Blood clot in Regenerative Endodontic Therapy of Immature Permanent Teeth: a literature review

Uso de coágulo sanguíneo en terapia Endodóntica Regenerativa en Dientes Inmaduros Permanentes: una revisión de la literatura

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Abstract:

Pulp necrosis is diagnosed when the pulp is presented asymptomatic in response to pulp tests. Its conventional management in immature permanent teeth is by apexification. However, this treatment has the disadvantage of weakening the root walls, tending to root fracture, and pulp does not revitalize. To promote the regeneration of the dentin-pulp complex, as well as root growth, maturation, and apical closure, it is advisable to use regenerative endodontic treatments. This involves utilizing stem cells and growth factors to replace cells within the root tissue, with clot formation serving as a scaffold to stimulate tissue regeneration using stem cells from the apical papilla. This review aims at showing and revise the available information about using blood clots in this treatment and contribute to the possibility of further creating a protocol consensus for this treatment.

Keywords: Blood clot, Regenerative endodontics, Pulp revascularization, Immature permanent teeth, Pulp necrosis

Resumen:

La necrosis pulpar se diagnostica cuando la pulpa se presenta asintomática sin respuesta a pruebas pulpares, su manejo convencional en dientes permanentes inmaduros es la apexificación. Sin embargo, este tiene la desventaja de debilitar las paredes radiculares tendiendo a la fractura y no generan una revitalización pulpar. Es por eso que se han recomendado el uso de tratamientos endodónticos regenerativos los cuales tienen como objetivo regenerar el complejo dentinopulpar, así como el crecimiento radicular, maduración y cierre apical a través del reemplazo celular dentro del tejido radicular utilizando células madre y factores de crecimiento. La formación del coágulo se usa como un andamio para que las células madre que van desde la papila apical estimulen la regeneración tisular. El objetivo de esta revisión es mostrar y revisar la información disponible respecto al uso del coágulo en este tratamiento y para contribuir a la posibilidad de la creación de un futuro consenso de protocolo de este tratamiento.

Palabras Clave: Coágulo Sanguíneo, Terapia endodóntica regenerativa, revascularización, dientes permanentes inmaduros, necrosis pulpar

INTRODUCTION

Pulp necrosis is defined as the death of the dental pulp, according to the American Association of Endodontists. It is diagnosed when the pulp is asymptomatic and doesn’t respond to pulp tests. The cause of this pathology may be due to dental trauma or cavities.1

The conventional management of this pathology in immature permanent teeth is apexification. This process involves the formation of a calcified barrier in the open apex of the immature tooth through the periodic placement of calcium hydroxide or a single application of MTA inside the root canal. This stimulates calcification and apical closure.2,3

To determine permanent tooth immaturity, it is important to understand the stage of development the tooth is in. Cvek proposed a classification system of 5 stages of development of the tooth where stage I is less than half of the root formation with open apex, stage II is half of the root formation with open apex, Stage III is 2/3 of the root formation with an open apex, on the stage IV the root is almost complete, but has an open apex and stage V is the complete root with a closed apex (Figure 1).4

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Conventional treatments for pulp necrosis in immature teeth, such as apexification, have the disadvantage of weakening the walls of the root canal, tending to fracture, and compromising root development. Therefore, pulp vitality is not achieved. That is why it is recommended to use regenerative treatments as an alternative treatment for pulp necrosis in immature permanent teeth. Table 1 briefly describes the advantages and disadvantages of apexification and blood clot in regenerative endodontics.

Endodontic regenerative treatments are based on the 1966 research by Nygaard-Ostby, who evaluated the effects of provoked bleeding during over-instrumentation of a root. Ever since, this procedure has gone through modifications for its current success and aims to regenerate the dentine-pulp complex by obtaining immune and nociceptive competence in addition to root growth, maturation, and apical closure through cell replacement within injured root tissue using stem cells and growth factors.

