Histologic Analysis of the Influence of a Gelatin-based Scaffold in the Repair of Immature Dog Teeth Subjected to Regenerative Endodontic Treatment

Clacir de Lourdes Dotto Londero, PhD, Claudia Medianeira Londero Pagliarin, PhD,† Mara Cristina Santos Felippe, PhD,‡ Wilson Tadeu Felippe, PhD,‡ Cristiane Cademartori Danesi, PhD,§ and Fernando Branco Barletta, PhD||*

Abstract

Introduction: Regenerative endodontic treatment is a new and promising approach to manage immature teeth with necrotic pulps and apical periodontitis. The use of scaffolds is essential to treatment success, but many materials are difficult to acquire and have a high cost. This study assessed tissue repair in immature dog teeth with necrotic pulps and apical periodontitis after using a gelatin-based scaffold (Gelfoam; Pharmacia & Upjohn Co, Kalamazoo, MI). **Methods:** Apical periodontitis was induced in 20 immature dog teeth. After disinfection with triple antibiotic paste for 2 weeks, canals were irrigated, dried, and filled with a blood clot alone (10 teeth) or combined with Gelfoam (10 teeth). Another 10 teeth were used as negative controls (no intervention). After 7 months, the dogs were euthanized. Histologic sections were stained with hematoxylin-eosin and analyzed in relation to tissue repair. Categoric data were analyzed using the Fisher exact test ($P < .05$), numeric data (histomorphometric analysis), and the Mann-Whitney *U* test. **Results:** Histologic analysis revealed a higher percentage of roots with new cementumlike mineralized tissue and connective tissue inside the canal in the blood clot + Gelfoam group ($P < .001$). Histomorphometric analysis showed a higher area of mineralized tissue in the same group ($P = .029$). Apical extension of root and inflammation were similar between the experimental groups. The new tissue formed onto canal walls and in the root canal space showed characteristics of cementum and periodontal ligament, respectively. **Conclusions:** The use of a gelatin-based scaffold (Gelfoam) combined with a blood clot improved repair in immature dog teeth with apical periodontitis subjected to regenerative endodontic treatment. *(J Endod 2015;41:1619–1625)*

Key Words

Apexification, revascularization, scaffold, tissue engineering

Tissue engineering is a multidisciplinary field that combines principles from engineering, biology, and clinical sciences to develop biological substitutes that can maintain, restore, or improve the function of organs and tissues (1). In this context, regenerative endodontics is an emerging field that involves restoration of the tooth structure and revitalization of the root canal space (2). These procedures are particularly useful in the treatment of necrotic teeth with incomplete root formation in which physiological root development is interrupted because of a necrotic pulp. For many years, the treatment of choice for this clinical condition was apexification with calcium hydroxide (3, 4). More recently, however, the use of mineral trioxide aggregate (MTA) has been indicated (5, 6). Even though these 2 treatment approaches have had their effectiveness demonstrated, in both cases teeth remain with short roots and thin root walls, leading to an unfavorable prognosis because of the high rate of fractures over time (7).

Recently, revitalization of the root canal space has been proposed or indicated to allow continued root development in both length and wall thickness (8, 9). Moreover, according to some authors, this approach would also help restore pulp vitality (7).

Among the factors that contribute to the success of regenerative endodontic procedures are stem/progenitor cells, morphogens (signaling molecules), and biocompatible scaffolds. Scaffolds are necessary to promote a 3-dimensional support for cell adhesion, migration, and proliferation, all paramount for tissue regeneration (10).

