Regenerative Endodontic Procedures Posttrauma: Immunohistologic Analysis of a Retrospective Series of Failed Cases

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Abstract

Introduction: Regenerative endodontic procedures (REP) are a novel treatment modality to restore the function of necrotic pulp tissue via stimulation or transplantation of stem cells into the root canal. This study aimed to investigate the immunohistologic outcome of 3 extracted teeth because of sequelae of trauma and unsatisfactory REP outcomes.

Methods: Three immature permanent maxillary central incisors of 3 female patients (6–9 years) were extracted 5.5–22 months after REP. Additionally, 1 sound permanent immature central maxillary incisor of 1 of the included patients was extracted for orthodontic reasons. The teeth were immunohistologically stained with Masson's trichrome, neurofilament (NF), pan cytokeratin, dentin sialophosphoprotein, and Gram+/-.

Results: The REP-teeth presented intracanalar vascularized connective/mineralized reparative tissue (RT), which was less organized than the pulp tissue of the sound tooth. Moderate to considerable calcification was observed below the Portland cement used during REP. In 1 case, the RT was NF+; in the 2 other cases, the periodontal ligament and apical granuloma/papilla were NF+. All teeth were Gram+/- negative; nevertheless, inflammatory cells were present in 2 cases. The pan cytokeratin and dentin sialophosphoprotein stainings were not specific enough for 2 cases.

Conclusions: This immunohistologic study of failed REP cases resulted immunohistologically in bacteria-free intracanalar reparative tissue containing inflammatory cells and biomaterial-induced calcification. Cone-beam CT scanning and multidisciplinary close consultation are important for treatment planning, especially in case of REP failure.

Key Words
Angiogenesis, cone-beam computed tomography, dental pulp, histology, stem cell(s)

Conservative and minimal invasive approaches with the implementation of biomaterials are promoted in vital pulp therapy (1). Nevertheless, conservative treatment of infected immature permanent teeth remains a challenge. Regenerative endodontic procedures (REP) aim to restore the function of the necrotic pulp via stimulation or transplantation of stem cells into the root canal (2). Although clinical and radiographic trials present favorable outcomes in terms of periapical bone healing and resolving clinical symptoms (3–5), immunohistologic studies report repair rather than regeneration of the pulp-dentin complex (6–10). Generally, thickening and lengthening of the root canal’s walls seem to go slow (11), and it is unknown whether the apposition of reparative and calcified tissue will strengthen these fragile teeth. Nonetheless, dental trauma affects children the most (12), and orthodontic therapy might be necessary in a growing dentition. Because only a few clinical trials report long-term survival of REP teeth (3–5, 13), the following questions remain unanswered:

1. Are these teeth strong enough to endure orthodontic load?
2. Is there any chance of a periapical flare-up during orthodontic treatment? and
3. Will these teeth remain functional until complete skeletal growth?

The lack of evidence makes multidisciplinary decision making difficult. However, studying treatment failure may help to evolve REP as well. Hence, this study aimed to investigate the immunohistologic outcome of 3 extracted teeth after failed REP.

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Figure 1. Case 1: a 6-year-old girl with avulsion of the right maxillary central incisor. (A) Clinical and radiographic: (a) directly after trauma; (b) after repositioning and splinting of the right maxillary central incisor; (c) at the end of the second REP session; (d and e) 5 months post-REP; (f) the extracted right maxillary central incisor; (g) the nano–computed tomographic scan of the extracted right maxillary central incisor showing resorption on the external root wall and intracanalicular from coronal to apical, GIC, MPC, calcified bridge (CB), and calcium hydroxide (CH) remnants; (h and i) the extracted left maxillary central incisor and the nano–computed tomographic scan; and (j) 5 months postextraction of the maxillary left and right central incisors. (B) Immunohistologic: (a) the REP tooth with intracanalicular MPC and RT; (b) the sound tooth; (c) the REP tooth with inflammatory fluid (IF) pressed into the dentin tubuli; (d and e) the sound tooth showing the pulp and dentinal walls; (f) the sound tooth and pulp (the arrow points at an axon stained brown by NF); (g and j) the arrow points at epithelial cell rests of Malassez in the periodontal ligament of the (g) sound and (j) REP tooth; (b) the REP tooth showing apoptotic neutrophils in the RT; (i) the REP tooth (the arrow shows a neurovascular bundle); (k) the REP tooth (the arrow points at NF+ staining in the RT); (l) the REP tooth showing reparative mineralized tissue apposition (MT) by the RT against the root canal wall (the arrow points at the internal root canal wall resorption); (m) the REP tooth showing MT apposition against the root canal wall (the black arrow points at a mineralization nucleus in the RT, and the white arrow points at a blood vessel in the RT); and (n) the REP tooth showing MPC in the coronal part of the root canal. Staining: (a, b, d, m, and n) Masson’s trichrome, (e and l) DSPP, (i, j, and g) pan cytokeratin, (b) Gram+/−, and (c, f, and k) NF. Scale bars: (a and b) 5000 µm, (d and n) 500 µm, (e, l, and m) 200 µm, (c, f, g, i, j, and k) 50 µm, and (b) 10 µm.
Materials and Methods