### Table 1. Advantages and disadvantages of apexification and blood clot in regenerative procedure

<table>
<thead>
<tr>
<th>Procedure type</th>
<th>Advantages</th>
<th>Disadvantages</th>
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</table>
| Apexification  | ● Biocompatibility of MTA and high success rate  
● Low cost when calcium hydroxide is used  
● Gold standard treatment for immature teeth with pulp necrosis | ● Demands several appointments  
● Shall only be performed on Cvek’s stage 4  
● Associated with root fracture during the treatment  
● Time-consuming  
● Calcium hydroxide increases dentin fragility when placed for long periods  
● Does not promote apical healing  
● MTA often spreads beyond the apex  
● Does not produce revascularization or root maturation |
| Blood clot in Regenerative endodontics | ● Promotes apical healing  
● Increases root length and thickness  
● Does not need further endodontic treatment when asymptomatic  
● Can be performed from Cvek’s stage 1 to 4 | ● No reported consensus on the protocol  
● Relatively new procedure  
● Hard to obtain the necessary amount of blood in the root canal  
● Expensive  
● Requires clinical expertise  
● Requires TPA, and it produces stains on teeth |

Since the 90s and until the late 2000’s, an emergence of a regenerative approach for endodontic treatment in several publications occurred. In 2001, revascularization was introduced to manage immature permanent tooth with apical periodontitis and sinus tract by Iwaya et al. The term adopted by the American Association of Endodontists (AAE) is “regenerative endodontic procedures” in 2007 for these procedures, while the European Society of Endodontics (ESE) referred to these treatments as “revitalization” in 2016. Procedures vary depending on the irrigant that is used, the scaffold or the clot protector that is used during the procedure.  

### Biological bases of regenerative endodontic therapy

Endodontic regeneration and tissue engineering is based on three main elements for its implementation:

1. Stem cells that differentiate into primary odontoblasts-like, including dental pulp stem cells, stem cells of the apical papilla, periodontal ligament stem cells, inflammatory periapical progenitor cells and bone marrow stem cells.
2. Growth factors and bioactive molecules that promote cell migration. The dentin matrix is considered a reservoir of growth factors and play a key role in cell recruitment, proliferation, differentiation, and promoting tissue regeneration.
3. Scaffolds provide an adequate environment for cellular development and a way to spread the medication to be applied.

A scaffold is an essential element for tissue engineering to guide stem cells and regulate proliferation and metabolism, they also regulate stem cell differentiation by the release of growth factors.
INDICATIONS
To perform this treatment, there are some indications to follow 7,9,10:
1) Necrotic permanent immature teeth with open apex (incomplete formation of the root apex) regardless of whether there are perirapical lesions or not
2) Teeth that don’t need a post for their final restoration
3) Patients and parents compliant with treatment
4) Patients not allergic to the medications used in the treatment
5) ASA I and II patients

CONTRAINDICATIONS
Criteria considered against regenerative endodontics7,9,10:
1) Deciduous teeth
2) Recently reimplanted teeth after an avulsion, since revascularization can occur spontaneously
3) Teeth that cannot be subjected to isolation with a rubber dam
4) Teeth with extensive coronary destruction with the need for a post-restoration
5) Teeth with periodontal lesions
6) Patients allergic to any medication or irrigant used in the protocol
7) Patients ASA III or higher, since it is difficult to control infections in the root canals on immunologically compromised patients

This treatment can be viable on teeth with an apex diameter of 0.24 mm or more. It can be applied in the first three stages of root development. This treatment can be used at stage 4 of root development, and apexification can be considered. This demonstrates another advantage against apexification since the regenerative treatment is viable from the first stage of root development. Endodontic regeneration can be performed at any age, between 8-18 years old.7,13

