Pulp Revascularization on Permanent Teeth with Open Apices in a Middle-aged Patient

Yu Wang, DDS, * Xiaofei Zhu, DDS, † and Chengfei Zhang, DDS, $PhD^{\sharp J}$

Abstract

Pulp revascularization is a promising procedure for the treatment of adolescents' immature permanent teeth with necrotic pulp and/or apical periodontitis. However, the ability to successfully perform pulp revascularization in a middle-aged patient remains unclear. A 39-year-old woman was referred for treatment of teeth #20 and #29 with necrotic pulp, extensive periapical radiolucencies, and incomplete apices. Pulp revascularization procedures were attempted, including root canal debridement, triple antibiotic paste medication, and platelet-rich plasma transplantation to act as a scaffold. Periapical radiographic and cone-beam computed tomographic examinations were used to review the changes in the apical lesions and root apex configuration. The patient remained asymptomatic throughout the 30-month follow-up. Periapical radiographic examination revealed no change in the apical lesions of either tooth at 8 months. The periapical radiolucency disappeared on tooth #20 and significantly decreased on tooth #29 by the 30-month follow-up, findings that were also confirmed by cone-beam computed tomographic imaging. No evidence of root lengthening or thickening was observed. Successful revascularization was achieved in a middle-aged patient's teeth. (J Endod 2015;41:1571-1575)

Key Words

Apical periodontitis, middle-aged patient, pulp revascularization

0099-2399/\$ - see front matter

Copyright o 2015 American Association of Endodontists. http://dx.doi.org/10.1016/j.joen.2015.04.022

Dulp revascularization is widely performed in the treatment of immature permanent teeth with necrotic pulp and apical periodontitis. Successful cases exhibited thickening of the canal walls, closure of root apices, continued root development, and recovery of a positive response to electric pulp testing in some cases (1, 2). Regenerative endodontic procedures have routinely been recommended for adolescent patients between 8 and 16 years old (3). Sparse reports are available on pulp revascularization in the mature permanent dentition. Although some researchers claim that the treatment outcome from regenerative endodontics does not vary according to patient age (4), the upper limit of participant age was only 28 years in a systematic review that included 51 clinical studies and case reports from January 1993 to December 2013 (5). Therefore, it is still unknown whether patient age is a critical factor in the success of pulp revascularization. Studies on bone marrow mesenchymal stem cells suggest that the total number and osteoprogenitor (CFU-F/ALP+) colonies of mesenchymal stem cells decrease with age (6, 7). Bone marrow mesenchymal stem cells and dental stem cells (including those of the dental pulp, periodontal ligament, and apical papilla) share some similarities (8); the outcome of dental pulp revascularization may also be affected by patient age.

The principles of revascularization include the use or recruitment of stem cells, growth factors, and biologic scaffolding (9). Platelet-rich plasma (PRP) has been suggested as an ideal biomaterial to improve treatment results because it releases many growth factors and acts as a scaffold (10, 11). Successful revascularization of infected immature teeth after autologous PRP transplantation has been reported (12–14).

In the present case report, we describe a PRP-supplemented revascularization of 2 nonvital permanent teeth with periapical lesions and open apices in a 39-year-old woman.

Case Report

A 39-year-old woman was referred to our department for treatment of her right and left second mandibular premolars (teeth #20 and #29). The patient visited her general dentist 1 week earlier with sharp pain of 3 days' duration in the left second premolar. She received access opening, medication with camphor phenol, and temporary seal. The dentist documented no caries but found fractured dens evaginatus in both mandibular second premolars. Her medical status was noncontributory.