Study Design

This study was designed as a retrospective case series and was conducted according to the principles of the 1996 Declaration of Helsinki on good clinical practice standards and with the approval of the Medical Ethics Committee of the UZ/KU Leuven (Leuven, Belgium; registration number S60773). This article was written according to the CARE (Case REport) checklist of 2013 (English, 2016 revisions) (14).

Clinical Procedure

Case 1. A 6-year-old girl consulted the dental emergency unit after a bike accident (Fig. 1Aa). She presented with an avulsed, immature permanent maxillary right central incisor that was kept dry for 1.5 hours and afterward shortly stored in milk. The endodontist on call (O.S.) cleaned the tooth with saline, replanted it into the socket, and placed a flexible splint (Fig. 1Ab). According to the guidelines of the International Association of Dental Traumatology (IADT) (15) for a tooth with an open apex and an extraoral dry time greater than 60 minutes, root canal treatment can be performed before replantation or later. Nevertheless, the IADT guidelines do also state that for replanted immature teeth root canal treatment should be avoided unless there is clinical or radiographic evidence of pulp necrosis. Amoxicillin, ibuprofen, and a chlorhexidine mouthwash were prescribed, and the patient was advised to avoid hard food and contact sports for at least 2 weeks. Four weeks posttrauma, at the splint removal session, the patient presented an acute apical abscess on the maxillary right central incisor. The tooth was tender to percussion, did not react on an electric pulp test (Pulppen DP2000 Digital; Dental Electronic, Ballerup, Denmark) or carbon dioxide snow (CDS), and there was a vestibular swelling of the gums. The splint was removed, and REP were performed by another endodontist (N.M.) relying on the European Society of Endodontology (ESE) position statement for revitalization procedures (16). After administering 1.8 mL local anesthesia with a vasoconstrictor (Lignospan; Septodont, Saint-Maur-des-Fossés, France), the tooth was isolated, and a ninja access was made with a sterile endodontic access bur under water cooling. Vital tissue was visible via the operating microscope and was sensible by probing with an endodontic file at 15 mm from the incisal border. The root canal was disinfected 2 mm above the vital tissue by means of sodium hypochlorite (1.5%, 20 mL) and saline (20 mL). Sterile paper points were used to dry the root canal, and a calcium hydroxide paste (UltraCal XS; Ultradent Products Inc, Orange, CA) and glass ionomer cement (GIC [Fuji 2 LC]) were placed into the root canal 2 mm above the vital tissue for 14 days to prevent aggressive infection-related resorption. A sterile cotton pellet was placed on the calcium hydroxide, and the tooth was temporarily sealed with glass ionomer cement (GIC [Fuji 2 LC; GC America, Alsip, IL]). During the second session, 1.8 mL local anesthesia without a vasoconstrictor (Scandondent 3%; Septodont, Saint-Maur-des-Fossés, France) was administered. Like in the first session, the entire second session took place under field isolation and microscopic magnification. After rinsing the root canal with EDTA (17%, 30 mL), 2 mm above the vital tissue, a blood clot was triggered periapically by means of an apically present size 35 Hedstrom file. Afterward, CollaPlug (Zimmer Biomet, Berlin, Germany) was placed as a scaffold for radiolucent Pure Portland Cement Med-PZ (MPC; Medcem GmbH, Weinfelden, Switzerland). Subsequently, the tooth was covered with GIC (Fuji 2 LC) and composite (Fig. 1Ac). Nevertheless, 3 months posttreatment, the REP tooth presented already ankylosed and in obvious infraclosure of about 1.5 mm (Fig. 1Ad and Ae). The tooth was still negative for all sensitivity tests described previously, and the patient presented an Angle class II malocclusion orthodontically. After multidisciplinary discussion between the orthodontic, pedodontic, and endodontic departments, it was decided to remove both maxillary central incisors to allow accelerated symmetrical eruption and spontaneous mesialization of the maxillary lateral incisors and canines. After informed consent and approval of the patient and her parents were obtained, the teeth were removed 5.5 months after REP (Fig. 1Af and Ai) and prepared for tissue processing. Further follow-up of the orthodontic development of the patient was performed by an orthodontist (Fig. 1Af).