PROTOCOL
The most commonly used protocol in endodontic regeneration is the creation of a blood clot, which consists of stimulating bleeding from the apex to form a natural scaffold derived from blood. The scaffold contains and attracts undifferentiated mesenchymal stem cells from the apical papilla.14 The American Association of Endodontists and the European Society of Endodontics each have clinical considerations for endodontic regenerative procedures. However, there is no clinical practice guide or consensus-based guidelines or criteria for these procedures.9,10 A review investigated the effectiveness of 12 protocols performed on human teeth based on removing the necrotic pulp through minimal or no mechanical instrumentation. They did not find a consensus regarding irrigating material or intracanal medication.5 The clinician must have legal written authorization from the minor's parents or guardians.9 Parents or guardians must be informed, verbally and in writing, about the current condition of the tooth to be treated and the procedure to be performed. The informed consent must contain specific and general information about the existing pathology; the procedure to be performed and its advantages and disadvantages; treatment alternatives such as apexification, extraction or no treatment, along with their advantages and disadvantages; duration of the procedure and follow-up appointments; usage of materials and medications; adverse effects such as discoloration of the root or crown, lack of response to treatment, pain or infection; and costs.7 Before starting treatment, it is necessary to perform a sensitivity test, besides an oral examination and x-rays to evaluate the tooth condition.9 The main points of the general protocol include minimal or no instrumentation of the dentinal walls, disinfection with ideal irrigants, application of an intracanal medication, provocation of bleeding and clot formation, placement of a matrix with biocompatible materials, and an effective coronal seal.10 The American Association of Endodontists and the European Society of Endodontics agree on managing this treatment in 2 appointments. The first appointment consists of the removal of the necrotic tissue in the pulp chamber, followed by the disinfection by irrigating the root canal with NaOCl, saline water, and EDTA and the further collocation of either calcium hydroxide or triple antibiotic paste (TPA) in the root canal and sealing with a temporary restorative material.9,10 The second appointment should be performed 1 to 4 weeks after the first appointment. It consists of the removal of the temporary seal, followed by irrigation with EDTA and the further induction of bleeding using a file or an endodontic explorer by over-instrumenting the apical foramen and rotating the instrument until filling the canal with blood below the cement-enamel junction and waiting for 15 minutes for the formation of the clot After that, both the AAE and the ESE recommend the placement of a resorbable matrix to increase the chances of root development. The use of a matrix will be discussed later in this article. The last step is the placement of MTA over the matrix and the coronal sealing with an adhesive material such as glass ionomer or resin.9,10 The protocol steps are detailed in Table 2 and presented in Figure 2 and Figure 3.

BLOOD CLOT
The clot formation aims at using it as a scaffold for promoting growth factors and stem cells that travel from the apical papilla towards the root canal and stimulate tissue regeneration. Although different materials can be used as a scaffold (such as platelet-rich fibrin, platelet-rich plasma or autologous fibrin matrix), the blood clot is the most used scaffold.7,15 An ideal scaffold must allow cell attachment and localization, provide growth factors, and be biodegradable along with the regulation of cell differentiation, metabolism, and proliferation.15 The use of blood clots has been challenged due to different issues, such as the poor ability to cause adequate bleeding to cover the necessary volume in the root or that apical irritation by causing the clot to affect the apical tissue. In addition, the amount of growth factors contained in the clot compared to other materials is argued.16
Table 2. Proposed synthesis of the Regenerative Endodontic Protocol using blood Clot as a scaffold based on 2 different guidelines.9,10

<table>
<thead>
<tr>
<th>First appointment</th>
<th>Second appointment</th>
</tr>
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<tbody>
<tr>
<td>1) Local anesthesia and absolute isolation with a rubber dam</td>
<td>1) 1-4 weeks after the first appointment</td>
</tr>
<tr>
<td>2) Local disinfection with 2% chlorhexidine</td>
<td>2) Repeat the first appointment procedure if there are signs of persistent infection symptoms. The use of systemic antibiotics is considered when there are signs such as fever or dysphagia</td>
</tr>
<tr>
<td>3) Dental Access preparation and removal of necrotic tissue in the pulp chamber, determination of working length</td>
<td>3) Local anesthesia without vasoconstrictor, absolute isolation with a rubber dam, and removal of temporary restoration</td>
</tr>
<tr>
<td>4) Avoid mechanical root instrumentation or perform minimal instrumentation with the Hedström file without dentin removal</td>
<td>4) Irrigation with 17% EDTA 20 ml/root canal for 5 min with lateral exit needle above the vital tissue followed by irrigation with saline solution and drying with paper tips</td>
</tr>
<tr>
<td>5) Disinfection by irrigation with 20 ml/root canal of NaOCl (1.5-3%) for 5 min with lateral exit needle above 1-2 mm from the apex</td>
<td>5) Induction of bleeding with a 40 Hedström file or an endodontic explorer by over-instrumenting after 2 mm of the apical foramen and rotating with the previously curved file until the canal is filled with blood, below the CEJ</td>
</tr>
<tr>
<td>6) Irrigation with saline water 20 ml/root canal for 5 min with lateral exit needle above 1-2 mm from the apex. Dry with paper tips</td>
<td>6) Wait 15 min for the Clot formation</td>
</tr>
<tr>
<td>7) Irrigation with 17% EDTA 20 ml/root canal for 5 min with lateral exit needle above 1-2 mm from the apex. Dry with paper tips</td>
<td>7) Place a resorbable matrix such as CollaPlug™, Collacote™, or CollaTape™ over the Clot with a diameter larger than the coronal portion of the root canal or place TPA under CEJ. Both are placed using a syringe</td>
</tr>
<tr>
<td>8) Place calcium hydroxide homogeneously in the root canal or place TPA under CEJ. Both are placed using a syringe</td>
<td>8) Placement of MTA on top of the matrix 2 mm below the CEJ and apply a 3-4 mm layer of photocurable glass ionomer over the MTA</td>
</tr>
<tr>
<td>9) Seal with a temporary restorative material such as Cavit™, IRM™ or glass ionomer. Restoration should be 3-4 mm wide</td>
<td>9) Refresh the walls in the coronal cavity with a diamond bur and place a permanent adhesive restoration</td>
</tr>
</tbody>
</table>