Upon clinical examination at our clinic, temporary fillings were found in the center of occlusal surfaces of teeth #20 and #29, with slight tooth discoloration and no evidence of sinus tract stomata (Fig. 1*A*–*D*). These 2 teeth were not sensitive to percussion or palpation and did not respond to thermal or electric pulp testing (Vitality Scanner; SybronEndo, Glendora, CA). Periapical radiographic examination revealed extensive periapical radiolucencies and incomplete apices on both left and right second mandibular premolars (Figs. 2*A* and 3*A*). Cone-beam computed tomographic (CBCT) images (NewTom VG; Quantitative Radiology, Verona, Italy) further characterized the extent of the bony defects and the large diameter of roots and apices (Figs. 2*D*–*F* and 3*D*–*F*). The diagnosis of necrotic pulp and symptomatic apical periodontitis was made for both second mandibular premolars.

The patient was informed that the goal of treatment was to initiate healing of the bony defects and to stimulate further root lengthening and thickening and that the proposed treatment might not be successful. The decision was made to perform an

From *Paediatric Dentistry and [†]VIP Dental Service and Geriatric Dentistry, School and Hospital of Stomatology, Peking University, Beijing, China, and [†]Shenzhen Institute of Research and Innovation and [§]Comprehensive Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong, China.

Address requests for reprints to Dr Chengfei Zhang, Comprehensive Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong, China. E-mail address: zhangcf@hku.hk



Figure 1. Preoperative photographs of teeth #20 and #29. (*A* and *C*) Buccal view photographs showed slight discoloration with no sign of sinus tracts or swelling. (*B* and *D*) Occlusal view photographs showed temporary fillings.

endodontic revascularization procedure with the aid of PRP. Written informed consent was obtained from the patient.

The clinical procedure generally followed consideration of the guidelines proposed by the American Association of Endodontists (http://www.aae.org/regenerativeendo). At the initial appointment, the temporary fillings and cotton pellets in the pulp chambers of teeth #20 and #29 were removed under local anesthesia and rubber dam isolation. Upon entry to the root canal system, no hemorrhage or necrotic pulp was noted. Each canal was irrigated with approximately 20 mL 2.5% sodium hypochlorite followed by saline (20 mL/canal). After drying with paper points, the root canals were medicated with triple antibiotic paste (equal parts ciprofloxacin, metronidazole, and minocycline to a final concentration of 0.1 mg/mL) and sealed with Cavit (ESPE, Chergy Pontoise, France).

At the 2-week recheck, the patient reported no symptoms. Both teeth were asymptomatic to percussion and palpation. A 10-mL sample of venous blood was obtained from the patient's left arm in a plastic dipotassium EDTA tube (Vacuette; Greiner Bio-One, Kremsmünster, Austria) for PRP preparation. The involved teeth were anesthetized, isolated with a rubber dam, and reaccessed. The antibiotic paste was flushed out with sterile saline irrigation. The canals were dried with paper points. During root canal irrigation and drying, the patient's blood sample was first centrifuged at 2400 rpm for 10 minutes to separate PRP and platelet-poor plasma from the red blood cells and then at 3600 rpm for 10 minutes to separate the PRP from the platelet-poor plasma. The PRP was then injected into the canal space up to the level of the cementoenamel junction, and several minutes were allowed for clot formation. White mineral trioxide aggregate (Dentsply Tulsa Dental, Johnson City, TN) was placed directly over the PRP clot. Moist cotton pellets were placed over the mineral trioxide aggregate, and the teeth were restored with Cavit. The patient returned to our department 48 hours later and was asymptomatic. The temporary fillings and cotton pellets were then replaced with composite (P60; 3M Dental Products, St Paul, MN).

Results

At follow-up appointments at 8 and 30 months, the patient remained asymptomatic. Percussion, palpation, thermal, and electric pulp tests were all negative. Periapical radiographic examination revealed no change in the apical lesions of either involved tooth at the 8-month follow-up (Figs. 2B and 3B). Thirty months after the initial treatment, periapical radiographs showed disappearance of the periapical radiolucency on tooth #20 and a significant shrinkage of the periapical lesion on tooth #29 (Figs. 2C and 3C). CBCT imaging at the 30-month follow-up further confirmed the findings on periapical radiographs (Figs. 2G–I and 3G–I). Bone healing around the root apex of tooth #20 was clearly observed (Fig. 2G–I). Although a defect remained in the buccal alveolar bone adjacent to the root of tooth #29, bone reconstruction and radiolucency reduction were apparent compared with the earlier images (Fig. 3D–I). No evidence of root lengthening or thickening was found on the CBCT scan.

