Pulp Revascularization of Immature Dog Teeth with Apical Periodontitis Using Triantibiotic Paste and Platelet-rich Plasma: A Radiographic Study

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Abstract

Introduction: This study evaluates radiographically 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 untreated as negative controls; the other 36 teeth were infected to develop pulp necrosis and AP following different treatment protocols. Four teeth were left untreated and assigned to the positive control group, and the last 28 teeth were randomly assigned into 4 experimental groups of 8 teeth: A1, sodium hypochlorite (NaOCl) + a blood clot; A2, NaOCl + platelet-rich plasma (PRP); B1, NaOCl + modified triantibiotic paste (mTAP) + a blood clot; and B2, NaOCl + mTAP + PRP. Teeth were monitored radiographically for 6 months regarding healing of periapical radiolucencies, thickening of the dentinal walls, and apical closure of roots. Results: Significant differences (P < .05) between the 4 groups were evident in the percentage of teeth showing improvement of periapical radiolucencies (62.5%), continued radiographic thickening of radicular walls (53.1%), radiographic apical closure (43.8%), and deposition of hard tissue on radicular dentin walls (53.1%). Group B2 showed maximal improvement in the 3 variables assessed (P < .05). Group A1 showed the minimum percentages in the 3 parameters assessed (P < .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. (J Endod 2015; ▶:1–6)

Key Words

Apical negative pressure irrigation, immature tooth, mineral trioxide aggregate, platelet-rich plasma, pulp regeneration, pulp revascularization, scaffold, triantibiotic paste

In the last years, revascularization procedures have been proposed to treat immature permanent teeth with necrotic pulp tissue and/or apical periodontitis/abscess (1). Revascularization 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 (2). In this endodontic therapy, the immature permanent tooth is not mechanically cleaned to its full length but is copiously irrigated and dressed with antimicrobial agents, which is the most important aspect of the revascularization procedure (1).

Several disinfection protocols have been proposed in the revascularization treatment including conventional irrigation with sodium hypochlorite (NaOCl) in combination of triantibiotic paste (TAP) (a mixture of ciprofloxacin, metronidazole, and minocycline) (3, 4) and the intracanal dressing with calcium hydroxide (5). Most recently, irrigation with apical negative pressure (ANP) has shown similar bacterial reduction to conventional irrigation with NaOCl plus intracanal dressing with TAP (6, 7).

After disinfection of the root canal, the induction of a hemorrhage to form a blood clot into the canal to act as a scaffold aids the ingrowth of new tissue into the empty canal space (4). The blood clot serves as a matrix for the migration of progenitor cells from the apical papilla into the canal (4). Other potential scaffolds for regenerative endodontic treatment regimens have been proposed such as collagen solutions (4, 8) and platelet-rich plasma (PRP) (9, 10).

Previous radiographic studies have shown thickening of the root canal walls and subsequent apical closure (1, 5, 9, 11–13) of immature permanent human teeth with apical periodontitis after a systematic disinfection of the canal space and filling with a scaffold.

This study aims to assess radiographically the ability of 4 different protocols, combining 2 type of scaffolds (blood clot and PRP) and 2 disinfection procedures (NaOCl with ANP using the EndoVac system [Discus Dental, Culver City, CA] and TAP) to obtain revascularization of necrotic-infected immature dog teeth with apical periodontitis.

Materials and Methods

This study was approved by the ethical committee of our 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 radiographed (Kodak RVG 6100 Digital Radiography System; Carestream Health, Inc, Rochester, NY) using radiograph paralleling devices (Dentsply Rinn, Elgin, IL) 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 [Zolelt; 100; Virbac España, SA, Esplugues de Llobregat, Barcelona, 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, SA, 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 (14). The pulps were mechanically exposed using a #12 diamond bur in a high-speed handpiece 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 (butorphanol tartrate [Torbugesic 0.2 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA]) postoperatively after 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 performed.

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 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 (butorphanol tartrate [Torbugesic 0.2 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA]) postoperatively after 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 performed.

