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CASE REPORT/CASO CLINICO

Regenerative endodontic procedures: a review of the literature and a case report of an immature central incisor

Procedura di rigenerazione endodontica: revisione della letteratura e caso clinico di un incisivo centrale immaturo

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KEYWORDS
Biodentine;
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Abstract
Background: Trauma of developing teeth may lead to pulpal necrosis with subsequent arrestment of root development, making them more susceptible to fracture. Regenerative endodontic procedures induce maturogenesis in necrotic immature permanent teeth in order to promote continuation of root growth. Mineral trioxide aggregate (MTA) is widely used as a blood clot protecting material, although it presents a potential drawback of discoloration. Biodentine is a tricalcium silicate cement with adequate bioactive properties that solve the problem of discoloration.
Case report: The current case report demonstrates a maturogenesis of an upper central incisor with chronic apical abscess. Calcium hydroxide was used as intracanal medicament for a week. After a blood clot was formed, Biodentine was placed over it. Periapical healing and root growth were evident at 6 months follow-up. Cone Beam Computed Tomography (CBCT) confirmed apical closure and complete healing at 1 year.
Key-learning points: Apical closure of necrotic immature permanent teeth is possible by means of regenerative endodontic procedure.Regenerative endodontic procedure with Biodentine has

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some advantages over that performed with MTA: No tooth discoloration, hort setting time, asy manipulation. CBCT is the best technique to evaluate root canal growth (length and wide).

PAROLE CHIAVE
Biodentine;
Denti permanenti immaturi necrotici;
Trattamento di rigenerazione endodontica.

Riassunto
Background: Il trauma di elementi dentari in via di sviluppo può in alcuni casi esitare in necrosi pulpare con successivo arresto della crescita radicolare e maggiore suscettibilità alle fratture. Tale procedura di rigenerazione endodontica induce la maturazione degli elementi al fine di promuovere il proseguimento della crescita radicolare (maturogenesis). Nonostante la presenza di potenziali svantaggi in termini di discolorazione, il mineral trioxide aggregate (MTA) viene comunemente utilizzato come materiale a protezione del coagulo ematico. Biodentine è un cemento a base di tricalcio silicato con adeguate proprietà bio-attive capace di ovviare le problematiche di discolorazione.
Caso clinico: Il seguente caso studio dimostra lo sviluppo radicolare (maturogenesis) di un incisivo centrale superiore affetto da ascesso apicale cronico. Come medicazione intracanalare è stato usato l'idrossido di calcio per una settimana. A seguito della formazione del coagulo ematico, è stato successivamente posizionato al di sopra Biodentine. Si è riscontrata evidente guarigione e crescita radicolare a 6 mesi di osservazione. La tomografia computerizzata cone beam (CBCT) ha confermato la chiusura apicale e completa guarigione ad 1 anno.
Punti chiave: La chiusura apicale di elementi dentari permanenti immaturi e necrotici è possibile tramite la procedura di rigenerazione endodontica. La procedura di rigenerazione endodontica attraverso l'utilizzo di Biodentine ha mostrato alcuni vantaggi quando confrontata con l'MTA: assenza di discolorazione dentale, tempo di indurimento ridotto, facilità di utilizzo. La CBCT rappresenta la miglior tecnica per valutare la crescita del canale radicolare (in lunghezza e larghezza).

Introduction

The majority of traumatic injuries to young permanent teeth occur before root formation is complete.[1] The most frequently affected teeth are the central and lateral maxillary incisors, located in a highly aesthetic zone, with a 20—30% prevalence in young patients.[2] Up to half of these traumatized teeth may result in pulpal necrosis, but only 8.5% will exhibit signs and symptoms of disease.[3] If total destruction of Hertwig's epithelial root sheath occurs, arrestment of normal root development leads to divergent dentinal walls and absence of the apical stop, which poses clinical challenges for conventional root canal treatment.

