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Case Report

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Clinical, Radiographical and Histological Evaluation of An Immature Permanent Molar with Pulp Necrosis after Revascularization/ Regeneration Procedures-Aase Report

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Abstract

Revascularization/regeneration procedures (REPs) in human immature permanent teeth with pulp necrosis have been shown to be a clinical challenge. The aim of this case report was to examine clinically, radiographically and histologically the outcome of REPs in an immature permanent molar with pulp necrosis. An immature permanent mandibular first molar with pulp necrosis in a 9-yr old girl was treated with REPs. At the 3-month follow-up visit the tooth was asymptomatic and functional. Radiographic examination showed that the thickness of the root structure and the root length had slightly increased. After 13 months the tooth fractured and was deemed non-restorable. The tooth was extracted and processed for histological evaluation. Microscopic observation revealed that the root canals were filled with loose fibrous connective tissue resembling periodontal tissue containing many spindle- shaped cells and newly-formed vessels. In the mesiobuccal canal, a mineralized tissue bridge lined by flattened cells was observed at the level of the coronal third. Nerve regeneration was visualized by a silver nitrate impregnation technique. A calcified structure containing irregular spaces filled with fibrous connective tissue was found at the level of the mid root of the distal canal. No calcified tissues were observed at the level of the open apices. The newly formed tissues inside the canals resembled a vascularized periodontal-like fibrous tissue and a cementum-like tissue which are comparable to the histologic findings of previous animal and human case reports.

Significance

This case report suggests that although the newly formed ingrown tissues in the root canals were not true pulp tissues, the tooth treated by regenerative Endodontics regained its vitality and function while also reestablishing its sensory function.

Keywords: immature permanent tooth; pulp necrosis; regenerative endodontics; reinnervation; scaffold

Introduction

Endodontic treatment of immature permanent teeth with pulp necrosis is a clinical challenge since the root canal walls are thinner and prone to fracture during instrumentation, while the apical third has a divergent configuration that complicates root canal obturation procedures [1]. Preservation of the pulp's vitality is of primary importance for a tooth to be healthy and durable. For this reason, there is a need for a clinical strategy that promotes regeneration of the pulp-dentin complex when these tissues have been lost. Revascularization/regeneration procedures (REPs) is an endodontic alternative to conventional apexification treatment that led to maturogenesis of necrotic immature teeth, with an increase in thickness of the root canal walls and a continuation of root development [2,3]. REPs are recognized as an effective method to treat necrotic immature permanent teeth with or without apical lesions [4,5]. Although this treatment has been successfully used for immature teeth [6-8], there is some evidence that it is also effective in adult teeth with mature apices [9-11]. As defined by the American Association of Endodontists [12], REPs intend to replace damaged tooth structures as well as cells of the pulp-dentin complex by the formation of new vascularized tissues within the root canal space. Further reinnervation may also occur, which is an observation that has been reported by several investigators [13-15]. Although histologic studies have shown that the newly formed tissues did not totally resemble the original pulp-dentin anatomy, teeth treated by REPs can regain vitality, immunology and functionality [16]. In spite of these favorable observations, it has been reported that periapical healing may not occur in a high percentage of cases or may occur without pulp tissue regeneration in the root canal [17-20]. The purpose of this report is to describe clinically, radiographically and histologically a case of a human immature permanent mandibular molar with pulp necrosis that has been treated by revascularization/regeneration procedures.

Case report

A 9- year-old white female with a non-contributory medical history was referred for evaluation and treatment of her right mandibular first molar. Clinical examination revealed the presence of a deep carious lesion. The tooth was asymptomatic. Periodontal probing and tooth mobility were within normal limits and the tooth was neither tender to percussion nor palpation. Radiographic examination showed roots with immature open apices, thin dentinal walls and no evidence of periapical pathology (Fig.1). Vitality tests were negative while the adjacent teeth responded normally without lingering. From the combined clinical and radiographic examinations, a diagnosis of pulp necrosis was arrived at. Based on the incomplete formation of the roots, REPs were recommended with particular emphasis on the importance of rendering treatment that promoted continued root development. After the risks and benefits of the treatment were explained, the parent of the patient signed an informed consent form.