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1. **Group A1**: Disinfection with NaOCl and a blood clot as the scaffold
2. **Group A2**: Disinfection with NaOCl and PRP as the scaffold
3. **Group B1**: Disinfection with NaOCl and a modified TAP (mTAP) dressing during 15 days and a blood clot as the scaffold
4. **Group B2**: Disinfection with NaOCl and mTAP dressing and PRP as the scaffold

**Group A1: NaOCl/Blood Clot**

The canals were disinfected with 20 mL 1.25% NaOCl (Sigma-Aldrich Química SA, Madrid, Spain) using the ANP irrigation Endovac system (Discus Dental). Taking into account that immature teeth with open apices were being treated, the ANP irrigation Endovac system was modified to avoid the extrusion of the NaOCl solution to the apical tissues as described by Cohenca et al (7). Canals were irrigated using the macrocannula only after being gauged to fit the apical size of the root.

The canals were left filled with NaOCl solution 1.25% for 3 minutes and then irrigated with 10 mL sterile saline solution to remove the rest of the NaOCl. Then, a final irrigation was accomplished using 1 mL 17% EDTA (Ultradent Products Inc, South Jordan, UT) for 60 seconds (8). The root canals were dried with sterile paper points (Protaper F3; Dentsply, Tulsa, OK), 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 cementoenamel junction by using a small cotton pellet soaked with sterile saline. After 10 minutes, the blood clot was formed. Over the clot, a collagen sponge (Collacote; Integra Lifesciences Corporation, Plainsboro, NJ) was set. Next, a 4-mm plug of mineral trioxide aggregate (MTA) (ProRoot; Dentsply Tulsa Dental, Johnson City, TN) was inserted into the canals using an MTA carrier (Hartzell & Son, Concord, CA) to seal the root canal at the cervical level. The MTA plug was verified radiographically. Then, a moist cotton pellet was placed over the MTA, and the access cavity was double sealed using IRM (Dentsply Caulk, Milford, DE) and glass ionomer cement (Vitrebond; 3M ESPE, Seefeld, Germany).

**Group B2: NaOCl/PRP**

The disinfection protocol and the final irrigation with EDTA were the same as for group A1. Then, the root canals were dried with sterile paper points ProTaper F3, and PRP was used as the scaffold instead of a blood clot. The PRP was obtained following the protocol described previously by Anitua (15). To prepare the PRP, a 5-mL sample of whole venous blood was drawn from the dogs. Then, the PRP was condensed into the canal until the cementoenamel junction using a hand plunger (Dentsply Maillefer). Over the PRP, the MTA placement and the coronal seal were set as described in group A1.

**Group B1: NaOCl/TAP/Blood Clot**

In this group, the teeth were disinfected in 2 sessions. In the first treatment session, the root canals were irrigated with NaOCl using the ANP-Endovac system as described in group A1. Then, the canals were irrigated with 10 mL sterile saline to remove the NaOCl and were dried with sterile paper points ProTaper F3.

After that, mTAP dressing was prepared immediately, mixing ciprofloxacin, metronidazole, and cefixime in sterile distilled water into a creamy mixture at a concentration of 20 mg of each antibiotic (16). The mTAP was delivered into the root canal with a sterile Lentulo spiral filler (Dentsply Caulk) following the technique described previously by Windley et al (3). All excess of the mTAP in the pulp chamber was removed, and a sterile cotton pellet was placed. The access cavity was then double sealed with Cavit (3M ESPE) and glass ionomer cement (3M ESPE). The intracanal dressing was left in the canals for 15 days.

At the second treatment session, the coronal seal was removed in aseptic conditions with a sterile high-speed diamond round bur under copious water cooling followed by flushing of the pulp chamber with 20 mL saline solution to remove the TAP intracanal dressing. Then, a final irrigation was accomplished using 1 mL 17% EDTA (Ultradent Products Inc) for 60 seconds.

The root canals were dried with sterile paper points ProTaper F3. In this group, a blood clot was used as the scaffold following the same protocol used in group A1, and the coronal seal was accomplished using MTA, IRM, and glass ionomer cement as described previously.

**Group B2: NaOCl/TAP/PRP**

The disinfection protocol was accomplished in 2 sessions as described in group B1. Then, after removal of the TAP, the canals...
were dried with sterile paper points, irrigated with 1 mL EDTA for 60 seconds, and once again dried with sterile paper points. In this group, PRP was used as the scaffold instead of a blood clot following the same protocol used in group A2, and the coronal seal was accomplished using MTA, IRM, and glass ionomer cement as described previously.

