Parameters evaluated

5.2.1 Type of teeth evaluated

Table 1 presents data extracted from the chosen case reports. The total number of teeth evaluated in the selected studies was 39, with patient numbers ranging from 1 to 20. Incisors were the most frequently evaluated teeth (n=27), followed by premolars (n=7), with only two studies examining molars (n=5) (45,47). Only one study evaluated more than five teeth, with Bukary S et al. (45) examining 28 teeth and showing that incisors have the best prognosis of 80,9% success.

Table 2 presents the extracted data from the selected clinical studies. With a number of patients ranging from 12 to 118, the studies evaluated a total of 237 teeth. Most teeth evaluated were incisors (n=98), followed by premolars (n=79). Notably, none of the studies included molars in their evaluation. Shivashankar VY et al. (52) did not report the tooth type. Nagata JY et al. and Saoud TMA et al. (50,51) only included incisors in their studies, while others evaluated both incisors and premolars.

5.2.2 Etiology

As listed in Table 1 and Table 2, the three main etiologies for pulpar necrosis are trauma, caries, and anatomical abnormality. As mentioned earlier caries is the most prevalent of the 3, however only 9 cases of caries were presented in the studies because the infection spreading makes it unfavorable for revascularization treatment in the studies.

Pulp necrosis with immature apex remains rarely detected in young patients because they remain asymptomatic. The main etiology encountered at the clinic is trauma, from which pulp necrosis is the main post-event complication (54). Trauma can produce pulpar necrosis but without a pulpar exposition it remains free of bacteria at first; all 123 teeth with trauma tested were incisors. Nonetheless, it could still induce root resorption and cause damage to the HERS as well as the SCAPs, resulting in the failure of regenerative therapy as mentioned by Lin J et al. (53).

Anatomical abnormality is represented by 30 teeth in the studies, mainly dens invaginatus and dens evaginatus. Lin J et al. (53) study showed a better prognosis for the dens evaginatus group compared to the trauma one, however, the latter study excluded the patient with the periapical lesion, which differs from the reality. Furthermore, Bukhari S et al. (45) observed more success in case of initial periapical lesions.

A metanalysis from Koç S et al. (61) demonstrated that the outcomes of the regenerative treatment show no difference between etiologies.

5.2.3 Criteria of evaluation

When evaluating the outcome of pulp revascularization treatment, several parameters are commonly measured to assess the success of the procedure.

Assessing clinical signs and symptoms is imperative through the follow-up in all the studies. Evaluate changes in the patient's clinical symptoms, such as pain, swelling, tenderness, and sensitivity, to determine if the treatment has been effective for eliminating or reducing these symptoms.

Together with clinical evaluation, radiographic observation or measurement is mandatory. Radiographs are taken before, during, and after the treatment to evaluate the changes in the root canal and surrounding bone structure. Parameters such as root length, thickness of dentinal walls, and surrounding bone density are measured to determine whether the treatment resulted in adequate root development and periapical healing. For the case report, the lack of standardization of procedures makes a comparison of data impossible. In the study from Lin J et al. (53) the periapical healing wasn't evaluated to compare the outcome of revascularization and apexification which makes it non-representative of the general population as the periapical lesion is one of the main complications after pulp necrosis (49,51,54). To evaluate root morphology a classification of 4 types was settled by Lin J et al. (53) (Annexes, Table 5). To evaluate the apical morphology some authors took the classification of Chen MYH et al. (62) as a reference (47,52) (Annexes, Table 6). It is mandatory to standardize the radiological analysis to be able to compare the results (55).

Tooth vitality: the necrosis led to a negative response to the vitality test, the hope is to restore the tooth vitality as in Kang KZ et al. (48) where the vitality was restored even with a deep MTA plug; the use of blood-derived product showed a better probability of positive response to vitality testing in the study of Shivashankar VY et al. (52) compared to induced bleeding.

Histological evaluation: Histological analysis can be performed on extracted teeth to examine the quality and quantity of new tissue formation and to assess the presence of inflammation or infection, while mostly done in animal research it's sometimes analyzed in humans for exceptional reasons as in Becerra P et al. (28). The results obtained from this study should be taken carefully, nevertheless the correspond to the animal studies, where fibrous tissue similar to periodontal ligament and cement, and hard tissue similar to the bone have been observed. While Antunes LS et al. (55) also states that revascularization is closer to a healing process than regenerative one, further studies need to be done to understand what histological mechanisms happen during revascularization.