Case 2. The right central maxillary permanent incisor of a 9-year-old girl suffered a complicated crown fracture (Fig. 2Aa and Ab). Under general and local anesthesia, the root canal was disinfected, and the fractured coronal part was repositioned by the dentist on call (no treatment specifications mentioned in the record). One month later, the patient consulted a pediatric endodontist because of an abscess and detachment of the fractured coronal part. A REP was performed following the same protocol as in case 1. However, during the first REP session, the coronal segment was reattached with composite resin (Fig. 2Ac–Af), and 3% sodium hypochlorite was used (instead of 1.5%). In this case, zinc oxide eugenol cement was used as a temporary filling (instead of GIC). Vital tissue was sensed during disinfection in this case as well.

At the 6 month recall, the patient complained about some pressure sensibility, and a cone-beam computed tomographic (CBCT) scan showed a persistent periradicular lesion (Fig. 2Ag). Orthodontically, the patient presented with an Angle class II malocclusion and crowding in the upper and lower front region. After multidisciplinary discussion between the orthodontic, endodontic, and pedodontic department, extraction of the REP tooth and mesialization of the teeth in the first quadrant (Fig. 2Ah–Ak) seemed to give a better long-term prognosis. After informed consent, the tooth was extracted 7 months after the REP (Fig. 2Ab).

Case 3. The right and left central maxillary permanent incisors of an 8-year-old girl suffered an uncomplicated crown fracture (Fig. 3Aa) (ie, no pulp was exposed). During the emergency session, a pediatric endodontist covered the teeth by means of an indirect pulp capping technique (17), namely with a calcium hydroxide liner (Life; Kerr, Orange, CA) and glass ionomer cement (Fuji 2 LC). In a following session, the teeth were restored with composite (Fig. 3Ab), and they reacted positively on CDS. Nevertheless, 8 months posttrauma, the left central maxillary permanent incisor reacted negatively on CDS and presented a vestibular abscess. The tooth was treated with the same protocol as in case 2. Even if vital tissue was sensed, triggering of the blood clot was painful and slow in the present case. No CollaPlug was used, and a zinc phosphate cement (instead of GIC) was placed underneath the composite restoration (Fig. 3Ac). During all recall sessions, there were no complaints, and the tooth did not react on percussion, CDS, or electric pulp testing. At the recall session, 20 months after the REP (Fig. 3Ad), the periapical lesion was healed, and there was apexogenesis; nevertheless, because of the fact that the MPC was pushed too far apically, there was not much root canal wall thickening (Fig. 3Ae–Ag). After multidisciplinary discussion between the orthodontic, endodontic, and pedodontic department, it was decided to remove this tooth rather than extracting a sound premolar to create space. Orthodontically there was crowding in the upper and lower front regions and an Angle class I malocclusion. Twenty-two months after the REP, the tooth was extracted. Seven months later, the right upper central incisor was extracted as well to obtain symmetry, and, subsequently, the lateral upper incisors were mesialized (Fig. 3Af).
Figure 2. Case 2: a 9-year-old girl with a complicated crown fracture of the right central maxillary permanent incisor. (A) Clinical and radiographic: (a) the complicated crown fracture; (b) the vestibular abscess; (c and d) the REP; (e–g) the persistent periapical lesion 7 months post-REP; (h) after extraction, the apical granuloma; (i and j) the rapid maxillary expansion with replacement of the right central maxillary permanent incisor; and (k) the orthopantomographic radiograph taken 15 months postextraction of the REP tooth. (B) Immunohistologic: (a) Masson’s trichrome staining (TCM): *apical granuloma. **reparative connective tissue, ***calcification induced by MPC (****); (b) TCM: *reparative mineralized tissue apposition against the inner root canal wall; (c) TCM: a closer look at the calcification, looks like day/night rhythm apposition (circadian clock); (d) NF+ staining (brown) in the apical papilla; (e) a part of the apical granuloma with lymphocytes (arrows) and plasma cells (circles); and (f and g) Gram+/- negative dentin tubules. Scale bars: (a) 1000 μm, (b) 50 μm, (c, e, and g) 200 μm, (d) 20 μm, and (f) 10 μm.
In a following stage, these teeth will be esthetically remodeled with a composite restoration.