The apexification technique with calcium hydroxide, and more recently with MTA-like materials, has traditionally been the clinician's first choice. Although this technique has a high success rate regarding periapical healing, it does not allow the root to growth nor in both length and or width or thickness, leaving the tooth with short roots, and with thin walls that are prone to fracture.[4]

Regenerative endodontic procedures (REP) were proposed to overcome the drawbacks related to the clinical management of necrotic immature permanent teeth (NIPT)[5] and are gaining prominence over traditional apexification among researchers and clinicians.

REP are described as 'biologically based procedures designed to replace damaged structures, including dentine and root structures, as well as cells of the pulp—dentine complex'.[6]

Different terminologies have been used for REP. At present, the term *revascularization* is broadly widely used in the current literature, but many authors challenge its use. Initially, Trope choose this term because the nature of the tissue that formed after the treatment within into the root canals was unpredictable, and the only certainty was the presence of a blood supply.[7]

Wigler et al., in contrast, claimed that 'procedures designed to promote continued root development in NIPT should be described as *maturogenesis*, rather than *revascularization*', because it describes clinically and radiographically the apical maturation in NIPT.[8] In a letter to the editor of the Journal of Endodontics, Huang and Lin agreed that revascularization was more applicable to events following dental trauma than to endodontic procedures. Moreover, the term revascularization is imprecise because it only considers one aspect of the newly formed tissues.[9]

Iwaya et al. (2001)[5] and Banks and Trope (2004)[10] were the first to publish a REP case report (called *revascularization*) on a necrotic mandibular central incisor and on a necrotic immature permanent mandibular second premolar respectively. According to their reports, after provoking intracanal bleeding, the blood clots were covered with calcium hydroxide and MTA respectively. The findings of both studies showed a thickening of the root canal walls and continued root development.[5,10]

All regenerative endodontic procedures are based on the research published by Nygaard Ostby (1961), which demonstrated that vasculature could be established to support new

tissue growth in the empty root canal through the formation of a blood clot in the pulp space.[11] According to this principle, the periapical area is intentionally damaged to promote bleeding and a fibrin clot, which begins healing after tissue injury. Fibrin, a biopolymer secreted to promote haemostasis and wound healing, is responsible for blood clot formation and stabilization, which serves as a scaffold for stem cells deriving from apical papilla (SCAP).[12,13]

SCAP provides a source of primary odontoblasts that promotes the continuation of root development and, as a result of proximity to the periodontal blood supply, can survives pulp necrosis even in the presence of periradicular infection.[13]

Research into the nature of the tissues developed inside empty root canals after REP has become the order of the day. Numerous histological and immunohistochemical studies are broadly in agreement that tissues formed inside empty root canals after REP are mainly cement-like, bone-like and connective tissue.[14]

Recent studies have confirmed that these tissues become reparative instead of regenerative after the death of the primary odontoblasts.[15] In fact, a fibrous non-mineralized connective tissue with varying degrees of inflammation and dystrophic calcifications has been observed inside root canals.[16]

These tissues have been observed to stain positively for bone sialoprotein (BSP) but negatively for dentine sialoprotein (DSP), the latter being proteins that have a role in dentinogenesis.[16,17] Other investigations report the absence of both polarized cells resembling odontoblasts or pulp-like tissue inside the canals after REP.[18,19] Furthermore, collagen fibrils secreted by undifferentiated dental pulp stem cells after treatment did not resemble the dentine extracellular collagen matrix.[20] Some authors have concluded that continued root development is mostly provided by cementum and poorly mineralized bone.[16,17]

On the other hand, studies in animals have identified the immunohistological expression of vascular endothelial growth factors (VEGF) and factor VIII, the two main factors implicated in angiogenesis. These studies found newly formed connective tissues, vessels and hard-mineralized tissues.[21] Research in this area continues.

