CASE SERIES

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Regenerative endodontic treatment in permanent immature teeth: Case series

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Regenerative endodontic treatment is a biologically based procedure primarily indicated for the management of necrotic immature permanent teeth. Beyond achieving the resolution of periapical radiolucencies and the elimination of clinical symptoms, RET aims to promote continued root development, including the thickening of dentinal walls and apical closure, and ideally, the re-establishment of pulp vitality. This case report presents two clinical cases treated with regenerative endodontic protocols, with follow-up periods of 30 months and 12 months, respectively. The first case involves a maxillary lateral incisor that underwent a repeated regenerative procedure, while the second case pertains to a maxillary central incisor with a history of dental trauma and prior conventional root canal treatment. The irrigation protocol consisted of 1.5% sodium hypochlorite, followed by distilled water and 17% ethylenediaminetetraacetic acid. Calcium hydroxide was employed as the intracanal medicament. Biodentine (Septodont, France) was used as a coronal barrier, and definitive restorations were completed with composite resin. During the follow-up period, a secondary regenerative procedure was performed for Case 1 at the 18-month recall. At the 30-month follow-up for Case 1 and the 12-month follow-up for Case 2, both cases exhibited radiographic evidence of periapical healing and remained asymptomatic. However, both teeth yielded negative responses to pulp vitality testing and cold stimuli. Regenerative endodontic treatment is considered a promising alternative to apexification therapy for immature and necrotic teeth.

Keywords: Platelet-rich fibrine; regeneration; revascularization.

Introduction

The treatment of permanent teeth with necrotic pulp, periapical lesions, and immature root development presents a significant challenge in clinical endodontics. Consequences of incomplete root development include thin dentinal wall, open apex, and inadequate root length, which predispose the teeth to a fracture (1). The absence of an apical constriction complicates endodontic therapy, as it hinders the effective sealing of the root canal system using conventional obturation techniques (2).

Historically, apexification using calcium hydroxide over multiple visits was the preferred approach to induce the formation of an apical hard tissue barrier in such teeth (3). However, with the introduction of mineral trioxide aggregate (MTA), studies have demonstrated its ability to form a well-sealed, high-quality barrier in the apical third of the root with a significant success rate (4). While apexification can achieve apical closure through calcific barrier forma-

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tion, it does not facilitate continued root development or reinforcement of root structure (2).

As an alternative approach, biologically based endodontic procedures have been developed with the goal of regenerating a functional pulp–dentin complex (5). Commonly referred to in the literature as revascularization or Regenerative endodontic treatment (RET) (6), this approach aims to stimulate the ingrowth of stem cells from the apical papilla, facilitating a pulp-like tissue formation within the root canal system. In addition to alleviating pain and inflammation, RET promotes healing of periapical lesions (7).

Recent advances in tissue engineering have emphasized three essential components for successful regeneration: Adult stem cells, signaling molecules, and a three-dimensional (3D) scaffold capable of supporting cellular growth and differentiation (8). Traditionally, the formation of a blood clot—stimulated by deliberate instrumentation beyond the open apex of an immature root—serves as the primary scaffold in RET (9). In this context, biological scaffolds such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) have been extensively investigated due to their ability to promote healing, support tissue regeneration, and modulate immune responses (10).

The steps in regenerative endodontics typically include: (i) Minimal or no mechanical instrumentation of dentinal walls, (ii) chemical disinfection using irrigants, (iii) intracanal medication, (iv) induction of intracanal bleeding to form a blood clot, (v) sealing with a hydraulic calcium silicate-based cement, and (vi) placement of a coronal restoration to ensure an effective seal (6).

This case report aimed to present the management of two clinical cases with regenerative endodontic procedures and follow-up periods.

Case Presentations

Case 1

A 15-year-old systemically healthy female patient referred with a complaint of fistulization and purulent discharge in tooth #12. Clinical examination revealed the presence of a sinus tract, with no tenderness to percussion or palpation. Radiographic evaluation showed an open apex, a periapical radiolucent lesion associated with tooth #12, and the presence of Type II dens invaginatus (Fig. 1a) (Panoramic radiography device (J. Morita MFG. CORP., Kyoto, Japan), periapical radiography device (J. Morita MFG. CORP., Kyoto, Japan), CBCT (NewTom VGIevo, Cefla, Imola, Italy)). Written informed consent was obtained from the patient's family.

