

PULP REVASCULARIZATION USING PRF AND CERVICAL SEALING WITH BIO-CERAMIC CEMENT IN A TOOTH WITH INCOMPLETE RHIZOGENESIS AND PULP IN THE NECROSIS PROCESS

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ABSTRACT

Therapy for necrotic teeth with incomplete rhizogenesis has long been performed through the apoxy technique with constant changes of intracanal calcium hydroxide-based medication, in order to form a mineralized apical barrier. Another option is the MTA plug, which aims to reduce the number of sessions of apicification, through the making of an MTA plug in the apical region and subsequent filling. However, in both techniques, the roots remain porous, fragile and susceptible to fractures. The third option for cases of immature teeth in a situation of pulp necrosis is pulp revascularization, a technique that advocates the regeneration of biological tissues inside the canals, without necessarily replicating the pulp dentin complex, with the purpose of continuing physiological apicogenesis, thus providing root development with thickening of the root walls and apical closure. The objective of this study was to report the clinical case of a tooth with incomplete rhizogenesis and in the process of necrosis, using the pulp revascularization technique with platelet-rich fibrin (PRF) and bioceramic cement as cervical sealing. Conclusion: It is concluded that the treatment of pulp revascularization with PRF and cervical sealing with bioceramic, in this case, was clinically and radiographically successful 10 months after the first intervention.

Keywords: Pulp revascularization. Platelet-rich fibrin. Bioceramic cements.

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INTRODUCTION

The endodontic treatment of teeth with incomplete rhizogenesis represents a great challenge for endodontists, as extensive caries or trauma can cause the interruption of apicogenesis, a physiological process in which the pulp has the function of producing dentin for root and apical development¹.

Thus, mechanical cleaning with instruments that remove the dentin becomes pernicious, as it further weakens the already thin root walls², increasing the risk of fractures, in addition to making it difficult to determine the working length and the hermetic apical sealing, since they have a thin dentin structure, very wide canals, and divergent walls for apical³.

Elements whose apices histologically do not have cementum-covered dentin are considered to be considered in this condition, and radiographically, when the apex has not reached Nolla stage 10. In these cases, when endodontic intervention is necessary, there are two possibilities: Teeth with pulp vitality and teeth in the process of necrosis⁴.

In vital teeth, when accidental pulp exposure occurs, direct pulp capping can be performed with calcium hydroxide, aggregate trioxide material (MTA), biodentine, or bioceramic cements, due to the biocompatible and repairing characteristics of these materials⁵. However, when these elements are contaminated by caries, it is necessary to perform a more invasive decontamination intervention, which may not be as successful according to the state of inflammation of the pulp⁶.

Teeth with pulp vitality are considered to be those that present characteristics such as firm consistency, bright red color, and the existence of active bleeding⁷. In these cases, pulpotomy is indicated, a procedure that recommends the removal of the inflamed coronary portion and the preservation of the root portion to continue physiological apicogenesis. After performing the pulpotomy, a repairing lining material, as one of the options mentioned above, is deposited in the coronary chamber to seal the mouth of the canals and build a repair-inducing base⁸.

When the pulp tissue does not show characteristics of vitality, it is considered that a transition or necrosis process is already installed. In these cases of teeth with pulp necrosis and immature apex, the traditional treatment proposed for a long time and still used today is apicification. A technique that consists of periodic intracanal medication (MIC) changes with calcium hydroxide.

This procedure can last from 9 to 24 months, depending on the clinical evolution, which becomes a disadvantage due to the possibility of recontamination, increased cost and patient dropout. In addition, the hygroscopic and proteolytic action of calcium



hydroxide increases the susceptibility to fractures of the root walls and the apical barrier formed is porous and fragile^{9,10}.

Another alternative option to apexification is the MTA buffer, which aims to promote a barrier of 2 to 4 mm in the apical region, in order to avoid extravasation of the filling cement and/or gutta percha and thus reduce the treatment time, since it is performed in one or two sessions.

However, with the performance of this apical plug, there is also no continuity of root development and the roots remain thin, fragile and susceptible to fracture¹¹.

A third treatment option for necrotic immature teeth is pulp revascularization, a conservative biologically-based procedure that aims to stimulate the closure of the apical region and thicken the dentin walls of the root canal¹².