One point that hasn't been studied by the researchers was to conduct the specific microbial analysis to determine the presence of bacterial strains in the root canal system before and after the treatment, to adapt and evaluate the effectiveness of disinfection procedures.

Collectively, a combination of these parameters is used to assess the outcome of pulp revascularization treatment and to determine procedure's success and restoring tooth vitality. Nevertheless, in most studies, success is typically defined as the tooth being asymptomatic and maintained in the mouth (45).

5.2.4 Complications

There have been reports of difficulty in tracking disease progression due to non-attendance or discontinuation of treatment. In Lin J et al. study (53), 15 cases were lost during the study with a majority from revascularization. As confirmed in Table 4, only a few studies followed their patient for more than 18 months.

During the clinical and Radiological Examinations signs and symptoms of failure were observed in only a few studies regarding periapical state; however, an important number of studies reported the non-closure of apex at the end of the follow-up; nonetheless, Kahler B et al.(49) reported that further apical closure was observed after

36 months. Additional studies should be done to observe the long-term outcome of revascularization, especially in older patients (45).

After completion of the treatment, the maintained tooth can present canal abnormality classified by Chen MYH et al. (62) (annexes, Table 6) reported as the second most frequent type of complication after discoloration.

Coronal discoloration was observed mainly due to the TAP but also from a coronally placed MTA (44,45,47).

5.3 Pulp revascularization and apexification

5.3.1 External factors that influenced the dentist

The investigation of St Paul A et al. (54) in Table 3 confirmed that several factors could influence the choice of the dentist apart from the patient: dentists are not taught as much about revascularization compared to apexification which has been thoroughly tested through decades of use. It's only recently that revascularization has been accepted as a treatment alternative. When it comes to choosing regenerative treatment, the age of the dentist also has an influence: younger practicians tend to use more regenerative techniques, especially when apical closure and further root development are needed. Despite the fact the current treatment is favoring the use of reparative over regenerative techniques for the management of open apex necrotic tooth, because of the lack of resemblance between original and regenerated tissues, it is however a field of improvement showing better outcomes (7).

5.3.2 Factors associated with the case

Pulp revascularization and apexification differ in approach and results. While the latter treatment won't restore the tooth's vitality, only one session is needed for the procedure. Apexification is considered a predictable and reliable treatment option that promotes healing of the periapical tissue and creates a barrier against future infections in immature necrotic teeth. Due to the fewer number of appointments and shorter follow-up duration, apexification is the treatment of choice for uncompliant patients or patients with systemic diseases (22,54).

5.3.3 Outcome evaluation

When evaluating the success of the two procedures, different criteria are considered. Apexification aims to promote the formation of a calcified apical barrier, while revascularization aims to restore the vitality of the pulp tissue and promote root development. A radiological follow-up is needed for both techniques, nonetheless, the need for a longer follow-up in case of regenerative technique is undeniable (43).

Comparing pulp revascularization and apexification results, studies among Table 2 and Table 4 have reported similar success rates in tooth survival and periapical healing. However, pulp revascularization has shown superior results concerning root development, dentin wall thickness, and maintenance of tooth vitality (22,56,57).

6 CONCLUSIONS

- Despite the variations in its evolution, the revascularization protocol remained similar to the original: disinfection of the root canal system, induction of bleeding to promote tissue regeneration, and creation of a scaffold for tissue growth using the induced blood clot or additional biomaterials. Variability in different protocols comes from the use of different materials for disinfection as irrigant or intracanal medicine. Revascularization is known to produce favorable outcomes in improving clinical symptoms, root lengthening, and dentinal wall thickening, although tooth discoloration and calcification may occur. Further research is necessary to improve the procedure's outcomes and the use of new techniques and materials.

- Several parameters are commonly measured to evaluate the success of pulp revascularization treatment. These include clinical signs and symptoms, tooth vitality, radiographic changes, histological evaluation, microbial analysis, and tooth survival rate. These parameters are used collectively to determine the effectiveness of the treatment in promoting pulp regeneration and restoring tooth vitality. Nonetheless, success is typically considered achieved when the tooth is asymptomatic and maintained in the mouth. It is crucial to keep in mind that the various study outcomes cannot be accurately compared due to the patient selection criteria and protocol variability, and lack of uniformity for the evaluation of the results.