Discussion

We performed pulp revascularization on teeth #20 and #29 in a 39-year-old woman with PRP as a scaffold. The periapical lesion of 1 tooth had healed; another one was healing after the 2.5-year follow-up. In the current literature, pulp revascularization has primarily been conducted on immature permanent teeth in teenagers (15). The treatment protocol recommended by the American Association of Endodontists is now routinely followed to maximally achieve continued root development in immature permanent teeth. However, there is no consensus on whether pulp revascularization is practical in the management of teeth with apical periodontitis in adult patients. Clinically, in teenagers, periapical radiolucency disappeared within 12 months after successful pulp revascularization (12, 16–18). In the present report, no sign of periapical healing was detected on radiographs at 8 months. After 2.5 years, tooth #29 displayed only partial bone

Case Report/Clinical Techniques

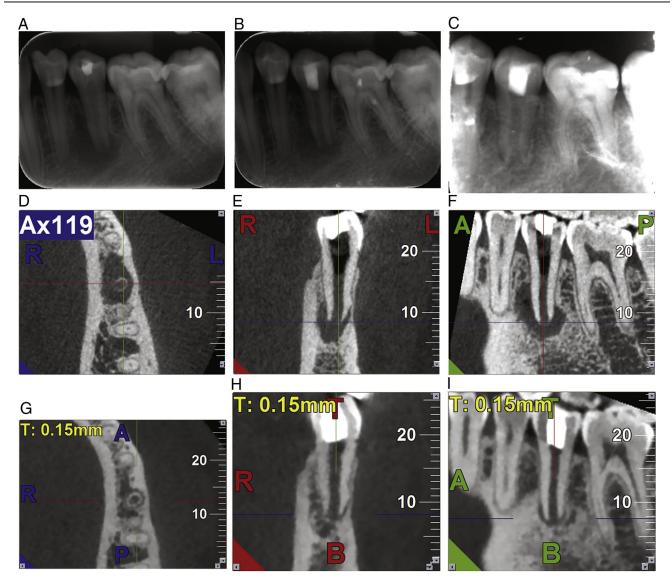


Figure 2. Periapical radiographs and CBCT sections of tooth #20. (*A*) Preoperative periapical radiograph showing extensive periapical radiolucency and open apex. (*B*) Radiograph taken at the 8-month recall. The periapical lesion has not changed in size. (*C*) Periapical radiolucency resolved 30 months after the initial treatment as shown on this periapical radiograph. (D–F) Preoperative CBCT scan. Images from different angles showed the extent of bone defect. (G–I) Thirty-month follow-up CBCT scan revealed bone healing around root apex. Root length and thickness remain unchanged compared with preoperative CBCT examination. The *yellow* T indicates image thickness; the *red* T indicates top aspect. A, anterior aspect; B, bottom aspect; P, posterior aspect; R, right side.