The procedure consists of chemical disinfection of the canal through the use of irrigating solutions (sodium hypochlorite and EDTA or chlorhexidine, usually) and intracanal medication (MIC), which can be a calcium hydroxide paste or a combination of antibiotics manipulated at the time of treatment. This paste is composed of ciprofloxacin, metronidazole and minocycline, compounded with glycerin and is known as "triantibiotic paste"¹³. Then, originally, bleeding is induced, promoting an over-instrumentation and the formation of a clot, rich in stem cells from the apical papilla, associated with the growth factors present, probably in the dentin and platelets, which will initiate the formation of a new tissue inside the root canal, which will provide stimuli for the end of root formation and closure of the apex¹⁴.

In addition to the conventional protocol of pulp revascularization, there are variations in the technique that have been developed to improve the predictability of the results, especially with regard to the histological characteristics of the tissues formed in the pulp space after the procedure. Such as the non-induction of bleeding for clot formation, light instrumentation in the cervical and middle thirds¹⁵, the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF)¹² and the performance of the revascularization technique in a tooth with a closed apex, inducing bleeding with foraminal enlargement^{16,17}. Therefore, there is still no definition of a standardized technique¹⁸.

PRP and PRF promote the onset of vascularization, improved healing of soft and hard tissues, have the ability to induce cell differentiation, and provide the continuation of root development. In addition, they are capable of developing a three-dimensional fibrin matrix that has a scaffold effect, i.e., they function as a framework for tissue development¹⁹.

For revascularization to be successful, it is also important to have a well-adapted coronary seal, thus avoiding reinfection. MTA is usually used to seal the entrance of the

channels, followed by glass ionomer and composite resin, as restorative materials¹⁴. Other options for the use of MTA are bioceramic cements, such as MTA Plus, NeoMTA Plus, Biodentine and *Endosequence*, which have demonstrated promising repairing capabilities superior to MTA for cervical sealing, as they promise to optimize the characteristics of biocompatibility, antibacterial activity, repairing action, radiopacity, good sealing and physicochemical stability¹³.

Thus, the objective of this study was to describe the treatment of pulp revascularization in a tooth with incomplete rhizogenesis and pulp in the process of necrosis, using platelet-rich fibrin and bioceramic cement as cervical-coronary sealing material.

CLINICAL CASE REPORT

The 11-year-old female patient A. V. C. B. was referred by the public health service of Teixeira de Freitas (ESB), accompanied by her guardian (mother), to the Endodontics specialization clinic of the Bahia Institute of Dental Research (IBPO) for evaluation and therapeutic conduct in element 46. The patient already had a digital periapical radiographic examination.

Clinically, the tooth in question presented an extensive temporary restoration with intermediate restorative material (IRM® - DENTSPLY Indústria e Comércio Ltda. Petrópolis, RJ, Brazil) with the presence of non-spontaneous painful symptoms. Radiographically, the image suggested the presence of extensive caries already involving the pulp chamber region, wide root canals, and incomplete apical region, with divergent walls (Figure 1), suggestive of Nolla stage 9.

Figure 1: Initial x-ray of the patient



Thermal sensitivity tests were performed with a rubber cup (MICRODONT-Indústria e comércio AS, Brazil) in the heat test and Endo ice (MAQUIRA Indústria de Produtos odontológicos LTDA, Maringá-PR, Brazil) in the cold test. The percussion test was



performed using the operator's right index finger. The tooth did not respond to either the heat or the cold

There was no horizontal response to the percussion test and there was a slight response to vertical percussion.

From then on, the treatment options for element 46 were clarified to the person in charge, among which apicification, MTA apical packing and pulp revascularization were considered.

Among the options, it was explained that the apexification would require several sessions to change the calcium hydroxide and that there was also a risk of tooth fracture due to the presence of thin dentin walls.

It was explained to the person in charge that in the MTA apical plug technique, the number of sessions would be reduced, however, the risks of imminent fracture would continue due to the paralysis of root development.

The mother chose as an alternative the pulp revascularization therapy with the use of PRF (Platelet Rich Fibrin) after being instructed that there would be a smaller number of sessions and advantages of trying to continue the physiological apicogenesis, so that there would be less risk of fracture of the walls with the possibility of developing these roots.

After signing the free and informed term, the procedure began.