Although there are no evidence-based guidelines to support a standard protocol to achieve the most favourable outcome in REP, a number of regenerative endodontic treatment protocols have been proposed depending on the type of the irrigant[10]; the irrigation method[22]; the intracanal dressing[23]; the type of scaffold[24]; and the blood clot-protecting material.[20]

Mechanical instrumentation of the root canal is contraindicated in REP due to the thin walls of teeth, which could further increase the risk of root fracture.[4] Therefore, disinfection of the root canal system depends solely on the irrigant's effectiveness. The most widely used disinfection irrigant is sodium hypochlorite (NaOCl), in different concentrations, including 6%, 5.25%, 2.5%, 1.25% and 0.5%.[25]

Furthermore, ethylenediaminetetraacetic acid (EDTA) has been shown to promote the release of growth factors embedded in dentine that participate in both the regenerative processes as well as stem cell proliferation, migration, and differentiation.[26]

Due to its extended residual antimicrobial properties, chlorhexidine has been successfully associated with NaOCl as a disinfecting agent.[27] Some authors have combined NaOCl with 3% hydrogen peroxide as a disinfection solution that has provided successful results.[28]

Periapical extrusion of NaOCl has cytotoxic effects and provokes pain, localized, or widespread swelling and ecchymosis.[29] In cases of an immature apex, special care should be taken to avoid irrigant extrusion. The negative-pressure irrigation technique may deliver the irrigants up to the working length, avoiding extrusion to the periapical space.[30]

Regarding the intra-canal dressing, triple antibiotic paste (TAP) and calcium hydroxide are the most widely used medicaments. According to the latest published review, TAP and its modifications were applied in 86% of studies, while calcium hydroxide was used in 13.5%.[31] Some authors have used formocresol as inter-appointment medicament, but it has been demonstrated that formocresol causes minimum improvement in root length and thickness.[32]

Although TAP has been shown to be more effective in eradicating bacteria,[33] it has the potential for tooth discoloration, which results from contact between minocycline and the root canal walls during the REP.[34] Exclusion of minocycline (known as double antibiotic paste)[5] or substitution of minocycline by amoxicillin, doxycycline, clindamycin, tetracycline or cefaclor has been reported to solve this problem.[31]

Platelet-rich plasma (PRP), the most widely used blood venous derivatives, is a mass of autologous plasma with a high platelet concentration, which is recommended as a scaffold for its abundance of growth factors (GFs).[21]

Some authors have used platelet-rich fibrin (PRF) as a scaffold with embedded growth factors. However, the results have shown only minor improvements in periapical healing, dentinal wall thickening, root lengthening and apical closure.[35]

A study in animals showed increased the success rate of REP in cases in which modified TAP (Ciprofloxacin, Metronidazole and Cefixime) was used as an intracanal dressing and PRP as a scaffold instead of a blood clot.[36]

The main drawbacks to PRP and PRF are related to the need for centrifugation, mainly to obtain PRP, and the need to collect venous blood from young patients.

During REP, a protective material is placed coronal to the blood clot to prevent recontamination and to induce differentiation of mesenchymal stem cells to produce new dental tissues. There is one clinical study that reports successful results with the use of glass ionomer as a protective material.[37] Different silicate calcium cements are more often used to this end, such as Calcium-enriched mixture (CEM) cement[38] and TheraCal LC.[20]

To date, MTA has been the material of choice for vital pulp capping and REP procedures due to the survivability of undifferentiated dental pulp stem cells (DPSC) after their exposure to MTA, which has the capacity to form new hard tissues.[39]

However, in addition to its ease of displacement within the clot during condensation, this material has other drawbacks, among them, long setting time, difficult handling[20] and potential tooth discoloration.[40] Because anterior teeth are more susceptible to trauma, it is important for aesthetic reasons to use calcium silicate materials that do not cause tooth discoloration after REP.

To avoid these shortcomings, a number of different materials have been developed, such as Biodentine (Septodont, Saint Maurdes-Fosses, France),[20] which is a calcium-silicate material that sets in approximately 12 min, does not wash out easily, is simple to handle[41] and does not cause discoloration.[40]

Accordingly, in the present case report, the authors described a regenerative endodontic procedure (REP) to treat an upper central incisor with a chronic apical abscess.

Report

An 8-year-old boy suffered a trauma to the upper left central incisor tooth, but he did not receive any treatment for it until one year later, when he came to the Department of Endodontics (Universitat Internacional de Catalunya) complaining of a sinus tract. On clinical examination, the tooth was not tender to percussion or palpation. Diagnosis was completed with radiographic examination, which showed a large periapical lesion in both the periapical radiograph and the cone beam computed tomography (CBCT) scan (Fig. 1).