Due to the immature root structure and the anatomical complexity posed by the invaginated portion of the tooth, which could complicate apexification with MTA, RET was planned. Following informed consent, access cavity preparation was performed under rubber dam isolation. The main canal was reached through the center of the invaginated area, and working length was established. Without any mechanical instrumentation, irrigation was carried out using 20 mL of 1.5% sodium hypochlorite (NaOCl), 20 mL of distilled water, and 10 mL of 17% ethylenediaminetetraacetic acid (EDTA) (11). The canals were dried with sterile paper points, and calcium hydroxide was placed as the intracanal medicament. Temporary restoration was completed with conventional glass ionomer cement.

At the second visit, three weeks later, the sinus tract had resolved and the tooth was asymptomatic. Local anesthesia without vasoconstrictor (3% Safecaine) was administered. Irrigation was repeated using 20 mL of 17% EDTA and 20 mL of distilled water. After drying the canal, apical pa-

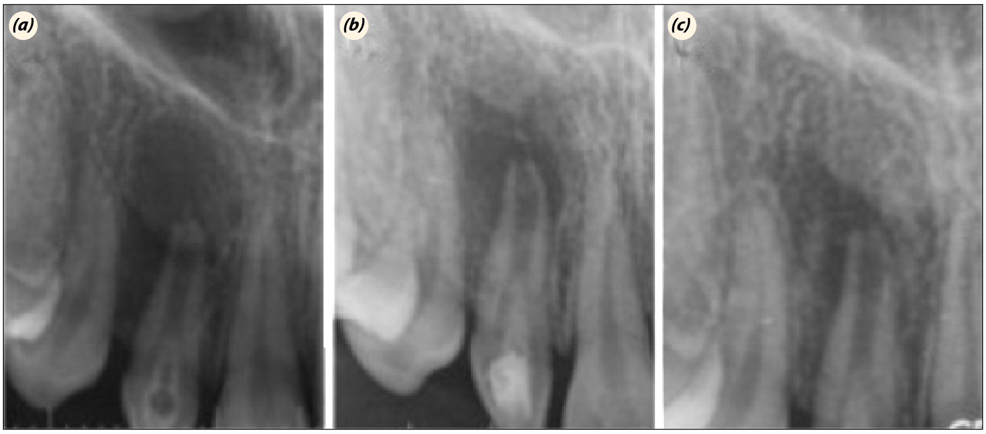

Fig. 1. Radiographic images of Case 1: **a)** Pre-treatment radiograph, **b)** 18-month follow-up radiograph, **c)** 30-month follow-up radiograph.

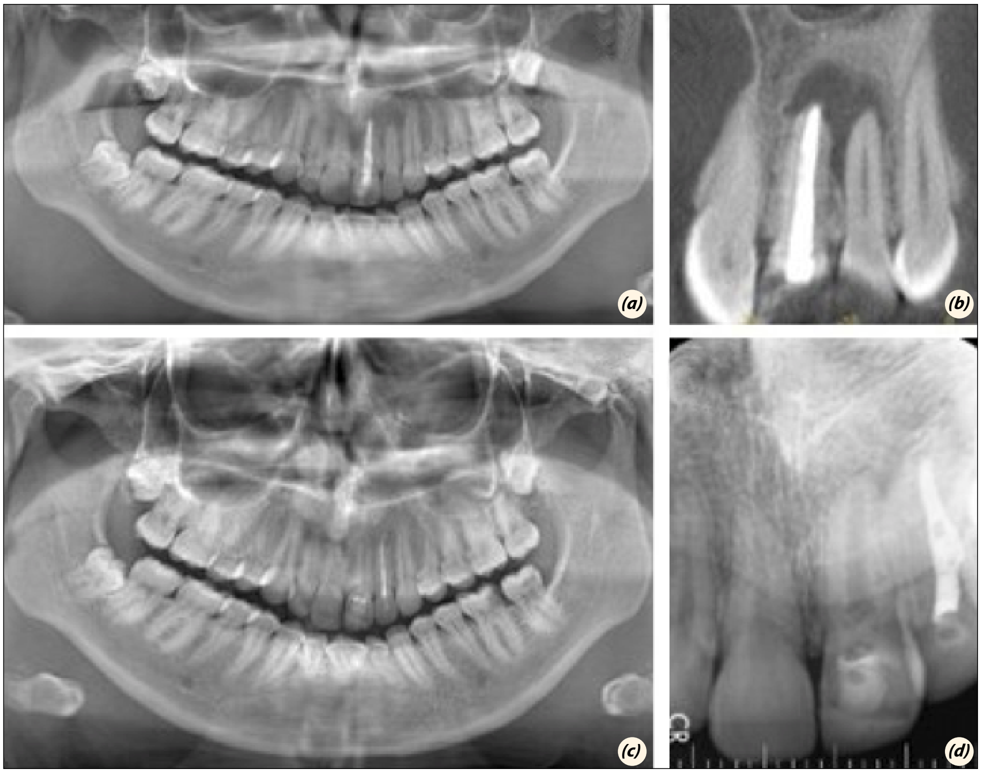

Fig. 2. Radiographic and tomographic images of Case 2: **a)** Pre-treatment panoramic radiograph, **b)** Pre-treatment CBCT, **c)** 12-month follow-up panoramic radiograph, **d)** 12-month follow-up periapical radiograph.