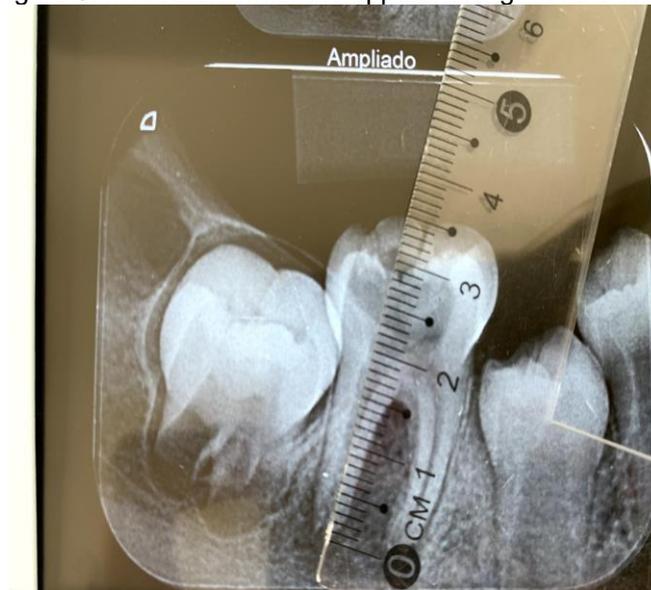
In the first session, local anesthesia was performed using the indirect pterygomandibular technique with 2% lidocaine with epinephrine 1:100,000 (Alphacaine®, Nova DFL, Rio de Janeiro, RJ, Brazil). The tooth was then isolated with a rubber dam (Madeitex, São Paulo, SP, Brazil) and a 200 clamp (Stainless Steel, São Paulo, SP, Brazil), complemented with a top dan barrier (FGM, Joinville, SC, Brazil).

Cariouss tissue was removed with a dentin spoon and spherical drills 6 (Maillefer, São Paulo, SP, Brazil) at low rotation.

The access to the chamber was made with a 1014 drill bit at high rotation (Maillefer, São Paulo, SP, Brazil) and compensatory wear with an Endo Z drill (Angelus, Londrina, PR, Brazil) with high-speed pens and abundant irrigation with water.

The measurement of the tooth and root canals was made with an X-ray and a transparent school ruler (Figure 3), due to the impossibility of using the foraminal locator, due to the open apices and, therefore, lack of impedance.

Figure 3: Measurement of the apparent length of the tooth



An inspection with a Kerr #15 and then #20 file (TDK, Curitiba, PR, Brazil) was performed in the mesiobuccal, mesiolingual, and distal canals.

Irrigation was then performed with 10 ml of 2.5% sodium hypochlorite, 3 ml in each canal and 1 ml in the coronary chamber (ASFER – Industria Química LTDA, São Caetano do Sul, São Paulo, SP, Brazil), with a disposable syringe (Saldanha Rodrigues LTDA, Manaus, AM, Brazil) in three twenty-second cycles, with a navi tip needle (Indaiatuba, SP, Brazil).

Irrigation was potentiated with *Easy clean* (Easy, MG, Brazil), (renewing 1ml of hypochlorite at each potentiation). Light instrumentation was performed with an X1 reciprocating instrument (MKLIFE, Porto Alegre, RS, Brazil) in the canals, without touching the walls, in the middle and cervical thirds, due to the presence of large contamination of carious origin.

Again, 10 ml of hypochlorite was irrigated with 3 ml in each canal and 1 ml in the coronary chamber.

Then, 3ml of 17% EDTA (ASFER – Indústria Química LTDA, São Caetano do Sul, SP, Brazil) was irrigated in each channel with easy clean potentiation. Irrigation was then repeated with 3ml of 2.5% sodium hypochlorite (ASFER – Industria Química LTDA, São Caetano do Sul, São Paulo, SP, Brazil) in each channel following the same protocol.

At no time was blood, pulp debris or exudate secreted. They were then dried with a Large absorbent paper tip (MKLIFE, Porto Alegre, RS, Brazil) for the insertion of calcium hydroxide paste (MAQUIRA, Maringá, PR, Brazil) and lubricant (K MED, São Paulo, SP, Brazil) manipulated on a glass plate in a one-to-one ratio, which was used as intracanal medication (MIC).



A Lentulo drill (MKLIFE, Porto Alegre, RS, Brazil) was used at the apparent working length for the introduction of this medication, which remained for 30 days. A sterile cotton ball (Cremer – São Sebastião do Paraíso, MG, Brazil) with a drop of camphor paramonoclophenol (Bio dinâmica, Ibiporã, PR, Brazil) was placed at the entrances of the root canals and the coronary portion was sealed with glass ionomer cement (CIV) (FGM, Joinville, SC, Brazil).

Then, after drying the IVD, occlusal adjustment was performed with a 3118 flame-shaped spherical drill (Maillefer, São Paulo, SP, Brazil).