After obtaining written informed consent from the patient's parents, we began the treatment. Following rubber dam isolation, the pulp cavity was accessed and the working length was determined using a K-type file (Dentsply Maillefer, Baillagues, Switzerland) 1 mm shorter than the root apex. Without instrumentation, the tooth was irrigated with 30 ml of 5.25% NaOCl with the aid of the Endovac system (SybronEndo, Orange, CA). The canal then was irrigated with 10 mL of sterile saline and dried with sterile paper points. Subsequently, creamy calcium hydroxide was delivered into the canal with a lentulo spiral instrument and the tooth was temporized with Cavit G (3M ESPE, St Paul, USA) for 1 week (Fig. 2).

By the following appointment after 1 week the sinus tract had healed. The patient was administered a local infiltration of Mepivacaine 3% without epinephrine. The pulp space was flushed with 10 ml sterile saline to eliminate calcium hydroxide and finally irrigated with 1 mL 17% EDTA for 60 s. The root canal was dried with paper points and a sterile #30 K-file was used to stimulate blood clot formation. Bleeding was stopped at the level of the cementoenamel junction (CEJ) and a blood clot formed after 10 min. Subsequently, a 3 mm-Biodentine barrier was placed over the clot to seal the root canal at the CEJ level and was verified radiographically. After waiting 12 min for the Biodentine to set, the definitive restoration was performed (Fig. 2).

The patient returned for follow-ups at 4 weeks, 3 months, 6 months and 1 year; examination included evaluation of clinical signs and symptoms, root development, periapical health and pulp vitality. At 2 years (Fig. 3), a CBCT scan revealed increased root length and dentine wall thickness as well as apical closure. Moreover, periapical radiolucency had almost completely disappeared. Sensibility test showed no response to either cold or electric pulp test. Finally, no signs of discoloration were observed.

Discussion

As discussed in the introduction to this article, the term *revascularization* is debatable. Many authors claim that it

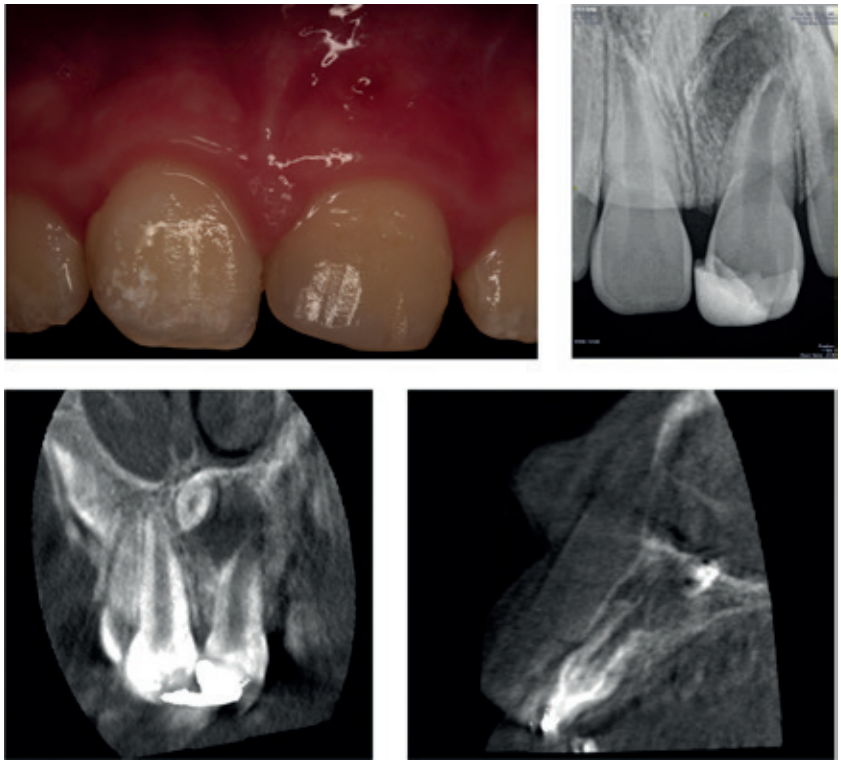

Figure 1 Initial radiographic and clinical examination. Periapical X-ray and CBCT showed open apex and large radiolucency.