Radiographic Evaluation
Teeth were monitored radiographically on a monthly basis for 6 months before the animals were sacrificed. Each individual root was taken as the unit of measure. Radiographic evaluation was performed according to the method described previously by Thibodeau et al (4). Briefly, after a training session explaining the gold standard of the 3 evaluation parameters, 2 examiners evaluated the radiographs independently regarding healing of radiolucencies, thickening of the dentinal walls, and apical closure of the roots. Digital radiographs were saved to a computer in jpg format, and the examiners, blinded to the experimental groups, separately viewed the image exposed preoperatively with an image exposed postmortem. Each examiner graded each root for the following 3 parameters:
1. Diminished size or absence of periapical radiolucency (PAR)
2. Presence or absence of continued thickening of radicular walls
3. Presence or absence of apical closure

When there was not agreement between both evaluators, a discussion was undertaken until a consensus was reached. The kappa statistic values comparing the 2 evaluators were as follows: 0.80 for evaluation of PARs, 0.72 for evaluation of thickness of radicular walls, and 0.68 for evaluation of apical closure, all indicating good agreement between the 2 evaluators.

Statistical Analysis
The data were analyzed with chi-square and analysis of variance tests, with the level of significance set at $P > .05$.

Results
No animal showed any established signs of undue distress from the treatment procedures. Because a gross mobility occurred after the initial infection procedure, 1 animal (positive control group) lost 2 experimental teeth during the course of the study, and another animal (positive control group) also lost 1 experimental tooth.

Radiographic Analysis
After 6 months, the radiographic examination of all the untouched negative control teeth revealed normal development of roots (Fig. 1A and B). On the contrary, all positive control teeth showed an arrest of root development and PAR (Fig. 1C and D).

Some teeth from all 4 experimental groups showed radiographic evidence of healed or healing PARs, thickening of root canal walls, and apical closure (Fig. 1E–H).

Radiographic Healing of PARs
The percentage of teeth in all experimental groups showing improvement of PARs was 62.5%, with significant differences between the 4 groups ($P = .014$) (Fig. 1I). Group B2 showed the maximal percentage of healing of PARs (87.5%) and group A1 the minimum (37.5%).

When the individual experimental groups were compared with each other using chi-square tests in $2 \times 2$ tables with 1 degree of freedom for periapical healing, there were significant differences between groups A1 and B1 ($P = .035$), groups A1 and B2 ($P = .003$), and groups A2 and B2 ($P = .022$). Comparing all teeth in groups B1 and B2 (disinfected in 2 sessions with TAP) with all teeth in groups A1 and A2 (disinfected in 1 session only with NaOCl), there were significant differences, with a higher percentage of periapical healing in the teeth in groups B (81.25%) compared with the teeth in groups A (43.75%) ($P = .002$). Comparing all teeth from groups 1 (blood clot) with all teeth from groups 2 (PRP as scaffold), there were no significant differences ($P = .302$). There were no other significant differences between the experimental groups with respect to periapical healing.

Increase in Root Thickness
The percentage of teeth in all experimental groups showing continued radiographic thickening of radicular walls was 53.1%, and there were significant differences between the 4 groups ($P = .013$) (Fig. 1II). Group B2 showed the maximal percentage of thickening of radicular walls (87.5%) and group A1 the minimum (37.5%).

When the individual experimental groups were compared with each other for thickening of radicular walls, there were significant differences between groups A1 and B2 ($P = .003$), groups A2 and B2 ($P = .005$), and groups B1 and B2 ($P = .022$). Comparing all teeth in groups B1 and B2 (disinfected in 2 sessions with TAP) with all teeth in groups A1 and A2 (disinfected in 1 session only with NaOCl), there were significant differences, with a higher percentage of thickening of radicular walls in the teeth of groups B (68.75%) compared with those of groups A (37.5%) ($P = .012$). On the other hand, comparing all teeth from groups 1 (blood clot) with all teeth from groups 2 (PRP as scaffold), there were no significant differences ($P = .133$). There were no other significant differences between the experimental groups with respect to thickening of radicular walls.