tency was extended by 3–4 mm using a #20 K-file due to the size of the lesion. Since effective intracanal bleeding was unlikely, platelet-rich fibrin (PRF) prepared prior to the procedure (Medifuge centrifuge (Silfradent S.r.l., Forlì, Italy), 2700 rpm, 12 minutes) was introduced into the canal space. The invaginated portion was sealed with Biodentine (Septodont, France), followed by placement of a resin-modified glass ionomer base, and the tooth was permanently restored with composite resin.

At follow-up visits, the tooth remained asymptomatic, and radiographic imaging showed a reduction in lesion size. However, no response was elicited to pulp vitality or cold testing. At the 18-month follow-up, a fistulization was detected. Given the previous reduction in lesion size (Fig. 1b), it was assumed that more effective bleeding could be achieved. Therefore, a second regenerative procedure was planned. Using a dental operating microscope (Leica Microsystems, Wetzlar, Germany) and ultrasonic tips (ED7, Guilin Woodpecker Medical, China), the Biodentine barrier was carefully removed. The same irrigation, medication, treatment, and obturation protocols were repeated.

At the 30-month follow-up, clinical examination revealed no sensitivity to percussion or palpation, and no signs of a sinus tract were observed. The size of the periapical lesion was remarkably reduced which was evident in radiographs (Fig. 1c). However, no significant thickening of the root canal walls was detected, and pulp vitality testing continued to yield negative results.

Case 2

A 22-year-old systemically healthy female patient referred with a complaint of pain in tooth #21. Medical history revealed a traumatic dental injury approximately 10 years earlier. Clinical examination showed tenderness to percussion in tooth #21. Radiographic evaluation revealed that tooth #21 was immature and previously treated endodontically, and there was a large periapical radiolucent lesion extending around the apices of both teeth #21 and #22 (Fig. 2a; Fig. 2b). Tooth #22 was nonresponsive to both cold and electric pulp tests. Written informed consent was obtained from the patient.

Given the incomplete root development of tooth #21, RET was planned, while conventional root canal therapy was indicated for tooth #22. During the first appointment, the existing root canal filling in tooth #21 was removed using mechanical techniques. An access cavity was prepared in tooth #22. Both teeth were irrigated with 20 mL of 1.5% NaOCl, 20 mL of distilled water, and 10 mL of 17%

EDTA, all activated ultrasonically. Calcium hydroxide was placed as the intracanal medicament, and the patient was scheduled for a second visit four weeks later.

At the second visit, both teeth were asymptomatic. For tooth #21, the same regenerative endodontic procedure, as described in Case 1, was performed under rubber-dam isolation. The root canal treatment for tooth #22 was completed using conventional techniques.

The follow-ups were performed at 1, 3, 6, and 12 month periods. At the 12-month follow-up, both teeth remained asymptomatic. Radiographic imaging showed resolution of the radiolucent lesion and apical closure of tooth #21 (Fig. 2c; Fig. 2d). However, no response to pulp vitality or cold testing was observed in tooth #21.

Discussion

Dental pulp necrosis may result from various etiological factors, including traumatic injuries, extensive carious lesions, and developmental anomalies (dens evaginatus/dens invaginatus). These insults can interrupt root development, leading to incomplete maturation of the affected teeth. Koç et al. (12), reported that there is no evidence that pulp necrosis etiology affected RET results. However, according to the findings of Hu et al. (13), teeth with developmental dental anomalies demonstrated a more favorable prognosis following RET compared to teeth with trauma-induced pulp necrosis (13). Geisler suggested that the outcomes of RET may vary depending on whether the affected teeth exhibit partial or total pulpal necrosis (14). Huang further proposed that the mode of pulpal regeneration is influenced by these distinct clinical conditions (15). In cases of partial necrosis, the remaining vital pulp tissue may hypothetically recover following disinfection and contribute to the regeneration of the damaged portion, thereby offering a more favorable prognosis. Conversely, in cases of complete pulpal necrosis, where de novo pulp tissue synthesis is required, the prognosis is generally less favorable and may necessitate the use of alternative protocols and scaffolding materials (14,15).