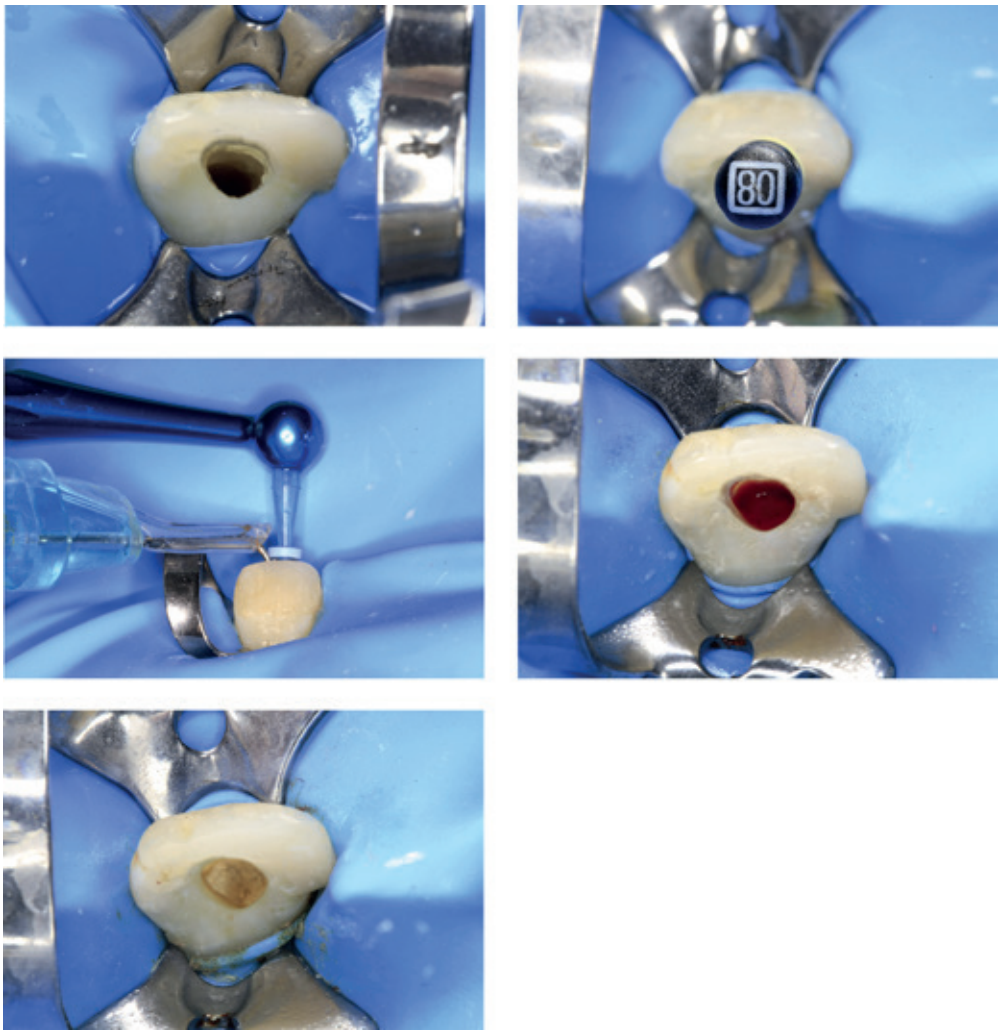

Figure 2 Clinical images of the first appointment: access cavity and work length establishment, and of the second appointment: root canal irrigation with EDTA 17% using Endovac system, blood clot formation and Biodentine placement.

should be used only for events related to dental trauma, considering the nature of tissues inside the root canal after treatment; therefore the current literature is replacing this term with *maturogenesis*.[8]