Tissue Processing and Immunohistologic Procedure

After extraction, the teeth were fixated with formaldehyde 6%. The tooth of case 1 was subsequently scanned by 1 of the authors (A.M.) with a Phoenix NanoTom S (7-μm pixel size, a 0.5-mm copper filter, 80 kV, and 320 mA), reconstructed using NRecon (version 1.6.9.8; Bruker microCT, Kontich, Belgium) software, and analyzed with Dataviewer (version 1.5.0.9; Bruker microCT) software (Fig. 1). The nano–computed tomographic scan was used to visualize nondestructively the 3-dimensional morphology of the root and observe whether there is any formation of reparative mineralized tissue. The preparation, processing, and immunohistologic staining of the teeth were performed as described by Meschi et al (6), with the only exception being that the coronal part of the teeth was separated before demineralization in order to reduce the demineralization time. After inspection of the slices stained with Masson’s trichrome (TCM), the following immunohistologic markers were chosen: neurofilament (NF), pan cytokeratin (PK), dentin sialophosphoprotein (DSPP), and Gram (+/-) positive/negative (Table 1).

Figure 3. Case 3: an 8-year-old girl with an uncomplicated crown fracture of the right and left central maxillary permanent incisor. (A) Clinical and radiographic: (a) directly after trauma, (b) 6 months posttrauma, (c) 8 months posttrauma the left upper central incisor received REP because of a vestibular abscess, (d) 20 months post-REP, (e–g) 22 months post-REP a persistent periapical lesion visible on the (f and g) CBCT scans, (h) after extraction the tooth was cut on the level marked by the yellow line on Fig. 3Ag, and (i and j) mesialization of lateral upper incisors 1 year postextraction of the left maxillary central incisor. (B) Immunohistologic: (a) TCM: *Portland cement, **calcification front because of the phosphate in Portland cement, ***bone-like tissue (ingrowth of periodontal tissue), and ****apical papilla; (b and c) arrows: NF+ in the (b) periodontal ligament and (c) apical papilla; and (d and e) Gram+/- negative (d) apical papilla and (e) dentin tubuli. Scale bars: (a) 500 μm, (b and c) 50 μm, (d) 20 μm, and (e) 10 μm.

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<th>Table 1. Primary Antibodies: Manufacturer, Clone, and Dilution</th>
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DSPP, dentin sialophosphoprotein; NF, neurofilament; PK, pan cytokeratin; RTU, ready to use.
repair/regeneration of the pulp-dentin complex, and the presence of bacteria were investigated.

**Case 1**

Five months postextraction of the maxillary right and left central incisors, both maxillary lateral incisors had erupted and mesialized spontaneously (Fig. 1Af). From coronal to apical, the immunohistologic stainings (Fig. 1Ba) and nano–computed tomographic scans (Fig. 1Ag) of the REP tooth presented a calcified bridge underneath the MPC (Fig. 1Ba and Bm), connective reparative tissue (RT) with blood vessels and mineralization nuclei (Fig. 1Ba and Bm), and mineralized reparative tissue against the internal resorbed root canal walls (Fig. 1Bl and Bm). The sound tooth presented vascularized pulp tissue (Fig. 1Bb and Bd) without resorption on the DSPP+ pulp-dentin surface (Fig. 1Be). The dentin, predentin, odontoblasts, and pulp show a more distinct DSPP expression in the sound tooth than in the REP tooth (Fig. 1Bl). The epithelial cell rests of Malassez in the periodontal ligament of both teeth were PK+ (Fig. 1Bg and Bj). The nerve fibers in the RT of the REP tooth (Fig. 1Bk) were much less NF+ (brown) than in the pulp of the sound tooth (Fig. 1Bf). There was also a neurovascular bundle visible in the REP tooth (Fig. 1Bi). The REP and sound tooth were G+/- negative. Nevertheless, apoptotic neutrophils (Fig. 1Bb) and inflammatory fluid in the dentin tubuli (Fig. 1Bc) showed the presence of an ongoing inflammation.