After 30 days, in the second session, a radiographic examination was performed, where a slight closure of the root apices of element 46 could be observed.

Anesthesia with 2% lidocaine with epinephrine 1:100,000 (Alphacaine®, Nova DFL, Rio de Janeiro, RJ, Brazil) was then performed only on the gingival papillae to position the absolute isolation clamp (as in the first session), CIV was removed and the intracanal medication was removed with the aid of the 17% EDTA solution (ASFER – Indústria Química LTDA, São Caetano do Sul, SP, Brazil) 3 ml in each canal, totaling 9 ml of solution, was potentiated again with the agitation of the *easy clean*, in order to help remove calcium hydroxide adhered to the dentin walls of the root canals and stimulate growth factors present in the region. This was followed by irrigation with 2.5% sodium hypochlorite (ASFER – Indústria Química LTDA, São Caetano do Sul, São Paulo, SP, Brazil), 3 ml in each channel with cycles of 1 ml in each channel and potentiation with *easy clean*.

Irrigation with 17% EDTA was performed again, with 3 ml distributed as follows: 1 ml in each channel and activation with *easy clean* for 20 seconds. The channels were dried with absorbent paper cones (MKLIFE, Porto Alegre, RS, Brazil).

At this time, blood was collected to prepare the fibrin-rich plasma. Thus, with the use of an 18G hypodermic needle and a sterile and disposable syringe (Descarpack Disposable from Brazil, São Paulo, SP, Brazil), 08 ml of intravenous blood was punctured from the patient's left arm (Figure 4).

Figure 4: Venipuncture for centrifugation



Soon after the puncture, the 08 ml of blood was transferred to a vacuum test tube, without any additives.

Then, the tube was correctly positioned in a centrifuge (CE1161, Centrilab – Vitehlab, Araras, SP, Brazil), and another tube with water in the same proportion in the opposite direction was used in order to maintain balance during centrifugation.

This occurred for 08 minutes at a speed of 1500 rpm. The centrifuge tube was removed, which contained dense-looking material and yellowish fluid (platelet-rich fibrin) (Figure 5).

Figure 5: Time and speed of blood centrifugation for the production of PRF (platelet-rich fibrin)



Figure 6: PRF produced after centrifugation



A needle was 3 ml of yellowish fluid material (liquid FRP) removed from the tube and deposited in the root canals with the aid of a syringe (Figure 6).

At that moment there was discomfort from the patient. Then, three fragments of dense yellowish material (consistent FRP) were sectioned with a scalpel from the tube and positioned approximately 1.0 mm at the mouth of each root canal (MV, ML and D) (Figure 7).

Figure 7: Sectioned PRF fragments (3 parts of 1 cm each)



Sealing was then performed with bioceramic repair cement (Biorepair Angelus) (Figure 8) in the cervical region of the canals, followed by glass ionomer cement (FGM, Joinville, SC, Brazil).

Figure 8: Bioceramic cement used to seal the mouth of root canals



At 30 days, the patient returned for composite resin restoration. He did not present painful symptoms. Six months after the revascularization procedure, a control X-ray was performed, where the thickening of the root walls and continuity of apical formation were observed. The patient was asymptomatic (Figure 9).

Figure 9: Radiograph of tooth 46 six months after the revascularization procedure



DISCUSSION

The traditional endodontic therapy for necrotic teeth with incomplete resorption is apicification, which consists of successive exchanges of calcium hydroxide-based MIC with the objective of closing the apex through the formation of a mineralized barrier for subsequent filling ²⁰.



This procedure, however, offers repair and not pulp regeneration, and root fractures may occur, as this barrier is fragile and porous²¹.

In the apical plug technique, it is possible to perform the treatment in one or two sessions through the formation of a plug that allows the filling without extravasation of the material, but in this technique there is also no formation of the root apex and there is still the disadvantage of the walls being fragile and thin, and therefore, vulnerable to fractures²².

Revascularization has been the most used treatment option due to its ability not only to repair, but also to revitalize pulps, through the use of the patient's own connective tissue. This aims to regenerate tissues from inside the root canal, without necessarily replicating the dental-pulp complex tissue³.

Several studies have shown that after pulp revascularization there was an increase in the thickness of the root walls, complete apical formation, and in some cases, the pulp returned to respond to pulp sensitivity tests^{23,24}.

In the present case, pulp revascularization was chosen because of the advantages of fewer sessions and the lower risk of root fractures through the continued formation of these thicker walls, the closure of the apex, and also the promising chance of no future obturator intervention.