The basic principle of maturogenesis is found in continued root development and hard tissue deposition of immature teeth reimplanted after traumatic or intentional avulsion.[42,43] Root canal maturogenesis is based on inducing a blood clot in the canal space by mechanically irritating the periapical tissues, which promotes mesenchymal stem cells within the root canal, which differentiate in order to stimulate root development.[12] Regardless of the type of tissue growth, REP promotes greater root development than conventional apexification methods do.[44]

Due to potential detrimental effects on stem cell survival, there is no consensus whether maximum NaOCl concentration should be used.[45] On the one hand, it has been reported that dentine conditioning with high concentrations of NaOCl resulted in lower survival rates of stem cells from apical papilla (SCAP). On the other hand, this effect can be reversed with the application of 17% EDTA after the use of NaOCl.[25] For this reason, 5.25% NaOCl was used, in accordance with Trevino, who observed a 74% survival rate of human stem cells exposed to 6% NaOCl, followed by 17% EDTA.[46] Considering the immature apex of the tooth, irrigants were flushed using the Endovac system to avoid irrigant extrusion through the apex.

There is no agreement about the use of inter-appointment medicaments. In one study, 90% of the cases that were administered TAP showed complete radiographic healing at 12 months follow-up,[47] although a more recent REP outcome study, in which calcium hydroxide was used, revealed the resolution of periapical disease in all cases except one (94.2%).[48] On the basis of these results, and in order to avoid tooth discoloration, we used calcium hydroxide as an inter-appointment medicament, as supported by the literature.[8]

The sealing properties and excellent biocompatibility of MTA makes it the material of choice for clot protection.[39] However, because anterior teeth are more susceptible to trauma, MTA, which may causes tooth discoloration,[49] is not the most indicated material for this type of tooth. Other MTA-like materials (or calcium silicate based materials) could be used for that purpose. Biodentine is a relatively new calcium silicate cement commercialized as a dentine substitute that

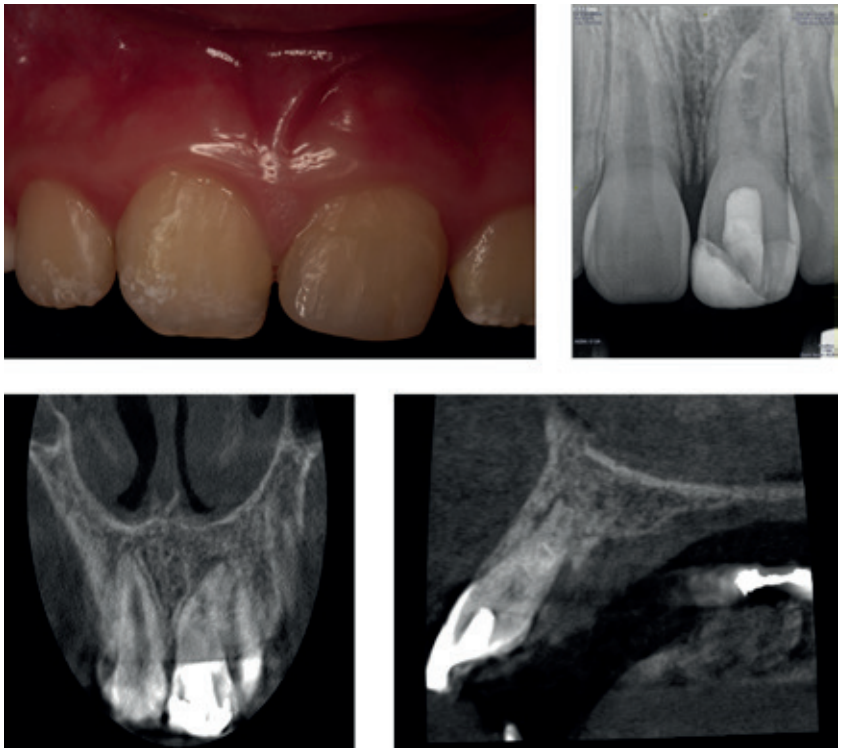

Figure 3 Two-years clinical and radiographic (CBCT) follow-up: an increase of root length and root canal thickness can be observed, as well as almost complete resolution of the periapical lesion.