**Case 2**

Figure 2B represents a combination of the TCM, NF, and G+/- staining of case 2. The PK and DSPP stainings were not specific enough for case 2. From coronal to apical, the following were visible (Fig. 2Bt and Bc): MPC, calcification beneath the MPC with a day/night rhythm apoposis (circadian clock) (18), RT, and an apical granuloma. Mineralized RT was deposited against the inner root canal wall (Fig. 2Bb). The expression of NF could only be found in the apical granuloma (Fig. 2Bd). Despite the presence of an apical granuloma, the sample was G+/- negative (Fig. 2Bf and Bg).

**Case 3**

Figure 3B represents a combination of the TCM, NF, and G+/- staining of case 3. The PK and DSPP stainings were not specific enough for case 3. From coronal to apical, the following were visible (Fig. 3Ba): MPC, a calcification front because of the phosphate in MPC, bonelike tissue (ingrowth of periodontal tissue), and the apical papilla. The periodontal ligament (Fig. 3Bb) and apical papilla (Fig. 3Bc) were NF+. The entire sample was G+/- negative (Fig. 3Bd and Bc).

**Discussion**

REP has been proposed as a treatment option by the American Association of Endodontists (AAE) (19) and the ESE (16) in order to maintain root development in infected immature permanent teeth. Even though long-term evidence is lacking (3), clinicians aim to preserve these teeth, and thus healthy alveolar bone, until at least complete skeletal growth (16, 19). Nevertheless, failures occur (10), and teeth get lost earlier than planned. This case series describes the histologic outcome of 3 failed REP cases.

The tooth loss described in case 1 was caused by sequelae of trauma, which still cannot be circumvented with the treatment modalities available nowadays. The IADT guidelines (15) were pursued. After delayed replantation of an avulsed tooth, ankylosis is almost unavoidable and must be taken into consideration. In case of an open apex, root canal treatment must be avoided unless there is clinical or radiographic evidence of pulp necrosis (15). Nevertheless, 4 weeks postreplantation, an acute abscess occurred. Because very immature infected permanent teeth are prone to cervical root fracture after endodontic treatment, REP is advised rather than apexification. Because ankylosis is unavoidable postavulsion, in a later stage, decoronation is performed much easier when the root canal is revitalized instead of filled (20). Nevertheless, in the current case, ankylosis occurred faster and more aggressively than expected, and decoronation was not a treatment option anymore. After extraction of the maxillary central incisors, an accelerated eruption and mesialization of the lateral incisors took place (Fig. 1Af). In due time, the crowns of the maxillary lateral incisors and canines can be restoratively and esthetically reshaped to become central and lateral incisors, respectively.

Regarding the immunohistologic outcome of case 1, RT with blood vessels and nervous tissue was detectable in the REP tooth (Fig. 1Ba and Bm). There was evidence for a reparative process in comparison with the sound tooth, namely mineralized tissue apposition against the inner root canal wall, mineralization nuclei, disorganized fibroblasts, and less nervous tissue than in the sound tooth (Fig. 1Bk-Bm). Nevertheless, whether this RT was (re)generated after the REP or if it was solely/partly a scar tissue remnant of the original pulp is uncertain. If this RT with calcifications would have strengthened the tooth is also not known. The calcified bridge underneath the MPC in the REP tooth was induced by MPC because it has cementoinductive and osseoinductive potential (21). MPC was applied instead of MTA to avoid discoloration (21, 22). The opaque (calcified) particles in the RT (Fig. 1Ba and Bm) were most probably remnants of the calcium hydroxide (Fig. 1Ag) that could not be flushed away when the ESE treatment protocol (16) was strictly applied.