Radiographic Apical Closure
The percentage of teeth in all experimental groups showing radiographic apical closure was 43.8%, with significant differences between the 4 groups ($P = .013$) (Fig. 1III). Group B2 showed the maximal percentage of radiographic apical closure (75.0%) and groups A1 and A2 the minimum (25.0%).

When the individual experimental groups were compared with each other for radiographic apical closure, there were significant differences between groups A1 and B2 ($P = .005$) and groups A2 and B2 ($P = .005$). Comparing all teeth in groups B1 and B2 (disinfected in 2 session with TAP) with all teeth in groups A1 and A2 (disinfected in 1 session only with NaOCl), there were significant differences, with a higher percentage of radiographic apical closure in the teeth in groups B1 and B2 (62.5%) compared with the teeth in groups A1 and A2 (25.0%) ($P = .003$). Comparing all the teeth from groups A1 and B1 (blood clot) with all the teeth from groups A2 and B2 (PRP as scaffold), there were no significant differences ($P = .313$). There were no other significant differences between the experimental groups with respect to radiographic apical closure.

Discussion
This study provides evidence showing that revascularization of necrotic-infected immature dog root canals with AP can be attained. A thorough disinfection of the root canal followed by the placing of a scaffold (blood clot or PRP) allows the formation of new hard tissue on the dentin walls and continued root development, with radiographic apical closure.
The dog has been selected as animal model because of its similarities in radicular structure to immature human teeth in their open apex characteristics (3, 4, 17–20) and its high growth rate that allows clinicians to obtain results in shorter experimental periods (21).

Radiologic findings have been evaluated by the method described previously by Thibodeau et al (4). The same method also has been used by others investigators (5, 9, 13, 17, 19, 20, 22).

In the total sample of infected root canals analyzed in this study, 62.5% of root canals presented healing/improvement of PARs, 53.1%

**Figure 1.** (A) A tooth from the negative control group (untouched) in the initial and final (B) stages of the experiment showing normal development of the roots with thickened root walls and apical development. (C) A tooth from the positive control group (untreated) in the initial and final (D) stages of the experiment showing the arrest of root development with no thickened root walls, no apical closure, and periapical radiolucency. (E) A tooth from group A2 in the initial and final (F) stages of the experiment showing normal development of the roots with thickened root walls and apical development. (I) The percentage of roots in experimental groups with and without improvement of PARs assessed radiographically. (II) The percentage of roots in experimental groups with and without the presence of thickened radicular walls assessed radiographically. (III) The percentage of roots in experimental groups with and without further apical closure assessed radiographically. (P values were calculated with chi-square and analysis of variance tests).
showed continued radiographic thickening of radicular walls, and radiographic apical closure was revealed in 43.8% of cases. These results are similar to those reported previously by others investigators. Khademi et al (20), in a study performed on mongrel dogs’ teeth, found apical healing and apical closure in 70% of the cases and thickening of the walls in 40%, and Thibodeau et al (4), in a study performed on mixed breed canine model dogs, found thickened walls in 43.9% and apical closure in 54.9%. However, recently Zhang et al (19) have found 78% of radiographic apical closure and 78% of root walls thickening.

In groups B1 and B2, taken together, these percentages were 81.25%, 68.75%, and 62.5%, respectively. The teeth in both groups B1 and B2 (TAP as the intracanal dressing) showed significantly higher percentages of improvement of PARs (P = 0.002), thickening of radicular walls (P = .012), and radiographic apical closure (P = .003) compared with the teeth of groups A1 and A2 (NaOCl alone). These results highlight the importance of disinfection in revascularization procedures. Moreover, these results suggest that the use of TAP as intracanal dressing (groups B1 and B2) provides a more complete disinfection of the root canals, helping to achieve a significantly higher percentage of revascularization. Similar results have been reported by Thibodeau et al (4), who found radiographic evidence of apical closure among roots of all teeth experimentally infected that were disinfected with the TAP, concluding that TAP can be relied on to consistently render infected canals effectively free of bacteria. This result is also in accordance with previous findings showing that TAP significantly reduces the bacteria in experimentally infected canals of immature dog teeth (3). Conversely, it has been communicated that root canals infected and not disinfected or treated further remained infected with a persistent lack of improvement of PARs, with absence of thickening of radicular dentin walls, and without apical closure (4, 24). However, it must be taken into account that the time frame of the present study may not have been long enough for complete radiographic healing of PARs because it occurred in other studies (4, 8).