The present case series examined the clinical efficacy of PRF as a scaffold in de novo regeneration scenarios, in addition to apical bleeding. In Case 1, recurrent fistulization was observed at the 18-month follow-up, which was attributed to inadequate apical bleeding likely caused by the extensive size of the apical radiolucency. After retreatment, all clinical signs and symptoms had resolved by the 30-month recall, and the tooth remained functional.

In Case 2, the RET was performed on a tooth with previous root canal treatment. At the 12-month follow-up, clinical symptoms had completely disappeared, while the tooth remained functional. In both cases, apical closure was observed; however, no significant increase in root wall thickness was recorded. Furthermore, electric pulp testing and cold stimulation tests demonstrated negative responses for both teeth.

Effective bacterial elimination from the root canal system plays a crucial role in the success of revascularization, as the presence of infection can disrupt the regenerative process (16). The local application of triple antibiotic paste (a combination of metronidazole, ciprofloxacin, and minocycline) was demonstrated to be effective in eliminating endodontic pathogens from infected root canals, both in vitro (17) and in vivo (18) conditions. Calcium hydroxide has also been shown to be an effective intracanal medicament (19). In both presented cases, calcium hydroxide was selected over triple antibiotic paste due to several disadvantages of the antibiotic paste including; the risk of tooth discoloration (20), potential for bacterial resistance (21), difficulty in complete removal from the canal system (21), and greater cytotoxicity toward stem cells (22).

Jadhav et al. (23) found PRP to be more effective than blood clots in regenerative procedures. The superiority to PRP's was related to collagen production stimulation, sustainable release of growth factors, and enhanced proliferation of undifferentiated mesenchymal and endothelial cells from the periapical area (23). In a clinical investigation involving 16 necrotic immature permanent maxillary incisors, Sharma et al. (24) evaluated the efficacy of RET. Their findings revealed significant improvements in periapical tissue repair, apical closure, root elongation, and increased dentinal wall thickness. Notably, the use of PRF yielded superior outcomes compared to the blood clot scaffold, particularly in promoting periapical healing, facilitating apical closure, and enhancing dentinal wall reinforcement. Platelet-rich plasma (PRP) is administered into the root canal space, where it subsequently undergoes coagulation. In contrast, platelet-rich fibrin (PRF) is derived from whole blood without the use of anticoagulants, platelet activators, or their antagonists, employing a single-step centrifugation process. During this procedure, the platelets are naturally activated and become incorporated into a developing fibrin matrix. The resulting fibrin clot is then harvested and introduced into the root canal system. Compared to PRP, PRF offers a more cost-effective and technically straightforward alternative, as its preparation does not require any exogenous biochemical agents. Due to the initiation of platelet activation during the centrifugation process, the preparation of platelet-rich fibrin (PRF) is considered technique-sensitive, requiring prompt clinical application of the final product to preserve its biological efficacy (9). In the present cases, due to the large size of the apical radiolucencies and the concern that adequate bleeding might not be achieved, PRF was prepared from the patient's blood prior to the procedure and introduced into the canal space as a scaffold. According to the 12 and 30 months follow-ups of both cases, the application of PRF demonstrated successful results and can be used in the treatment of necrotic and immature teeth similar to the PRP.

The RET aims to maintain the function of the involved tooth and demonstrate favorable healing outcomes. Several earlier studies have reported satisfactory root maturation despite the treated teeth exhibiting no response to pulp sensibility tests. This paradox may be attributed to histological evidence indicating that the tissue formed following regenerative endodontic procedures often resembles periodontal or bone-like structures, rather than a true pulp–dentin complex (25). Responses to vitality tests can be misleading depending on the use of EDTA and the thickness of the barrier material employed (26). It is important to note that the absence of a pulp response does not necessarily indicate the absence of vitality (27). Moreover, the newly formed pulp-like tissue may possess an immature or insufficiently developed neural network, potentially leading to a delayed or absent response during pulp sensibility testing (26).

The absence of CBCT at the follow-up sessions may limit the ability to comprehensively evaluate three-dimensional healing outcomes, such as root wall thickening or resolution of periapical pathology.

Conclusion

At the follow-up sessions both Case 1 and Case 2 represented radiographically periapical healing, teeth were asymptomatic and in function, on the other hand vitality test responses were negative. Although these procedures represent a promising alternative to apexification treatments, long-term follow-up and further evidence-based studies are necessary.

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