Nevertheless, the extractions in cases 2 and 3 were caused by unsatisfactory REP outcomes. Despite disinfection during the REPs, a slight (case 3) to severe (case 2) periapical inflammation persisted. A sodium hypochlorite concentration between 1.5% and 3% is prescribed by the ESE position statement on revitalization procedures (16), relying on the balance between enough disinfection and the preservation of stem cells (23). However, even if the sodium hypochlorite concentration in cases 2 and 3 was twice the concentration applied in case 1 and although the teeth in cases 2 and 3 were G+/- negative, the inflammatory reaction could only be caused by bacteria. This puts the disinfection protocols established for REPs (16, 19) into question. Additionally, because pulp necrosis posttrauma was the reason why the REP was performed, the failure of these 2 cases might also be caused by sequelae of trauma, even if the trauma in case 1 was more severe (24, 25). It has been described previously that the outcome of REP is reduced/less favorable when the etiology is trauma in comparison with anatomic anomalies (5, 26).

The intracanal part of the immunohistologic image of case 2 (Fig. 2B) is very similar to case 1, with the exception that the calcified bridge in case 2 is much larger. The calcified bridge in case 2 has a particular appearance; darker and lighter shades are systematically repeated as if a circadian rhythm (18) would be present in the MPC-induced hard tissue (Fig. 2B).

Concerning case 3, a critical note is that a light-curable GIC is recommended to be used as an intermediate layer between composite and hydraulic calcium silicate cements rather than zinc phosphate cement (16, 19). In case 3, MPC was pushed undeliberately too far apically, probably because of the fact that the blood clot was not hard enough, and importantly no CollaPlug was used as a scaffold for MPC. Clinically, the blood clot induction was more difficult than in cases 1 and 2. Hence, the introduction of mesenchymal stem cells into the root canal for pulp regeneration was not attained (27), and intracanal calcification was prominent. This calcification was partially induced by the MPC coronally and by the periodontal tissues apically, coinciding at
a calcification front (Fig. 3B). Nevertheless, the immunohistologic analysis of case 3 showed an apex with a vital apical papilla and thus growth potential. Taking into account that the periapical lesion was healed, at least the primary goal of the AAE clinical considerations for regenerative endodontics was achieved (19). Hence, case 3 would not be a “failed” case but rather one with an unsatisfactory REP outcome because there was no further root development in terms of root canal wall thickening and lengthening. However, as shown in other REP studies, obtaining at least 30% further root development takes 1–3 years (11). This burdens orthodontic decision making in cases with the following question: Should a sound premolar be extracted just to keep a weak immature incisor with an unknown prognosis? Additionally, the radiographic diagnosis of a (persistent) periapical inflammation is of utmost importance; in cases 2 and 3, it is difficult to tell on the 2-dimensional periapical radiograph (Figs. 2Ae and 3Ad) whether there was a periapical lesion or not (Figs. 2Ag and 3Ag). A CBCT scan is then recommended if the radiation dose can be held as low as possible (28). This CBCT scan might be used for multidisciplinary decision making, namely endodontic, pediatric, and orthodontic treatment planning. Chaniotis (29) based his decision making on periapical radiography. In his study, 2 sound permanent maxillary premolars were extracted for orthodontic reasons, and an REP-treated permanent maxillary central incisor with an assumed healed periapical lesion was kept and moved orthodontically. Even if the tooth endured the orthodontic forces without clinical symptoms, it is precarious to assume that the tooth is periapically healed without any accurate (3-dimensional) radiographic imaging (5).

Regarding the present and previous immunohistological studies (6–10), it can be concluded that REP based on the cell homing technique results in repair rather than regeneration, whether (6–9) or not (10) the treatment was successful. The disinfection protocols advised by the AAE (19) and ESE (16) were in the present study not effective enough to cure the periapical lesion and clinical symptoms, which was histologically proven by the presence of inflammatory cells (Figs. 1Bb and 2Be). Bacterial sampling for quantitative polymerase chain reaction analysis should be taken into consideration in future clinical trials in order to show more adequately the presence and species of bacteria that might be responsible for treatment failure (30). Moreover, CBCT imaging for multidisciplinary usage seems to be a valuable tool in comparison with 2-dimensional radiography (4, 5) for a more accurate diagnosis of bone healing and precise evaluation of root development/resorption. Because the replacement of front teeth in children is challenging, it is advisable to perform decision making and treatment planning in REP failures in a close multidisciplinary consultation of endodontists, pedodontists, orthodontists, and restorative dentists. A better understanding and timely anticipation of the biological interactions in REP are necessary in order to give this biologically based treatment a well-deserved place in the arsenal of endodontic therapy.

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