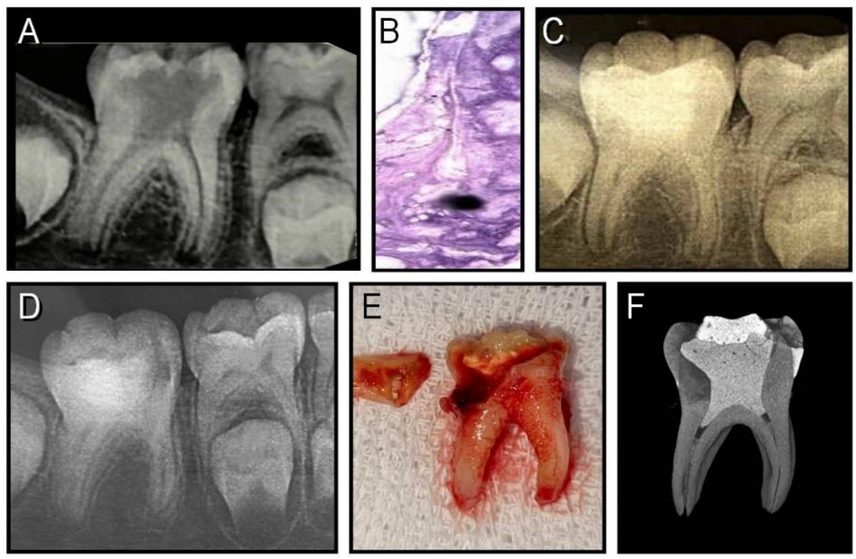

Figure 1A: Periapical preoperative radiograph of the mandibular right first molar showing an extensive carious lesion.
B: Histologic section of the necrotic tissue removed from the root canals. H&E stain; Original magnification x100.
C: Periapical postoperative radiograph obtained 3 months after REPs.
D: Periapical postoperative radiograph obtained 13 months after REPs. No periapical lesion is present.
E: Photograph of the extracted molar showing a transvers oblique fracture and the fractured coronal piece.
F: Microtomographic picture of the extracted molar. Note the level of the BD plug in the Di and Mb canals.

Treatment procedure

Mandibular block was established with 1.7 mL of 4% Articaine 1:100.000 L-adrenaline (Sidus SA, Buenos Aires, Argentina) and the tooth isolated with rubber dam. After the decay was removed a large pulp exposure was detected. Under copious irrigation with saline, the access cavity was completed, and three canals (one distal and two mesial canals) were identified. The content of the canals was removed with manually operated K-files (Dentsply/Maillefer, Ballaigues, Switzerland) and immediately fixed in 10% neutral buffered formalin. The canals were instrumented manually with K-files (Dentsply/Maillefer) taking care as to not to disturb the weakened dentinal walls. The canals were then irrigated and disinfected for 5 min with1.5% NaOCl followed by 17% EDTA and copious rinsing with saline. After the canals were dried with sterile paper points the working lengths were established radiographically. A creamy calcium hydroxide paste using propylene glycol as the vehicle was then placed in the canals and the access cavity was temporarily sealed with glass ionomer cement (Fuji II LC; GC Corp, Tokio, Japan). At the 2-week follow-up appointment the patient continued to be asymptomatic. In order to facilitate bleeding, the tooth was anesthetized with 3% Mepivacaine (Septodont, Cedex, France) without vasoconstrictor. After rubber dam isolation the access cavity was accessed once more and the calcium hydroxide paste removed from the canals under copious irrigation alternating 1.5% NaOCl with saline. The final step of the irrigation/disinfection procedure consisted of a 17% EDTA solution for 1 min followed by rinsing with saline. After the canals were dried with sterile paper points bleeding was induced by pushing sterile size #20-K files (Dentsply/Maillefer) beyond the apices. The subsequent intracanal hemorrhage was controlled by applying pressure for 10 minutes with a sterile saline soaked cotton pellet until a blood clot had formed approximately 1-2 mm below the cementoenamel junction. Biodentine (BDT; Septodont St-Maur-des-Fossés, France) was then prepared according to the manufacturer´s instructions and gently placed on the blood clot using an endodontic plugger followed by exerting light pressure with a saline moistened cotton pellet. The access cavity was then coated with Single Bond Universal dentin bonding agent (3M, St. Paul, MN USA) and Filtek XT composite resin (3M), both lights cured for 20s at an output of >750mW/cm using a Blue Phase C5 curing unit (Ivoclar Vivadent, Schaan, Liechtenstein). After 3 months the patient returned for a follow-up examination. The tooth continued to be asymptomatic and functional, while an examination showed a healthy periodontium without pocketing. Radiographic examination showed a slight increase in thickness of the root walls and a slight increase in root length. After 13 months following completion of REPs the tooth suffered an oblique coronal fracture causing the loss of the lingual aspect of the crown, which extended below the alveolar crest making the tooth unrestorable. At this time, cold and hot tests were negative. However, there was a positive reaction to the electric pulp test. After permission from the parents, the tooth was extracted and fixed in 10% neutral buffered formalin. To ensure adequate penetration of the fixative into the canals, the resin composite and the BD plug were removed prior to fixation.