healing. The comparatively delayed bone healing in our patient is likely related to her age. Two aspects of aging could affect tissue rehabilitation: intrinsic changes in stem cells and changes in the local environment that regulate the biological properties of stem cells (19). Adult progenitor cells, existing essentially in all tissues, modulate tissue homeostasis and regeneration. Depletion and/or senescence of these cells may result in age-related tissue degeneration as well as decreased regenerative potential (20, 21). Animal studies have shown significantly reduced bone regeneration capacity with aging (22, 23). When compared with 4week-old mice, middle-aged (6 months) and old mice (18 months) present less vascularized fracture callus and less bone regeneration (22). Up-regulation of the inflammatory response results in a lowlevel, long-lasting, systemic proinflammatory state, which underlies most age-associated diseases (24). Therefore, a proinflammatory state may also delay bone healing in older individuals. Aged mice have higher levels of interleukin 6 and tumor necrosis factor alpha and 60% less bone formation than young mice (25). In a recent published systematic review of 51 relevant publications, most teeth treated with regenerative endodontic procedures showed resolution of periapical radiolucencies, increased root length and root wall thickness, and apical closure during the follow-up period (5). Among these 51 publications, only 3 articles included patients who were over 18 years old, and no patient was older than 28 years. Our findings indicate that a longer period is needed for healing of periapical radiolucencies in middle-aged patients. Even the 2 teeth in this report exhibited different bone healing progress. Tooth #29, which had both cortical bone and cancellous bone loss, took longer to heal than tooth #20, which had only cancellous bone loss. Further reviews are ongoing.

In this case, there was no evidence of apical closure, root lengthening, root wall thickening, or pulp vitality. Similar results have been reported in some teenaged patients after revascularization procedures (26-28). Unsatisfactory treatment outcomes are often associated with

Case Report/Clinical Techniques

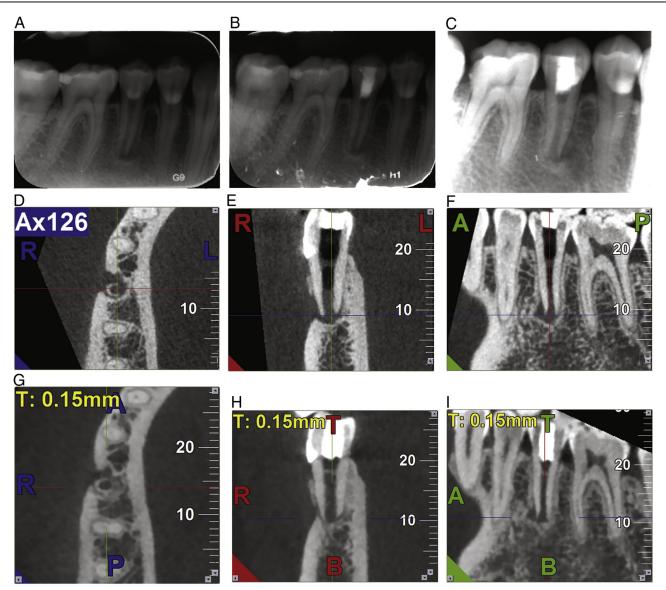


Figure 3. Periapical radiographs and CBCT sections of tooth #29. (*A*) Preoperative periapical radiograph showing extensive periapical radiolucency and open apex. (*B*) Radiograph taken at 8-month recall. The periapical lesion has not changed in size. (*C*) Periapical radiolucency reduced in size after 30 months. (D–F) Preoperative CBCT image. Images from different angles showed the extent of bone defect, especially cortical bone loss on the buccal aspect. (*G*–*I*) Thirty-month follow-up CBCT image revealed bone reconstruction and reduction of radiolucency. Root length and thickness remain unchanged compared with the preoperative CBCT scan. The *yellow* T indicates image thickness; the *red* T indicates top aspect. A, anterior aspect; B, bottom aspect; P, posterior aspect; R, right side.

long-standing periapical infection. Chronic periapical infections can compromise the vitality of the Hertwig epithelial root sheath, which is associated with root development (26-28). In another aspect, fewer stem/progenitor cells exist in mature teeth than in immature teeth (29). In the present report, immature apices implied that root development became arrested when the patient was approximately 12 years old. It is highly likely that necrosis and infection were present for over 20 years. In these cases in which Hertwig epithelial root sheath (HERS) vitality has been lost, root development ceases (30).

