Histologic Outcomes of Uninfected Human Immature Teeth Treated with Regenerative Endodontics: 2 Case Reports

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Abstract

A growing body of evidence exists showing the possibility of growing vital tissues in the root canal spaces of teeth with necrotic pulps and open apices. However, there is very limited histologic information regarding characteristics of tissues formed in the root canal space of human teeth after regenerative endodontics. The aim of this study was to examine clinically and histologically the outcomes of human immature teeth treated with regenerative endodontics. Two healthy birooted human maxillary first premolar teeth scheduled for extraction were included. Preoperative radiographs confirmed that these teeth had immature apices. Vitality tests showed the presence of vital pulps in these teeth. After receiving consent forms, the teeth were isolated with a rubber dam, and the pulps were completely removed. After the formation of blood clots in the canals, the teeth were covered with mineral trioxide aggregate. Four months later, the teeth were clinically and radiographically evaluated, extracted, and examined histologically. Both patients remained asymptomatic after treatment. Radiographic examination of the teeth showed signs of root development after treatment. Histologic examination of tissues growing into the root canal space of these teeth shows the presence of connective tissue, bone and cementum formation, and thickening of roots. Based on our findings, it appears that when canals of teeth with open apices are treated with regenerative endodontics, tissues of the periodontium grow into the root canals of these teeth. *(J Endod 2015;41:1725–1729)*

Key Words

Immature tooth, pulp regeneration, regenerative endodontics, stem cell

Pulpal necrosis in immature teeth often results in incomplete root development. These teeth often have thin root canal walls that are susceptible to fracture after treatment (1). Complete cleaning and shaping as well as obturation of these teeth are difficult or sometimes impossible. Apexification using a mineral trioxide aggregate (MTA) apical plug has been shown to produce successful outcomes (2). The main shortcoming of this procedure is the fact that is does not promote continuation of root development throughout the whole root, and these teeth remain susceptible to coronal root fracture. An ideal treatment outcome for these teeth is true pulpal regeneration and reestablishment of the pulp-dentin complex. The benefit of regenerative endodontics is not only revitalization of the tooth but also continued root development and, potentially, increasing fracture resistance. Regenerative endodontics has 3 critical steps: adequate disinfection of the root canal system, induction of bleeding to create a scaffold for stem cells, and coronal sealing of the blood clot with a biocompatible material (3, 4). Several case reports and case series have shown clinically successful outcomes and further root development after regenerative endodontic treatment (5, 6). Recent case reports and clinical studies have shown that root development as a possible indicator of pulp regeneration is not a predictable outcome (7–9). Among all aspects of root development (ie, increase in length, increase in root wall thickness, and apical closure), apical closure has been shown to be the most frequent finding (7), which could be unrelated to pulp regeneration (9). Hence, histologic analysis of tissues formed inside the root canal after regenerative treatment is important, especially regarding the differentiation of stem cells into odontoblasts. Histologic data on treatment outcomes in human teeth are limited to a few case reports. The available data show formation of mainly loose connective tissue in the root canal space and cementum/bonelike tissue deposited on the dentinal walls (1, 10–12) after the use of a blood clot or platelet-rich plasma (PRP) as a scaffold. The aim of this study was to examine clinically and histologically the outcomes of 2 noninfected human teeth treated with regenerative endodontics.

Materials and Methods

Two birooted fully erupted immature maxillary first premolar teeth scheduled for extraction as a part of orthodontic treatment from 2 patients aged 9 (a female) and 10 (a male) were included in this investigation. The study protocol was peer reviewed and approved by the Institutional Review Board at the Ghazvin University of Medical Sciences, Ghazvin, Iran (IRB# 28/20/9591; date of approval: June/02/2014). Written consent was obtained from the parents. Preoperative radiographs were taken to confirm the

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2015.05.004

presence of open apices and the absence of caries and restorations (Fig. 1*A1* and *B1*). Both teeth were treated by 1 clinician according to the following protocol:

After pulp testing using Endo Ice (Hygenic, Akron, OH) and an electrical pulp tester (Analytic Technology, Redmond, WA), local anesthesia was administered using 1.7 mL 3% Carbocaine without epinephrine (Novocol Pharmaceutical, Cambridge, Ontario, Canada). Each tooth was isolated with a rubber dam, and an access cavity was prepared using a high-speed bur and water spray. After establishing the working length radiographically 1 mm short of the open apex, the coronal thirds of the canals were enlarged using Gates Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland). All canals were instrumented using hand files (Dentsply Maillefer) to completely remove the pulp tissue. The canals in the 9-year-old patient were taken to a size 90 master file and those in the 10-year-old patient were taken to a size 60 master file. Between each instrument, the canals were gently irrigated with 1.25% sodium hypochlorite (NaOCl) at 1 mm short of the working length. After completion of instrumentation, all canals were irrigated with 5 mL 17% EDTA and dried with sterile paper points. Bleeding was induced by overextension of a size 30 hand file 2–3 mm beyond the working length. A blood clot was allowed to form for 10 minutes. MTA powder and liquid (ProRoot, Dentsply Tulsa Dental, OK) were mixed to a putty consistency, placed over the blood clot, and gently adapted to the dentinal walls. The access cavity of each tooth was filled with resin-modified glass ionomer cement (Fig. 1*A2* and *B2*).