could be used for all MTA indications. Its advantages include: no tooth discoloration, easy handling,[40] fast setting time (12 min) and promotion of bone regeneration, as confirmed by recent cell culture studies.[50,51]

Predictors of a clinically successful REP include lack of signs and symptoms of disease, such as pain, swelling, sinus tract, radiographic evidence of periapical healing, as well as increased root length and canal wall thickness, indicating continued root development,[47,48,52] and many authors include positive response to sensitivity testing.[44,47] The survival of a permanent tooth is particularly important in young patients undergoing continued cranioskeletal development because implants are contraindicated at this stage.

Unhealed periapical lesions (sinus tract or swelling recurrence, root resorption and larger or unchanged periapical lesion), fractures, and failure to induce periapical bleeding are the main causes associated with failures after RET.[53]

There is a discrepancy between the view of the patient and the clinician as to what a successful outcome is: for the patient it is one that prolongs the functional life of an asymptomatic tooth without discoloration. However, no prognostic study considers tooth discoloration as a clinical failure after REP.

In two retrospective analyses, REPs showed a higher tendency than apexification in eliminating apical lesions more rapidly. Nevertheless, in terms of radiographic healing, no statistical differences were identified between the two techniques.[47,48] Alobaid et al. observed a complete resolution of the radiographic lesions in 90% of the cases at one year REP. The remaining cases still showed radiographic evidence of disease but were clinical symptom-free.[47] According to these results, Nagy et al. found that both MTA apexification and REP promoted healing in 100% and 90% of the cases respectively.[54] Kahler et al. obtained similar results with 90.3% complete healing of periapical radiolucencies.[55]

Teeth with preoperative apical diameters wider than 1 mm have been reported to show a greater increase in root thickness, length, and apical narrowing. Moreover, patients younger than 13 years old show a notable decrease in root apical diameter.[52]

From all the treatment modalities described for the management of NIPT, including conventional root canal treatment, MTA or calcium hydroxide apexification and REP, Jeeruphan et al.[44] found that the percentage changes in root thickness and length were significantly greater in teeth treated with REP (28.2% and 14.9%) than with those treated with MTA apexification (0% and 6.1%) and calcium hydroxide apexification (1.5% and 0.4%). The survival rate was significantly higher in both the REP group and the MTA apexification group (100% and 95.5%) when compared to the calcium hydroxide apexification group (77.2%).[44]

Chen et al. reported that 96% of the cases treated by means of REP presented some degree of apexogenesis. In addition, 76.2% and 79.2% of the cases, showed increased root length and thickness, respectively, and 55.4% of the sample exhibited complete apical closure.[56] Similarly, Saoud et al. found complete apical closure in 55% of REP treated teeth, with all cases confirming at least a 20% decrease in apical diameter.[57] Kahler et al. concluded that apical closure after treatment in 47.2% of the cases was incomplete, compared to 19.4% cases of complete. In addition, increase in root length varied from 2.7% to 25.3% and root dentin

thickness, from 1.9% to 72.6%.[55] Accordingly, Nagy et al. observed an increase in root length and thickness of 11.8% and 12.7%, respectively, whereas no changes were detected in the MTA apexification group.[54] In the present case, a CBCT scan taken of the patient for orthodontic reasons showed increased root length and thickness and complete apical closure at 2-year follow-up.

Regarding pulp vitality, Chen et al. established that half of the sample responded to sensitivity tests (cold, heat, and electrical).[48] In contrast, Saoud et al. found that none of the teeth regained responsiveness to pulpal sensitivity tests during the follow up period.[57] Similarly, in the present case, the patient responded to neither cold nor electric pulp test, and no signs of discoloration were observed.

In spite of these findings, there is a need for long-term prognostic studies with larger samples in order to evaluate the prognosis of REP versus apexification.

Conclusion

The 2-year follow-up showed apical closure of an immature central incisor after REP with Biodentine.

Conflict of interest

The authors have no conflict of interest to declare.

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