One study compared the use of platelet-rich fibrin, the use of platelet-rich plasma, and the use of blood clot in teeth with pulp necrosis and open apex and found no significant difference between the 3 groups regarding the periapical outcome at 3 months after treatment, but at 6 and 12 months it was concluded that the group treated with platelet-rich plasma had a significantly better apical result. However, they concluded that because treatment with platelet-rich plasma requires the extraction of 15 ml of blood from the patient and requires biochemical processing, it is better to use blood clots as it saves time and resources.17

A systematic review concluded there is no superiority against using blood clots compared to platelet-rich fibrin or platelet-rich plasma both radiographically and clinically. Furthermore, it suggests that clot formation is an ideal scaffold for growth factors and stem cells derived from the apical papilla. It concluded that using blood clots offers similar results to platelet concentrates.14

A meta-analysis reported no significant difference in the resolution of periapical lesions using plasma concentrates compared to blood clots usage. Likewise, there was no significant difference concerning apical closure, and any scaffolds compared offered similar results.18

Another meta-analysis compared using platelet concentrates with using blood clots. It compared the thickness of the dentinal wall and root elongation between these scaffolds, finding no significant difference between them. In addition, it did not find relevant differences in treatment success between these two scaffolds.19

An in vitro study compared the pH and Calcium ion release from MTA on interaction with platelet-rich fibrin (PRF) and blood clot since an alkaline environment promotes osteogenic differentiation and bone formation and calcium ions act on osteoblasts and cementoblasts cells, the study found that 14 days into the trial, both blood clots and PRF recorded similar PH values, and calcium ions values were not significantly different between blood clots and PRF but with a significant difference
from the single use of MTA (control group) concluding that PRF and Blood clots influence pH, and Calcium ion release from MTA.\textsuperscript{20}

A randomized controlled clinical study evaluated the effect of a collagen membrane in promoting root development in immature teeth after regenerative endodontics. It consisted of two groups with regenerative therapy with a blood clot as a scaffold, one with a collagen membrane before placing MTA and one without it. The conclusion established it could promote dentin wall thickness increase in the root since it improved the deposition of new mineralized tissue in the root wall.\textsuperscript{21}

Figure 2. First appointment protocol\textsuperscript{9,10}

Figure 3. Second appointment protocol\textsuperscript{9,10}
IRRIGATORS
Regenerative endodontics depend on stem cells, a scaffold, and disinfection. The clinician must provide maximum disinfection without damaging stem cells. For an irrigator to be ideal, it must have an antibacterial effect, low cytotoxicity, and the ability to allow the release of growth factors. A single chemical solution is not sufficient to achieve proper disinfection. The most commonly used irrigant is sodium hypochlorite (NaOCl). The American Association of Endodontists and the European Society of Endodontics recommend using this irrigant in low concentrations (1.5-3%).
They also recommend using 17% EDTA or saline water after irrigation with sodium hypochlorite to minimize cytotoxicity towards stem cells in the apical tissues, since NaOCl affects their survival and differentiation capacity due to the toxicity of its molecule and irritating properties. And for EDTA’s capacity to dissolve inorganic components of the smear layer against NaOCl and its capacity to stimulate stem cell attachment.
Another widely used irrigant is chlorhexidine digluconate. Either alone or combined with NaOCl because it has lower cytotoxic effect. However, neither the AAE nor ESE mentions this irrigant in their protocols. Table 3 provides a comparison of three of the most commonly used irrigants.