Tissue processing

Demineralization was performed at 4°C in 10% EDTA. After decalcification, the roots were separated using a sharp cutting blade. They were then washed in running tap water for 48 hs, dehydrated in ascending grades of ethanol, cleared in xylene and embedded in individual paraffin blocks. Longitudinal serial sections of approximately 6 µm thick were obtained from each of the roots. Care was taken to assure that the sections passed through the center of the apical openings. Once the apex was identified the paraffin blocks were realigned to obtain longitudinal sections from the middle and coronal portions of the roots. Sections were stained with hematoxylin and eosin (H&E) for histopathologic conditions and alizarin red (ARs) for identification of calcified tissues. Other sections were stained with toluidine blue (TOb) and silver nitrate impregnation (SNi) to allow identification of nerve fibers. The soft tissues from the root canals were also fixed in neutral buffered formalin and imbedded in paraffin, followed by sectioning and staining with H&E.

Results

Due to technical problems the mesiolingual canal was lost during histological preparation. The tissue removed from the canals was necrotic which confirmed the clinical diagnosis. The histologic sections of the mesiobuccal (Mb) root were significant for the presence of loose fibrous connective tissue which upon careful analysis resembled periapical tissues that had grown inside the canal up to the BD plug (Fig.2). This tissue was remarkable for spindle-shaped fibroblast-like cells, blood vessels and abundant collagen extracellular matrix. At the level of the BD plug, AR-stained sections showed structures resembling a cementum-like tissue bridge lined with flattened cells that were connected with the dentin walls and gradually changed to dentin-like tissue. Thin black filament structures that were interpreted as nerve fibers were observed in most of the SNi stained sections. In the distal root (Di) a loose periodontal-like tissue without inflammatory cells was also observed in the apical portion (Fig.3). At mid third level, the canal contained newly formed cementum-like structures with different fused areas of calciotraumatic lines and irregular spaces filled with fibrous connective tissue. In both the Mb and Di roots, no calcified tissue was observed at the level of the open apices.