To enhance revascularization efficacy, PRP was used instead of a blood clot in our procedure. PRP contains several growth factors and forms a 3-dimensional fibrin matrix that acts as a scaffold (10, 11). PRP is extensively applied in regenerative dentistry and reported to be a suitable scaffold for revascularization of infected immature teeth (12-14). However, some studies have found that the effects of PRP

were minimal in enhancing dental pulp revascularization and bone regeneration (10, 31).

In the present case, we irrigated the canals with 2.5% sodium hypochlorite and medicated the root canals with triple antibiotic paste. Chlorhexidine, as an effective irrigant against microorganisms, is frequently used in nonsurgical root canal disinfection (32). However, we did not use it because chlorhexidine irrigant was reported to be detrimental to the survival of stem cells (33). When we conducted the revascularization procedures for this case in 2011, we did not use a final irrigation with 17% EDTA, which is highly recommended by the American Association of Endodontists in recent years (http://www.aae.org/regenerativeendo). It was reported that 17% EDTA irrigation could enhance the release of dentin-derived growth factors, which subsequently facilitated stem cell survival and function (33, 34). Triple antibiotic paste is a well-known and extensively used disinfectant for

pulp revascularization. It has been thought that triple antibiotic paste is more effective in killing bacterial than calcium hydroxide (35). In contrast, recent publications showed that high concentrations of triple antibiotics are detrimental to dental stem cell survival, whereas lower concentrations as well as $Ca(OH)_2$ at all tested concentrations favor stem cell survival and proliferation (36, 37). Therefore, intracanal medication with appropriate concentrations with an adequate bactericidal effect and minimal side effects on stem cell viability is a critical step for pulp revascularization. Furthermore, calcium hydroxide could be more effectively removed from root canals than triple antibiotic paste (38).

In conclusion, the pulp revascularization procedures performed on this individual middle-aged patient achieved healing of the periapical radiolucency by bone deposition.

Acknowledgments

Yu Wang and Xiaofei Zhu contributed equally to this study. Supported by the Program for New Clinical Techniques and Therapies of Peking University School and Hospital of Stomatology. The authors deny any conflicts of interest related to this study.

References

- Kahler B, Mistry S, Moule A, et al. Revascularization outcomes: a prospective analysis of 16 consecutive cases. J Endod 2014;40:333–8.
- Ding RY, Cheung GS, Chen J, et al. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. J Endod 2009;35:745–9.
- Garcia-Godoy F, Murray PE. Recommendations for using regenerative endodontic procedures in permanent immature traumatized teeth. Dent Traumatol 2012;28: 33–41.
- Jeeruphan T, Jantarat J, Yanpiset K, et al. Mahidol study 1: comparison of radiographic and survival outcomes of immature teeth treated with either regenerative endodontic or apexification methods: a retrospective study. J Endod 2012;38: 1330–6.
- Kontakiotis EG, Filippatos CG, Agrafioti A. Levels of evidence for the outcome of regenerative endodontic therapy. J Endod 2014;40:1045–53.
- Delorme B, Chateauvieux S, Charbord P. The concept of mesenchymal stem cells. Regen Med 2006;1:497–509.
- D'Ippolito G, Schiller PC, Ricordi C, et al. Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. J Bone Miner Res 1999;14:1115–22.
- Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 2009;88:792–806.
- 9. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. J Endod 2007;33:377–90.
- Zhu X, Zhang C, Huang GT, et al. Transplantation of dental pulp stem cells and platelet-rich plasma for pulp regeneration. J Endod 2012;38:1604–9.
- Hargreaves KM, Giesler T, Henry M, Wang Y. Regeneration potential of the young permanent tooth: what does the future hold? J Endod 2008;34:851–6.
- Jadhav G, Shah N, Logani A. Revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: a pilot clinical study. J Endod 2012;38: 1581–7.
- Martin G, Ricucci D, Gibbs JL, Lin LM. Histological findings of revascularized/revitalized immature permanent molar with apical periodontitis using platelet-rich plasma. J Endod 2013;39:138–44.

