Archives of Oral Biology 71 (2016) 122–128

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Histologic characterization of regenerated tissues after pulp revascularization of immature dog teeth with apical periodontitis using tri-antibiotic paste and platelet-rich plasma

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ARTICLE INFO

Article history:
Received 15 November 2015
Received in revised form 12 May 2016
Accepted 25 July 2016

Keywords:
Apical negative pressure irrigation
Immature tooth
Mineral trioxide aggregate
Platelet-rich plasma
Pulp regeneration
Pulp revascularization
Scaffold
Tri-antibiotic paste

ABSTRACT

Introduction: This study evaluates histologically the efficacy of 4 revascularization protocols in necrotic-infected immature dog teeth with apical periodontitis (AP).
Methods: Forty double-rooted immature premolar teeth from 4 female Beagle dogs aged 5 months were used. Four teeth were left untouched as negative controls; the other 36 teeth were infected to develop pulp necrosis and AP. Four teeth were left untreated and assigned to the positive control group. The last 28 teeth were randomly assigned into four experimental groups of 8 teeth, each one treated with a different treatment protocol: A1, sodium hypochlorite (SH) + blood clot (BC); A2, SH + platelet-rich plasma (PRP); B1, SH + modified tri-antibiotic paste (mTAP) + BC; B2, SH + mTAP + PRP. The animals were sacrificed, histologic sections were prepared and three parameters were assessed: (1) presence or absence of new hard tissue on the internal root dentinal walls, (2) presence or absence of continued apical closure, and (3) presence or absence of vital tissue within the canal space.
Results: Significant differences ($p < 0.05$) between the four experimental groups were evident in the percentage of teeth showing histological apical closure (34.5%) and vital tissue within the canal space (68.8%). Group B2 showed the maximal improvement in the three variables assessed ($p < 0.05$). Group A1 showed the minimum percentages in the three parameters assessed ($p < 0.05$).
Conclusions: These results suggest that an intracanal dressing of mTAP, and the use of PRP as scaffold, improves the success rate of the revascularization procedure.

1. Introduction

Apexification with a long-term calcium hydroxide application has been the most accepted treatment option for immature permanent tooth with pulp necrosis (Rafter, 2005). However, this treatment has several disadvantages: requires multiple visits during a long period, hindering patient's follow-up (Kleier and Barr, 1991). Moreover, long-term calcium hydroxide therapy may leave thin dentinal walls even more prone to fracture (Andreasen, Farik, & Munksgaard, 2002).

An alternative to conventional calcium hydroxide apexification was suggested by Torabinejad and Chivian (1999), who proposed to seal the open apex with mineral trioxide aggregate (MTA) in one visit. Nevertheless, this technique could promote apical repair but does not reduce the risk of future fracture because the root width will not increase (Jeeruphan et al., 2012).

Revascularization procedure (RP) is a conservative and effective method for inducing maturogenesis in necrotic immature teeth, increasing thickening of the canal walls by deposition of hard tissue and encouraging continued root development (Jadhav, Shah, & Logani, 2012). Revascularization has been proposed to treat immature permanent teeth with necrotic pulp tissue and/or apical periodontitis/abscess (Iwaya, Ikawa, & Kubota, 2001). In RP of immature permanent teeth the disinfection protocol is a main aspect because the tooth is not mechanically cleaned to its full length (Iwaya et al., 2001). A copiously irrigation of root canal and

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http://dx.doi.org/10.1016/j.archoralbio.2016.07.007
0003-9969/

dressing with antimicrobial agents are needed. Amongst the disinfection protocols proposed in RP are the following: conventional irrigation with sodium hypochlorite in combination of tri-antibiotic paste (TAP) (a mixture of ciprofloxacin, metronidazole, and minocycline) (Thibodeau et al., 2007; Windley et al., 2005) the intra-canal dressing with calcium hydroxide (Chueh and Huang, 2006), and most recently the irrigation with apical negative pressure (ANP) has shown similar bacterial reduction to conventional irrigation with sodium hypochlorite plus intra-canal dressing with TAP (Hockett, Dommisch, Johnson, & Cohenca, 2008; Cohenca et al., 2010). A problem that often accompanies the intracoronal use of TAP containing minocycline is dentin discoloration (Rodríguez-Benitez, Stambolsky, Gutiérrez-Pérez, Torres-Lagares, & Segura-Egea, 2015), but it has been confirmed that the incorporation of cefaclor in the TAP, instead of minocycline, avoids discoloration (Miller et al., 2012).

Initial studies on revascularization, after disinfection of the root canal, induced a hemorrhage to form a blood clot into the canal to act as a scaffold to aid the in-growth of new tissue into the empty canal space (Thibodeau et al., 2007). It is hypothesized that the blood clot serves as a matrix for migration of progenitor cells into the canal, possibly from the apical papilla (Thibodeau et al., 2007). Collagen solutions (Thibodeau et al., 2007; Yamauchi et al., 2011) and platelet–rich plasma (PRP) (Torabinejad & Faras, 2012; Torabinejad & Turman, 2011) have been mentioned as a potentially ideal scaffold for RP.

If a thorough disinfection of the canal space is accomplished before filling it with a scaffold, then successful revascularization of immature permanent human teeth with apical periodontitis has been demonstrated, just as it is for the uninfected canals in case of tooth avulsion (Banchs and Trope, 2004; Cvek et al., 1990; Petrino et al., 2010).