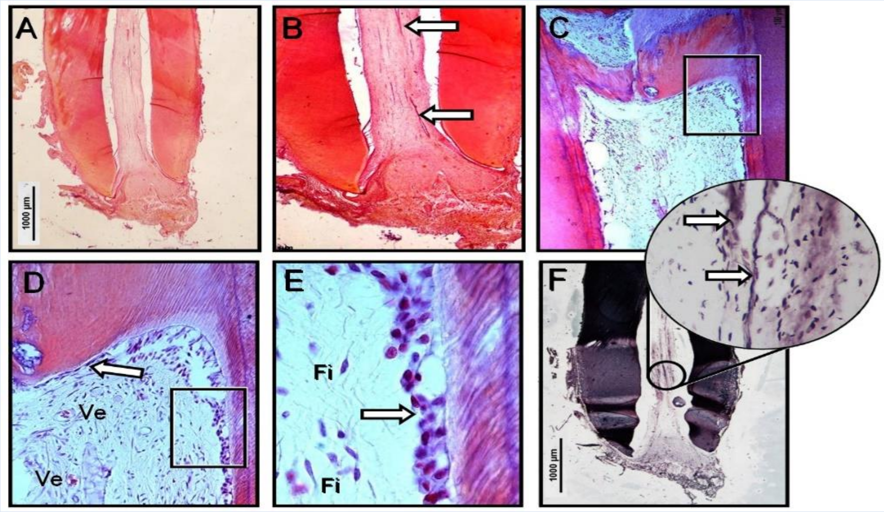

Figure 2A: Photomicrograph of the extracted MB root of revascularized/regenerated tooth. H&E stain; original magnification x20; Bar 1000 µm. The white space is a shrinkage artifact.
B: Detailed view of the apical portion in A. The root canal is filled with newly formed fibrous connective tissue ingrowth. Note the presence of newly formed blood vessels (arrows). H&E stain; original magnification x40.
C: Detailed view of the mid portion of the root showing a mineralized tissue bridge formation resembling cementum tissue lined by flattened cells in direct contact with the dentinal walls. H&E stain; original magnification x100.
D: High magnification of the square area in C. Note that the cementum-like bridge tissue lined by flattened cells (arrow) gradually changed to dentin tissue and that the flattened cells gradually changed to odontoblast cells. Ve: Blood vessels. H&E stain; original magnification x400.
E: High magnification of the square area in D. Note the presence of spindle-shaped fibroblasts (Fi) and odontoblast cells lining the dentinal walls (arrow).
F: Microphotograph of the same MB root stained by silver nitrate impregnation. Original magnification x20; Bar 1000 µm. The inset shows a detail of the area indicated by the circle. Note the presence of nerve fibers (arrows).

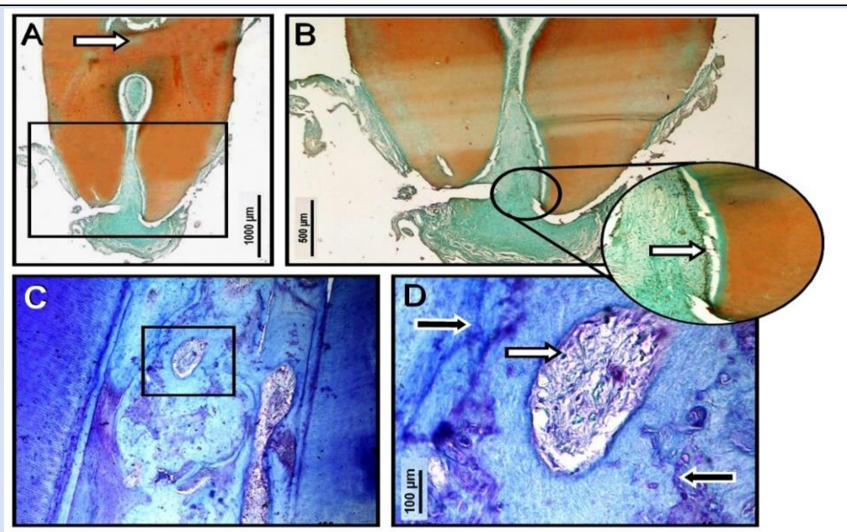

Figure3A: Photomicrograph of the extracted Di root of a revascularized/regenerated tooth. White arrow indicates a superposed root structure. Alizarin red stain; original magnification x20; Bar 1000 µm.
B: Detailed view of the square area in A. In the apical third the root canal was filled by a fibrous periodontal-like tissue ingrowth. Alizarin red stain; original magnification x40. Bar 500 µm. The inset shows a high magnification of the area indicated by the circle. At the level of the apical opening, the root canal walls are lined with cementoblasts-like cells. Alizarin red stain; original magnification x400.
C: At the mid portion of the root, the canal was totally filled with a mineralized cementum-like tissue connected to the dentinal walls. Note the presence of different fused hard tissue areas and spaces filled with fibrous tissue. Toluidine blue stain; original magnification x400.
D: Higher magnification from the square area in C showing a space filled by a dense fibrous connective tissue (white arrow). The presence of numerous calciotraumatic lines (black arrows) can be seen. Toluidine blue stain; original magnification x800; Bar 100 µm.