According to Lima *et al*,²¹ there is still no defined revascularization protocol, as several studies are being carried out with new techniques and materials constantly available on the market.

Decontamination during endodontic treatment must be very efficient for success. In cases of teeth with incomplete rhizogenesis, it should be very thorough. Many authors discuss the need for little or no instrumentation during the preparation and sanitation of the root canal system, due to the risk of fracture of the thin walls still in formation.^{25,26,27}

Root canal decontamination is mainly attributed to irrigation and auxiliary MIC²⁸. The most commonly used irrigating substance in pulp revascularization processes is sodium hypochlorite in various concentrations (0.5%, 1.0%, 2.5%, 5.0%), despite the risk of toxicity and extravasation at the apex still in formation, since *in vitro* studies have shown the ineffectiveness of 2% chlorhexidine in maintaining the vitality of cells important for revascularization and effective sanitation, since it does not mature organic matter. A protocol for preventing overflow is that irrigation is done 3 mm below the apex and in constant movement and without pressure²⁹.

In this case, hypochlorite was used at a concentration of 2.5% for its healing action, for the safety of a lower concentration and for the ease of finding it on the market. EDTA



has also been used to assist in the disinfection of the root canal system and for its signaling action for mesenchymal cells present inside the root canals, arising from remnants of the *Hertwig sheath*, according to studies carried out²⁸.

After the first session of revascularization, when abundant irrigation is performed, ICM insertion is performed with a stay of 7 to 30 days in order to contribute to the decontamination of the root canal system.

As minocycline causes tooth darkening, attempts have been made to reduce the permanence time of this MIC or to replace it with another antibiotic such as cefaclor or fosfomicin or even not to use it³⁰. Calcium hydroxide has been widely used as an alternative to triantibiotic paste because it does not cause browning and contributes to decontamination, since it has a high pH^{9,22}. Some authors advise against the use of calcium hydroxide in the pulp revascularization process, justifying that its high alkalinity can be harmful in the process of cell differentiation, in addition to making the dentin structure more fragile. In the present case, calcium hydroxide was chosen because of the simplicity of access to use, the time it should remain inside the tooth without causing darkening, and its bactericidal potential due to its high pH (alkalinity).

The Mineral Assaulted Trioxide (MTA) has been the most used material for cervical sealing in revascularization techniques, but it has some disadvantages such as difficult manipulation, long setting time and chromatic alteration of the tooth crown.

However, biomaterials are being developed with properties of high compressive strength, high microhardness values, good flexural strength, good sealability, easy handling, short setting time and the promise of not staining dental crowns.

Among these biomaterials we can mention, MTA Plus, NEO MTA Plus, Biodentine, Endosequence, which are being used for cervical sealing in pulp revascularization and have characteristics such as radiopacity, biocompatibility, physical and chemical stability and good sealing to maintain the appropriate environment for cell preservation and consequently tissue regeneration, characteristics that promise to be superior to MTA, since they penetrate the open dentin tubules, crystallizing when they intertwine with the dentin¹³.

Joshi *et al*³⁴ analyzed clinical cases in the literature with the potential effect of MTA and Biodentine when associated with platelet-rich fibrin in the treatment of revascularization of immature permanent teeth.

As a result, bone filling occurred faster since these materials helped in guided tissue repair and bone regeneration.

Investigations with PRP and PRF are increasingly frequent in cases of pulp revascularization, due to the positive results when used, mainly due to the functions of a



scaffold, induction of the proliferation of undifferentiated mesenchymal cells, possibly from the apical papilla, and the stimulation of the migration of growth factors probably from the dentin and platelets within the root canal system²⁵.

These will promote differentiation and then formation of new tissue inside the root canal¹⁹.

In this study, PRF was used for its cost-benefit ratio, with simplicity of technique and low cost, as it does not require chemical additives as in the PRP use protocols. Also for the positive results of the most recent research and studies.

The bioceramic was used as a promise of advantages compared to MTA. Although pulp revascularization has shown promising characteristics, it still needs more research and studies, as new materials and techniques have emerged on the market and a standard protocol has not yet been established.

According to Félix et al²⁸, one disadvantage of revascularization is the variants of a protocol that has not yet been defined in the technique.

CONCLUSION

This article concludes that there is still no defined revascularization protocol, that pulp revascularization treatment with cervical sealing using bioceramic promotes a cellular metaplasia in the space of the Root Canal System occupied by the blood clot and that, in this case, clinical and radiographic success was achieved 10 months after the first intervention, and that the use of PRF helps in root maturation.



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