Outcomes were assessed after 5 months in all experimental groups. In studies by Thibodeau et al (4) and Yamauchi et al (8), a 5-month follow-up period was used. By way of comparison, the pioneer study of Strindberg (25) revealed that in humans the complete healing of PAR lesions may take up to 4 years.

In the present study, the pulps of all experimental teeth were all necrotic and infected before disinfection, as shown in the teeth in the positive control group. Therefore, the healing/improving of PARs with continued thickening of root walls and apical closure evaluated radiographically, can only be explained because of ingrowth of progenitor cells from the periapical area (4, 25). As claimed by Thibodeau et al (4), this was taken as a surrogate outcome measure representing successful revascularization because the new hard tissue was produced by cells that grew in to repopulate the canal space. Recently, Flake et al (26) has proposed the radiographic root area, a measurement of the entire surface area of the root as observed on a periapical radiograph, as a valid method to measure radiographic outcomes of endodontic therapies on immature teeth.

In the revascularization protocols reported in previous studies, decontamination of root canals was performed with 20 mL NaOCl solution at level concentrations from 2.5%–6% (5, 12, 13, 27), without conventional mechanical instrumentation for the prevention of the destruction of cells that might be present in the apical part of the root canal (28). In addition to irrigation, the use of intracanal dressing materials, mainly those composed by TAP (metronidazole, ciprofloxacin, and minocycline), have shown antimicrobial activity against oral bacteria and its ability to decontaminate infected dentin (16, 29, 30). However, the use of antibiotics as intracanal dressing may promote some side effects such as coronal discoloration (27, 31, 32), bacterial resistance (33), and allergic reactions, as suggested by Reynolds et al (27). Thibodeau and Trope (34) reported substituting minocycline for cefaclor in the triantibiotic formula to avoid dentin discoloration. In the present study, mTAP was used, substituting minocycline with cefixime, a third-generation broad-spectrum cephalosporin.

In the recent review performed by Kontakiotis et al (35) analyzing the protocols that have been used in regenerative endodontic therapy including 60 clinical studies, 49 studies used antibiotics alone (n = 45) or in combination with calcium hydroxide (n = 4). The antibiotic mixture consisted of ciprofloxacin, metronidazole, and minocycline (TAP) is the most widely used intracanal medicament in regenerative endodontic therapy (36). In addition, the TAP has been shown to be biocompatible when placed in polyethylene tubes and implanted subcutaneously in rats (18) although it is important to note that it has been shown that TAP can reduce stem cell survival in a concentration-dependent manner (37); therefore, further investigation is needed to determine appropriate antibiotic formulations or search for other antimicrobial medications with similar useful properties to the tested TAP but without having its associated deleterious side effect.

The experimental protocol performed in group B2 in the present study, including disinfection using NaOCl with the ANP-Endovac system and an intracanal dressing of TAP (ciprofloxacin, metronidazole, and cefixime) followed by the use of PRP as the scaffold, obtained the highest percentages of improvement of PARs (87.5%, P = .014), thickening of root canal walls (87.5%, P = .013), and apical closure (75%, P = .013) compared with the other groups and a significantly higher percentage of thickening of root canal walls (P = .022) than group B1 (a blood clot as the scaffold). This result suggests that the use of PRP as the scaffold improves the success rate of the revascularization procedure.

Platelet-rich preparations constitute a relatively new biotechnology for stimulation and acceleration for tissue healing and bone regeneration (38). PRP has been proposed as a potentially ideal scaffold for regenerative endodontic treatment (39). PRP has been shown to contain growth factors and can stimulate collagen production (9). However, several studies have found that the use of PRP into root canals showed no enhancement in new tissue formation compared with induction of a blood clot into the root canals alone (40). The use of a scaffold of fibrin gel has been found to improve cell infiltration and cell-dentin interaction in pulpless immature human premolars implanted in rodents (41).

Conclusions

Present radiographic findings show that revascularization of necrotic-infected immature teeth with AP is possible. The use of TAP as the disinfectant and PRP as the scaffold seem to be useful in pulp revascularization procedures. However, long-term clinical trials and histologic studies are required to analyze the benefits of using TAP and PRP in revascularization procedures.

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References

Basic Research—Biology