Discussion

The treatment of immature permanent teeth with pulp necrosis has always been an endodontic challenge [18,19]. However, there are many studies reporting a good prognosis after REPs treatment [2-4,6,8]. For REPs, two different clinical procedures have been proposed: The cell transplantation and the cell homing techniques [9,21,22]. In the present case, a cell homing approach was used through the induction of a blood clot after producing bleeding from periapical tissues. According with previous reports [23,24], the blood clot acting as a scaffold plays one of the most important roles in the formation of new tissues in the canal space after REPs in immature necrotic permanent human teeth. Hargreaves et al [23]. and Lovelace et al. [24] suggested that in comparison to blood from the peripheral circulation, blood that originates from periapical tissues contains a high concentration of mesenchymal stem cells markers, platelet-derived growth factors and signaling molecules that are capable of promoting stem cell proliferation and differentiation. In addition to the blood clot, an effective irrigation/disinfection protocol and a plug material that is biocompatible are crucial when using REPs. Furthermore, an adequate coronal seal is mandatory to allow for the survival of stem cells that are needed for generation of new tissues. In this particular case, 1,5% NaOCl and 17% EDTA were used for irrigation/disinfection of the root canals. Trevino et al [25]. reported that a low concentration of NaOCl followed by 17% EDTA are effective irrigants that do not affect the survival of the stem cells from the apical tissues. El Ashry et al [26]. reported that EDTA as a dent in surface modifier before establishing the blood clot promoted new tissue in growth in the root canal space when compared to other irrigants. This can be explained since partial demineralization of dentin walls enhances the liberation of dentin growth factors along with stem cell proliferation and sialoprotein expression, while at the same time the deleterious effect of NaOCl is reduced [26,27]. BD was used for the plug for several reasons. It has biocompatibility [28] and bioactivity [29] properties as well specific modulating functions for the growth factor TGF-ß1 [30]. BD is easy to manipulate, offers improved physical properties and has a short setting time (approximately 12 min), [31,32]. which allowed for sealing the access cavity with a restorative material in the same appointment. The presence of mineralized tissue structures resembling calcified bridges connected to the dentin walls were not a surprising finding. These mineralized structures were similar to the cementum-like tissue bridges observed by Wang et al [31]. in immature dog teeth and Nostrat et al [34]. in humans after REPs. Deposition of these intracanal structures as has been suggested by several reportsmay be the result of the stem cells from the apical papilla which may persist after irrigation and disinfection which have a great potential to differentiate into osseo/cementum forming cells after receiving specific inductive signals in the absence of vital pulp tissue. Although reinnervation is still an issue of discussion [8,35.36]. In this patient the presence of nerve fibers after REPs were visualized by a silver impregnation method while they were also confirmed clinically by the reaction of the tooth to an electric pulp test. Nerves play a role in the control of the flow of blood, in the reaction to injuries and in the regulation of the immune responses [13]. Thus, their presence in the regenerated tissues after REPs is an indication that restoration of the sensory function may occur. Our results demonstrate that after 13 months revascularization/regeneration by means of a vital fibrous-like tissue ingrowth in a root canal can be achieved in immature permanent necrotic teeth with REPs. We can only speculate but do expect that further changes would have occurred if the tooth had not fractured after 13 months. Longer periods of time resulting in better defined anatomical pulpal features on more teeth would need to be evaluated to confirm the aforementioned speculation.

Conclusions

Based on the clinical, radiographic and histologic observations of the case presented here suggest that REPs can be recommended for the treatment of immature permanent teeth with pulp necrosis, especially since recuperation of the sensory functions appears to be possible. However, since this is a single case report with a relatively short follow-up period of 13 months, long-term randomized clinical trials with an adequate number of samples should be conducted before definitive conclusions can be reached. Given the nature of the problem this is a tall task.

Conflict of interest

There is no conflict of interest related to this case report.

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