The teeth were scheduled for extraction 4 months after treatment. Before extraction, a periapical radiograph was taken to evaluate the continuation of root development (Fig. 1*A3* and *B3*). Cold and electrical pulp vitality tests were performed, and results were recorded. After obtaining local anesthesia, the teeth were extracted. The teeth were immediately placed in 10% formaldehyde for fixation. Both teeth were decalcified in 7% formic acid. Complete decalcification of the specimens was confirmed radiographically. After decalcification, the specimens were rinsed with running tap water for 2 hours, dehydrated with ascending concentrations of alcohol (70%, 90%, and 100%), and embedded in paraffin. The glass ionomer restorations were removed gently before embedding. Five-micrometer-thick labiolingual sections were prepared serially, and specimens were stained with hematoxylin-eosin. Samples were evaluated microscopically (Zeiss, Goettingen, Germany) by 2 independent oral pathologists to determine the histologic features of tissues formed within the root canal spaces of teeth after regenerative endodontics.

Results

Clinical examinations of patients after 4 months showed that they were asymptomatic after regenerative endodontics. The 4-month follow-up radiographs showed progression of root development and maturation of the roots in both patients (Fig. 1*A* and *B*). Both teeth did not respond to cold and electrical pulp tests before extraction.

The sections from the first premolar in the 9-year-old female revealed the presence of soft and hard tissues within the 2 roots. The roots showed a well-developed dentin layer surrounded by a periodontal ligament (PDL) on the outer root margins. Foreign body material (MTA) was noted in the coronal portions of this tooth. A thick layer of hard tissue was observed beneath this material (Fig. 2*A*). There was a fibrotic collagenous soft tissue with hypercellular and hypervascualr connective tissue supporting hard tissue (Fig. 2*B*) consistent with osteocementum. The hard tissues within the root canals have numerous resting lines (Fig. 2*C* and *D*). Additionally, the osteodentin showed osteoblastic rimming in places (Fig. 2*C*). Several epithelial rests are also observed within the cellular fibrotic pulp chamber (Fig. 2*E*). No inflammatory cells were found within the canal spaces. The tissues within the roots of this tooth displayed features mimicking odontogenic fibroma–PDL–type histology.

The sections from the first premolar in the 10-year-old male revealed portions of roots showing well-developed dentin surrounded by PDL on the outer root margins. A well-preserved primary dentin layer was identified surrounding the canal spaces (Fig. 3*A*). The dentin layer in this tooth was much thicker than in the other specimen. Foreign body material (MTA) was also observed in the coronal portions of this tooth (Fig. 3*B*). The hard tissue juxtaposed to the foreign body material (MTA) was not as thick as that observed in the other specimen. There was a fibrotic connective tissue within the canal spaces and occasional

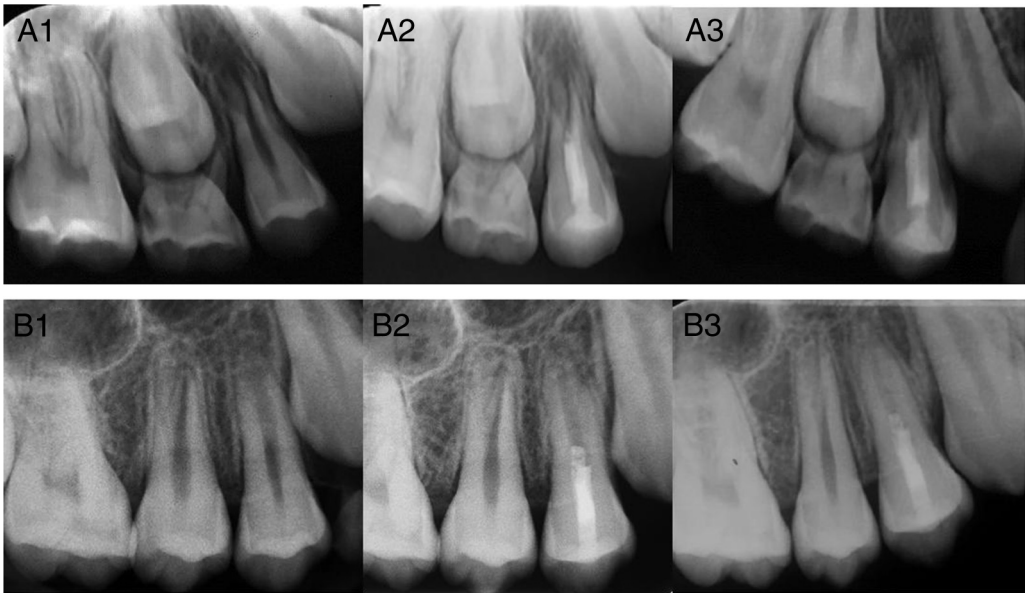

Figure 1. (*A*) Case #1. (*B*) Case #2. From left to right: (*1*) preoperative radiograph, (*2*) immediate postoperative radiograph, and (*3*) recall radiograph after 4 months before extraction. Root development is evident in both teeth.