- In terms of approach and results, apexification treatment and regenerative endodontic differ, with the former having been proven to promote healing of periapical tissue and only requiring one session, while the latter being able to promote continued tissue formation and requiring multiple sessions. While both treatments have similar success rates in tooth survival and periapical healing, pulp revascularization has shown superior results in terms of root development, dentin wall thickness, and recovering tooth vitality. Ultimately, factors such as patient age, tooth maturity, and operator experience and expertise may affect the technique's success, and the decision should be individualized.

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8 ANNEXES

Figure 6: Schematic diagram of regenerative endodontic procedure, 1st appointment (32).

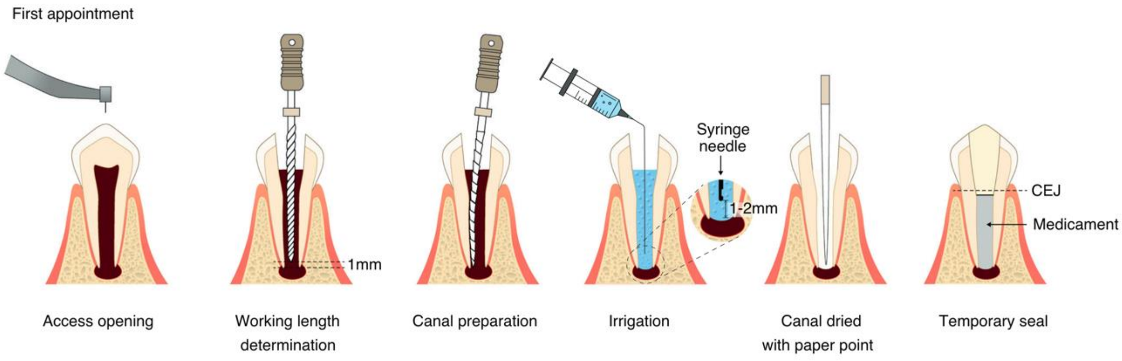

Figure 7: Schematic diagram of regenerative endodontics procedure, 2nd appointment (32).

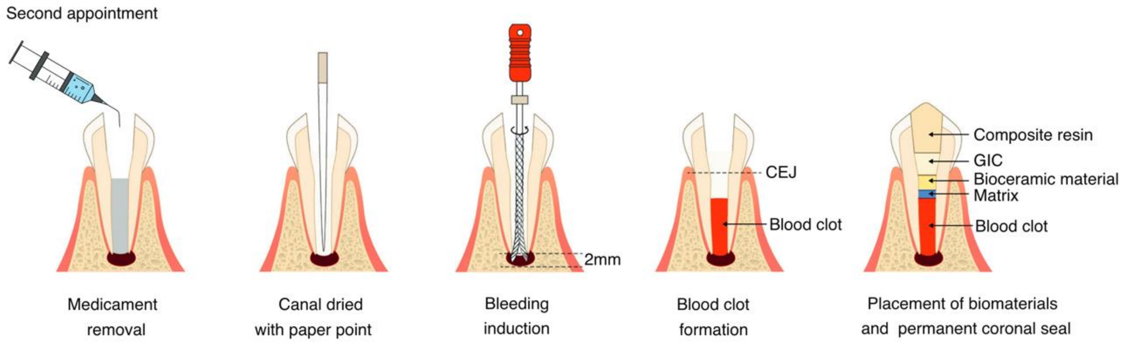

Table 5: Classification of Root Morphology by Lin et al. (22).

Type	Root lenght	Apical foramen size
type I	Increased	Decreased
type II	Increased	Unchanged
type III	Unchanged	Decreased
type IV	Unchanged	Unchanged

Table 6: Classification of apical morphology encountered after revascularization by Chen et al. (62).

1: increased thickening of the root canal walls and continued root maturation.
2: no significant continuation of root development with the root apex becoming blunt and closed.
3: continued root development with the apical foramen remaining open.
4: severe calcification (obliteration) of the root canal space.
5: hard tissue barrier formed in the canal between the coronal MTA plug and the root apex.