- Torabinejad M, Turman M. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: a case report. J Endod 2011;37:265–8.
- Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol 2001;17:185–7.
- Bezgin T, Yilmaz AD, Celik BN, Sonmez H. Concentrated platelet-rich plasma used in root canal revascularization: 2 case reports. Int Endod J 2014;47:41–9.
- Kim JH, Kim Y, Shin SJ, et al. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. J Endod 2010;36: 1086–91.
- Cehreli ZC, Isbitiren B, Sara S, Erbas G. Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: a case series. J Endod 2011;37:1327–30.
- Hadjiargyrou M, O'Keefe RJ. The convergence of fracture repair and stem cells: interplay of genes, aging, environmental factors and disease. J Bone Miner Res 2014;29:2307–22.
- Saeed H, Abdallah BM, Ditzel N, et al. Telomerase-deficient mice exhibit bone loss owing to defects in osteoblasts and increased osteoclastogenesis by inflammatory microenvironment. J Bone Miner Res 2011;26:1494–505.
- Richardson GD, Breault D, Horrocks G, et al. Telomerase expression in the mammalian heart. FASEB J 2012;26:4832–40.
- Lu C, Hansen E, Sapozhnikova A, et al. Effect of age on vascularization during fracture repair. J Orthop Res 2008;26:1384–9.
- Meyer MH, Meyer RA Jr. Genes with greater up-regulation in the fracture callus of older rats with delayed healing. J Orthop Res 2007;25:488–94.
- 24. Salvioli S, Capri M, Valensin S, et al. Inflamm-aging, cytokines and aging: state of the art, new hypotheses on the role of mitochondria and new perspectives from systems biology. Curr Pharm Des 2006;12:3161–71.
- Wahl EC, Aronson J, Liu L, et al. Restoration of regenerative osteoblastogenesis in aged mice: modulation of TNF. J Bone Miner Res 2010;25:114–23.
- Chen MY, Chen KL, Chen CA, et al. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. Int Endod J 2012;45:294–305.
- Nosrat A, Homayounfar N, Oloomi K. Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic immature teeth: a literature review and report of a case. J Endod 2012;38:1428–34.
- Nosrat A, Li KL, Vir K, et al. Is pulp regeneration necessary for root maturation? J Endod 2013;39:1291–5.
- Paryani K, Kim SG. Regenerative endodontic treatment of permanent teeth after completion of root development: a report of 2 cases. J Endod 2013;39:929–34.
- Lin LM, Rosenberg PA. Repair and regeneration in endodontics. Int Endod J 2011; 44:889–906.
- Ranly DM, Lohmann CH, Andreacchio D, et al. Platelet-rich plasma inhibits demineralized bone matrix-induced bone formation in nude mice. J Bone Joint Surg Am 2007;89:139–47.
- Gomes BP, Vianna ME, Zaia AA, et al. Chlorhexidine in endodontics. Braz Dent J 2013;24:89–102.
- 33. Trevino EG, Patwardhan AN, Henry MA, et al. Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. J Endod 2011;37:1109–15.
- Diogenes AR, Ruparel NB, Teixeira FB, Hargreaves KM. Translational science in disinfection for regenerative endodontics. J Endod 2014;40:852–7.
- Adl A, Shojaee NS, Motamedifar M. A comparison between the antimicrobial effects of triple antibiotic paste and calcium hydroxide against *Enterococcus faecalis*. Iran Endod J 2012;7:149–55.
- Ruparel NB, Teixeira FB, Ferraz CC, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod 2012;38:1372–5.
- Chuensombat S, Khemaleelakul S, Chattipakorn S, Srisuwan T. Cytotoxic effects and antibacterial efficacy of a 3-antibiotic combination: an *in vitro* study. J Endod 2013; 39:813–9.
- Berkhoff JA, Chen PB, Teixeira FB, Diogenes A. Evaluation of triple antibiotic paste removal by different irrigation procedures. J Endod 2014;40:1172–7.