Table 3. Most commonly used irrigants in regenerative endodontics.

<table>
<thead>
<tr>
<th>Irrigant</th>
<th>Properties</th>
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<tbody>
<tr>
<td>NaOCl</td>
<td>Most used antiseptic irrigant.</td>
</tr>
<tr>
<td></td>
<td>Effective against biofilm.</td>
</tr>
<tr>
<td></td>
<td>Less effective in immature teeth.</td>
</tr>
<tr>
<td></td>
<td>Cytotoxic effect on stem cells</td>
</tr>
<tr>
<td>EDTA</td>
<td>Capacity to dissolve inorganic components of smear layer against NaOCl.</td>
</tr>
<tr>
<td></td>
<td>Decalcifies dentin. Chelating agent.</td>
</tr>
<tr>
<td></td>
<td>High antibacterial effectivity.</td>
</tr>
<tr>
<td></td>
<td>Concentrations above 2% affect the viability and attachment of stem cells.</td>
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</tr>
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</table>

INTRACANAL MEDICATION
An ideal intracanal medication should not be irritating, should not stain the tooth, should be highly effective and biocompatible, and should have good results over an extended period, in addition to being an excellent repairer of injured peri-radicular tissue.

During the initial appointment, intracanal medication is administered as part of regenerative therapy. Both the American Association of Endodontists and the European Society of Endodontics recommend the use of calcium hydroxide and the use of triple antibiotic paste TAP (1:1:1 ciprofloxacin: metronidazole: minocycline).

TAP provides effective action against microorganisms due to the action of 3 different antibiotics and reduces the probability of developing bacterial resistance. The disadvantage of this paste is dental discoloration or staining, so it is necessary to consider this effect. The American Association of Endodontists recommends sealing the pulp chamber with a dentinal bonding agent to minimize the risk of dental staining and placing the paste under the CEJ.

Calcium hydroxide is recommended because it does not produce dental staining, has low toxicity towards stem cells and allows their proliferation. It also has high alkalinity, tissue dissolution capacity, neutralizes endotoxins and has antibacterial properties. This acts as a physical barrier that limits microorganism proliferation.

FOLLOW-UP
Treatment monitoring should occur at 6, 12, 18, and 24 months and then annually for five years. It should include radiological and clinical examinations evaluating the presence of pain, inflammation, resolution, and apical radiolucency, widening of the root walls, increase in root length, and response to vitality tests. The European Society of Endodontics recommends avoiding orthodontic treatment on teeth treated with revitalization.

SUCCESS CRITERIA
The European Society of Endodontics considers regenerative treatment to be successful when the following criteria are met:

1. No pain
2. No signs or symptoms of inflammation
3. Resolution of the pre-existing periapical bone lesion
4. Increase in root thickness and length
5. Positive response to vitality tests
6. Patient acceptance
7. Minimal dental stains
8. Radiographic detection of new pulp and dentin-like material within the root walls

On the other hand, the American Association of Endodontists categorizes treatment success into three aims to achieve:

1. Primary aim: elimination of symptoms with evidence of bone resolution
2. Secondary aim: increase in root width and/or increase in root length (this objective is desirable but not essential)
3. Tertiary aim: to observe a positive response to vitality tests (indicating more organized vital pulp tissue)

There is no significant number of systematic reviews regarding endodontic regeneration because endodontic regenerative treatment is a relatively recent therapy. A meta-analysis reported that the success rate of resolution of periapical pathology using regenerative treatment was...
equivalent to apexification or the use of MTA. However, comparing the achievement of apical closure, increase in root length and the formation of dentin, endodontic regeneration was more successful than other treatments. Another meta-analysis concluded that regenerative treatment success is high and that clinicians should be familiar with regenerative procedures as management in immature necrotic teeth. A meta-analysis of randomized controlled trials reported 95.6% success in immature teeth and concluded that regenerative endodontic treatment has high success rates and promotes root development; however, it stated that there is more evidence in mature teeth than in immature teeth. A systematic review reported that the most important variable in root development in endodontic regeneration is root canal disinfection and warned about the possibility of developing intracanal calcifications.