The aim of this study was to assess histologically the ability of four different protocols, combining two type of scaffolds (Blood Clot and PRP) and two disinfection procedures (Sodium Hypochlorite with ANP using the Endovac® system and Tri-Antibiotic Paste) to obtain revascularization of necrotic-infected immature dog teeth with apical periodontitis.

2. Materials and methods

2.1. Study groups

This study was made with the approval of the Ethical Committee of the University. Forty double-rooted premolar teeth from 4 female Beagle dogs aged 5 months were randomly divided into 4 experimental groups of 8 teeth each (16 roots), a positive control group (4 teeth, 8 roots) and a negative control group (4 teeth, 8 roots).

Before any interventions, the involved teeth were radiographically throughout (Kodak RVG 6100® Digital Radiography System, Carestream Health, Inc. Rochester, NY, USA) using radiograph paralleling devices (Dentsply Rinn, Elgin, IL, USA) to confirm incomplete root formation and open apices. These radiographic aids were used for all subsequent radiographs to improve the alignment and position of the films and x-ray beam for direct comparison of the radiographs with minimal distortion or magnification.

All interventions were made under general anesthesia (induction by Zolazepam hydrochloride [Zoletil 100, Virbac España, S.A., Spain] 0.1 mL/kg intravenously and intubation and maintenance with isoflurane [Isoflo, Abbott Laboratories Ltd., Berkshire, UK.]) supplemented with local anesthesia (Lidocaine 5%, B. Braun Medical, S.A, Barcelona, Spain).

In the first treatment session, the teeth of the negative control group were left untouched for natural development for comparison with the experimental and positive control teeth. The pulps of 32 experimental and 4 positive control teeth were infected according to the protocol described previously by Leonardo et al. (1993). The pulps were mechanically exposed using a #12 diamond bur in a high-speed hand-piece with copious saline solution. Then, each pulp was disrupted with a #20 sterile stainless steel endodontic hand file (Colorinox®, Dentsply Maillefer. Ballaigues. Switzerland). This procedure was repeated individually on each dog and the root canals were left exposed to the oral cavity for 7 days to allow microbial contamination. The animals were given analgesics (Torbugesic 0.2 mg/kg; Butorphanol Tartrate, Fort Dodge Animal Health, Fort Dodge, IA) postoperatively following this and all operative procedures and were monitored in the postoperative period. After 1 week, the coronal access was sealed with Cavit® (ESPE, Norristown, PA), without intracanal dressing. The teeth were monitored radiographically by using paralleling devices until there was radiographic evidence of apical periodontitis (AP), which occurred within 15–25 days. Once the injuries were radiographically visible, 32 teeth were randomly assigned into 4 groups of 8 teeth, each following different treatment protocols, and 4 teeth were assigned to the positive control group, in which no further treatment was carried out.

Under general and local anesthesia, all previously infected teeth were isolated with a rubber dam, and the operative field was disinfected with 30% hydrogen peroxide until no bubbling occurred. All surfaces were then coated with tincture of iodine and allowed to dry. The temporary restoration was removed with a sterilized round bur #12 in a high-speed handpiece. Using #40 K-file (Colorinox®, Dentsply Maillefer, Ballaigues. Switzerland), the working length (WL) was established radiographically 1 mm short of the radiographic apex. Then, four teeth were left with no further treatment as positive controls, and each experimental group was treated according to 4 different protocols, as follows:

Group A1: disinfection with sodium hypochlorite (SH) and blood clot (BC) as scaffold; Group A2: disinfection with SH and PRP as scaffold; Group B1: disinfection with SH and a modified tri-antibiotic paste (mTAP) dressing during 15 days, and BC blood clot as scaffold; Group B2: disinfection with SH and mTAP dressing, and PRP as scaffold.

2.1.1. Group A1: sodium hypochlorite/blood clot

The canals were disinfected with 20 mL of 1.25% NaOCl (sodium hypochlorite; Sigma-Aldrich Química SA, Madrid, Spain) using the apical negative pressure irrigation ANP-Endovac® system (Discus Dental, Culver City, CA, USA). Taking into account that immature teeth with open apices were being treated, the ANP-Endovac® system was modified to avoid the extrusion of the sodium hypochlorite solution to the apical tissues, as described by Cohenca et al. (2010). Canals were irrigated using the macro-cannula only after being gauged to fit the apical size of the root.

The canals were left filled with sodium hypochlorite solution 1.25% for 3 min and then irrigated with 10 mL sterile saline solution to remove the rest of sodium hypochlorite. Then, a final irrigation was accomplished using 1 mL of 17% EDTA (Ultradent Products Inc. South Jordan, Utah, USA) for 60 s (Yamauchi et al., 2011).

The root canals were dried with sterile paper points and a sterile #30 K-file (Colorinox®, Dentsply-Maillefer, Ballaigues. Switzerland) was used to stimulate bleeding for clot formation. The bleeding was stopped at the level of the cementum-enamel junction by using a small cotton pellet soaked with sterile saline. After 10 min, the blood clot was formed. Over the clot, a collagen sponge (Collacote®, Integra Lifesciences Corporation, Plainsboro, NJ, USA) was set. Next, a 4 mm plug of MTA (Proroot®, Denstply, Tulsa Dental, Johnson City, TN, USA) was inserted into the canals using an MTA carrier (Hartzell & Son, CA, USA) to seal the root canal at the cervical level. The MTA plug was verified radiographically.