Many materials have been used in regenerative endodontic procedures, including organic collagen-based scaffolds (11, 12), synthetic polymers (13, 14), calcium phosphate (15), platelet-rich plasma (16–19), and hydrogel (20). Notwithstanding, the use of these materials in the clinical setting has yet to be proven feasible and needs the support of a good manufacturing practice laboratory to supply clinical grade stem cells (21), not to mention the fact that they are very difficult to acquire and have a high cost. In this scenario, the Gelfoam gelatin sponge (Pharmacia & Upjohn Co, Kalamazoo, MI) has been proposed as a feasible alternative material for use in regenerative endodontics. Gelfoam is biocompatible, biodegradable, easy to acquire, and does not have any inhibitory effect on cell proliferation, as previously shown (22, 23). According to the manufacturer, the material is completely absorbed within 4 to

From the *Dental Service, †Department of Stomatology, and §Department of Pathology, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul; ‡Department of Dentistry, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina; and ||Graduate Program, School of Dentistry, Universidade Luterana do Brasil, Canoas, Rio Grande do Sul, Brazil.

Address requests for reprints to Dr Clacir de Lourdes Dotto Londero, Av Medianeira, 1782/302, 97060-002, Santa Maria, RS, Brazil. E-mail address: clacirlondero@gmail.com

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6 weeks. Moreover, recently, it has been shown that the combined use of Gelfoam and stem cells in the dental pulp of dogs allows for pulp and dentin regeneration (24).

The objective of this study was to assess tissue repair in immature dog teeth with necrotic pulps and apical periodontitis subjected to regenerative endodontic treatment with the use of a gelatin-based scaffold (Gelfoam).

Methods

The research project was approved by the Animal Ethics Screening Committee of the Federal University of Santa Catarina (Florianópolis, Santa Catarina, Brazil). The second, third, and fourth mandibular premolars and the second and third maxillary premolars (n = 60 roots) of 3 beagle dogs aged 5–6 months were used.

Animals received preanesthesia with 0.5 mg/kg acepromazine hydrochloride (Ouro Fino, Cravinhos, SP, Brazil) and 4 mg/kg pethidine (Cristália, São Paulo, SP, Brazil) via intramuscular injection. After 10 minutes, the animals were cannulated using a 22-G catheter and administered 0.9% saline solution as maintenance fluid at 10 mL/kg/h. Subsequently, anesthesia was induced using intravenous propofol (Cristália, São Paulo, SP, Brazil) at 5 mg/kg; animals were intubated using an endotracheal tube with cuff and maintained under anesthesia with isoflurane (Cristália).

Preoperative radiographs were obtained to confirm the presence of open apices (Fig. 1). After endodontic access of 20 teeth and working length determination (tooth length − 1 mm), pulp tissues were removed using #60 K-files (Dentsply Maillefer, Ballaigues, Switzerland). Subsequently, #60 Hedströen files (Dentsply Maillefer) calibrated at the same length were used to remove residual pulp tissues. Canals were irrigated with 2.5 mL distilled water. Once hemostasis was established, a cotton pellet was placed at the canal entrance. Teeth were left open (no coronal sealing) for 3 weeks to allow root canal contamination (Fig. 2).

At the end of 3 weeks, the animals were anesthetized once again as described previously and the canals re-entered under aseptic conditions of isolation and surface disinfection with 0.12% chlorhexidine and tincture of iodine (25, 26). Teeth were irrigated with 10 mL 2.5% sodium hypochlorite (Farmácia de Manipulação Nova Derme, Santa Maria, RS, Brazil) to remove debris accumulated inside the root canals. After irrigation, canals were dried using sterile paper points (Dentsply Maillefer) and filled with triple antibiotic paste (Farmácia de Manipulação Nova Derme) composed of ciprofloxacin, metronidazole, and minocycline (20 mg of each antibiotic per mL). The paste was applied to the cementoenamel junction using a 27-G needle calibrated to 2 mm short of the working length. A sterile cotton pellet was placed on top of the pastes, and teeth were temporarily sealed with intermediate restorative material (Dentsply Caulk, Milford, DE).