A systematic review of successful cases reported that 86% of the cases studied used blood clots as a scaffold. It also indicated the time of initiation of treatment and that success identification was between 2 months to 8 years, detecting 39% after 2.5 years from the start of treatment. It must be noted that 96% of cases reported the resolution and absence of periapical lesions as treatment success. According to Cvek’s classification, 45% of cases reported root development achieving a Cvek stage V (complete apical closure).

A systematic analysis of failed cases reported that 91% of the cases studied used blood clots as a scaffold. It also stated that the time of treatment initiation and identification of failed cases ranged from 3 weeks to 8 years. The majority identified more than one year after follow-up. Furthermore, 79% of the cases studied presented at least one sign of persistent infection. The reported causes of failure were fracture, dental staining, and coronal leakage.

Clinicians should rely on radiographic signs of healing that can include development of the dentin pulp complex, absence of clinical symptoms, increased dentinal wall thickness, continued root development, apical bone regeneration, regression of apical lesion, conical root apex, obliteration of the apical root canal. A retrospective study estimated quantitative differences in the development of root length and dentinal wall thickness of radiographs from 54 case-series of immature permanent teeth that were performed endodontic regeneration procedures with an image transformation and analysis program. It divided the cases into three groups treated either with TPA, Calcium hydroxide, or formocresol and found radiographic evidence of continued root development in all the groups studied. The TPA group also showed an increase in dentin wall thickness.

**COMPLICATIONS**

Common complications in regenerative endodontics can induce several symptoms. Endodontists should pay attention to these issues. Some complications may include:

1. Pain during or after the treatment due to mechanical stimulation on periapical tissue or residual infection within the canal root. It is essential to work with accurate root canal length, isolate with a rubber dam, disinfect the canal, and irrigate with proper irrigants.

2. Tooth discoloration may occur due to TAP medication, which contains minocycline and can cause dental stains. It is suggested to use a low concentration of TAP and keep it below CEJ, or to use a DAP (not containing minocycline) or instead, use calcium hydroxide.

3. Intra-canal calcification. It is uncertain the etiology of why this complication occurs. It is believed to be related to the ectopic bone formation and cementogenesis inside the root canals.

**HISTOLOGICAL OUTCOMES**

Since creating new tissue as functional as dentin and pulp is one of the main goals of this treatment, histological analyses should be performed. The number of reports on human teeth that have been histologically analyzed is limited compared to the ones performed on animals.

A systematic review revised 13 studies conducted in different animals including dogs, ferrets, and sheep. The protocol in every study used sodium hypochlorite, either with EDTA or without it, and some used a scaffold as blood clot and platelet-rich plasma; some didn’t use scaffolds at all. They found that every study lacked pulp formation, but the new tissues were cementum-like, some resembled periodontal ligaments, and some were bone-like. There was also the presence of dentin-like structures in the studies that used a scaffold. They reported that 80% of the studies that used blood clots presented the formation of intracanal hard tissue.

In a study involving 2 case reports in humans, blood clots were used as a scaffold. After four months the immature treated teeth were extracted and examined histologically. The study found a well-developed primary dentin layer surrounded by a periodontal ligament on the outer root margins, as well as a fibroblast collagenous soft tissue supporting hard tissue consistent with osteocementum. Epithelial rests were also found within the cellular fibrotic pulp chamber. The study concluded that the periodontal tissues were present and had grown into the root canals.

**CONCLUSIONS**

Regenerative endodontic procedures are biologically based treatments for immature permanent teeth diagnosed with pulp necrosis, whose main objective is to regenerate the pulp-dentin complex, prolong the time of the tooth in the mouth, and restore its normal function. Their success will depend on various factors, including clinical management, patient cooperation, adherence to an established protocol, etc. There are main points already mentioned above that indicate cases of success and/or failure. However, they are procedures that allow clinicians to have a resolution, which is why they continue to be the treatment of choice in necrotic teeth with immature apex.
REFERENCES