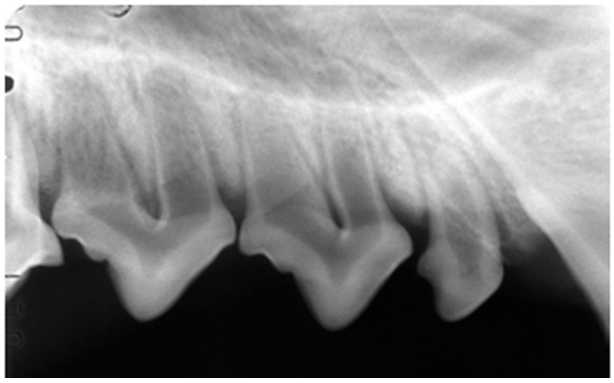

Figure 1. Preoperative radiograph showing open apices.

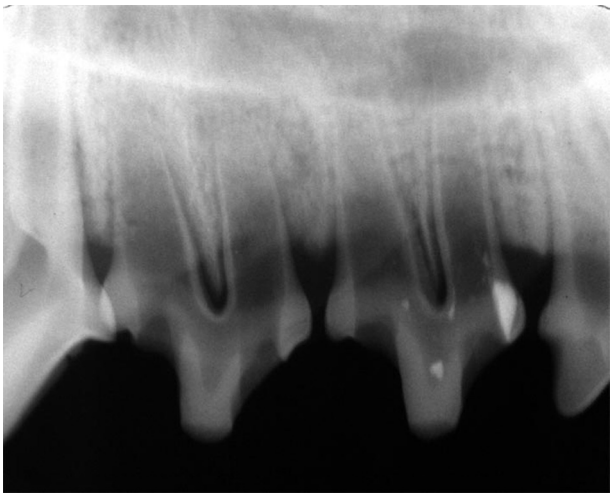

Figure 2. Radiograph showing root canals after 3 weeks with evidence of periapical lesion formation.

After 2 weeks, after the same anesthesia and asepsis protocols, temporary restorations were removed, and the teeth were irrigated with 10 mL 2.5% sodium hypochlorite followed by 10 mL sterile saline solution. Subsequently, canals were irrigated with 17% EDTA for 3 minutes and then dried with sterile absorbent paper points (Dentsply Maillefer). At this point, teeth were randomly divided into 3 experimental groups.

In the blood clot group (n = 10 teeth), a #30 K-file (Dentsply Maillefer) was advanced into the root canal to 2 mm beyond the root length to induce bleeding in periapical tissues and allow the formation of a blood clot inside the canal. With the aid of a sterile cotton pellet soaked in saline solution, the clot was gently pressed 1–2 mm apical to the cementoenamel junction for approximately 15 minutes.

In the blood clot + Gelfoam group (n = 10 teeth), a sterile, absorbable, gelatin-based sponge (Gelfoam) was used as a scaffold. Small pieces of Gelfoam were cut and placed inside the canals using a Paiva plugger (SS White, Rio de Janeiro, RJ, Brazil) until the canal was completely filled. Subsequently, bleeding was induced as described for the blood clot group, again limiting the blood clot to 1–2 mm apical to the cementoenamel junction. Once the canal was filled with blood, the file was withdrawn using counterclockwise rotary movements to avoid Gelfoam displacement.

In both groups, a 1- to 2-mm thick plug of white fast-setting MTA was placed on top of the blood clot (Angelus Indústria de Produtos Odontológicos Ltda, Londrina, PR, Brazil). Then, definitive amalgam restorations were performed (DFL Alloy Indústria e Comércio SA, Rio de Janeiro, RJ, Brazil). Figure 3 shows pre- and postoperative radiographs of the teeth.

The 10 teeth included in the negative control group were not subjected to any intervention to allow the physiological development of the root and comparisons with experimental teeth. The animals received analgesic medications after all surgical procedures and were constantly monitored by the team of veterinarians of the laboratory animal house.

After 7 months, animals were euthanized by perfusion, and the jaws were removed, dissected, and fixed in 10% formaldehyde. After decalcification in 50% formic acid and 5 N sodium citrate, specimens were diaphonized and reduced to obtain individual roots. Each root was then embedded in paraffin and longitudinally sectioned along the tooth's long axis until reaching the apical foramen. Subsequently,