Histologic Evaluation of Regenerative Endodontic Procedures with the Use of Chitosan Scaffolds in Immature Dog Teeth with Apical Periodontitis

Paulo J. Palma, DDS, PhD, João C. Ramos, DDS, PhD,* João B. Martins, DDS,* Anibal Diogenes, DDS, MS, PhD,† Maria H. Figueiredo, PhD,* Paula Ferreira, PhD,‡ Carlos Viegas, DVM, MSc, PhD,§ and João M. Santos, PhD**

Abstract

Introduction: The aim of this study was to evaluate histologically the newly formed tissues after regenerative endodontic procedures (REPs) in dogs using either a blood clot (BC) or 2 different formulations of a chitosan hydrogel as scaffolds. **Methods:** Apical periodontitis was induced by inoculating immature teeth with oral plaque in 4 beagle dogs. Teeth (n = 96) were divided into 2 control (n = 20) and 4 test groups (n = 76) according to the treatment: apexification and REPs with BC, sodium hyaluronate:chitosan (HA:CS) scaffolds, or pectin:chitosan (P:CS) scaffolds. All root canals were disinfected with 2.5% sodium hypochlorite and a triple antibiotic paste intracanal medicament before evoked bleeding, clot formation, or scaffold placement. Thirteen weeks after treatment, the animals were sacrificed and the jaw blocks harvested for histologic processing, histomorphometric analysis, and statistical analysis. **Results:** The lumens of the root canals were completely filled with mineral trioxide aggregate with evidence of a mineralized apical bridge between the root canal walls in 83% of the samples in the apexification group. Vital vascularized tissue was found in the REP groups; apical closure happened in 66.7% of these treatments, and root growth was detected more often as an increase in thickness (85.6%) than in length (45.6%). The greatest amount of mineralized tissue inside the canal was observed in the BC group, with statistical significance compared with the HA:CS and P:CS groups ($P < .05$). Further histologic evaluation revealed the presence of apical papilla. **Conclusions:** The addition of chitosan scaffolds to blood in regenerative procedures in dogs did not improve the formation of new mineralized tissues along the root canal walls or the histologic evidence of the regeneration of a pulp-dentin complex. *(J Endod 2017;■:1–9)*

Key Words

Apexification, apical papilla, chitosan scaffolds, immature permanent tooth, regenerative endodontics procedures, root canal, tissue engineering, tissue regeneration

Significance

We performed a histologic assessment of the regenerative potential of 2 chitosan-based scaffolds compared with blood clot in immature dog teeth with pulp necrosis and apical periodontitis. This study demonstrated the continued survival and differentiation potential of the apical papilla after infection.

During the development of permanent teeth, the occurrence of caries, trauma, or anatomic alterations is quite common and can jeopardize the pulp tissue and impair the pulp-dentin complex physiology and as a consequence normal root development. Premature loss of a functional pulp in immature teeth leads to the arrest of root dentin formation, resulting in a thin and functionally compromised canal wall (1). These anatomic and functional conditions are associated with greater predisposition to treatment failures and fractures and decreased tooth survival (2–4).

Regenerative endodontic procedures (REPs) have emerged such as revascularization and revitalization of pulp tissue in immature necrotic teeth with apical periodontitis (AP) to allow the reinforcement of root canal walls and sometimes the continuation of their development, thus opening new therapeutic possibilities in this field (5–7). The underlying strategies to promote growth of new tissues in pulp canal space are based on 4 fundamental assumptions:

1. Effective endodontic disinfection/antisepsis
2. Recruitment of undifferentiated mesenchymal stem cells (MSCs) from the apical region
3. Creation of a scaffold that allows growth of new tissue
4. Appropriate coronal sealing to prevent reinfection (8, 9)

There have been substantial advances in disinfection techniques that combine the balance between the antimicrobial effect and biocompatibility with MSCs. Also, it has been shown that intracanal bleeding evoked from the apical tissues brings substantial numbers of MSCs into the canal system (10). Although a blood clot (BC) has been traditionally used as a scaffold, it has several limitations that include undefined composition,

From the *Department of Dentistry, Faculty of Medicine and ‡Chemical Process Engineering and Forest Products Research Centre (CIEPQPF), Department of Chemical Engineering, University of Coimbra, Coimbra, Portugal; †Department of Endodontics, University of Texas Health Science Center at San Antonio, San Antonio, Texas; and §Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal.

Address requests for reprints to Prof Doutor Paulo J. Palma, Área de Medicina Dentária, Faculdade de Medicina da Universidade de Coimbra, Avenida Bissaya Barreto, Blocos de Celas–CHUC, 3000-075 Coimbra, Portugal. E-mail address: ppalma@fmed.uc.pt

0099-2399/$ - see front matter

Copyright © 2017 American Association of Endodontists.

http://dx.doi.org/10.1016/j.joen.2017.03.005

presence of immune cells, unknown breakdown kinetics, and its generation requires traumatizing the apical tissues. Other alternatives have been successfully used in the clinical setting including the use of platelet-rich plasma (11, 12), platelet-rich fibrin (13), and soluble collagen (14). Their clinical use allowed resolution of signs and symptoms of disease leading to the healing of AP and, in certain cases, appreciable radiographic evidence of root development.

The 3 key components for tissue engineering are stem cells, growth factors, and scaffolds. There are ongoing research efforts to develop scaffolds for tissue regeneration using either natural or synthetic materials (15). These alternative scaffolds have been evaluated in animal models of regenerative endodontics, including soluble collagen (16), platelet-rich plasma (17), and absorbable gelatin sponge (18). In these models, they failed to improve the histologic outcome, and, so far, only insoluble cross-linked collagen sponges were associated with better results (19, 20). To overcome some limitations presented by those materials, including a fast resorption rate and a lack of mechanical support for the coronal seal, we sought to test 2 new lyophilized 3-dimensional scaffolds based on chitosan that have been previously evaluated *in vitro* for their biocompatibility (21, 22).

This *in vivo* study assessed histologically the regenerative potential of 2 chitosan-based different scaffolds (hyaluronic acid:chitosan [HA:CS] and pectin:chitosan [P:CS]) compared with the use of an autologous BC in immature dog teeth with pulp necrosis and AP.

Material and Methods

Animals

The study protocol was approved by the Animal Welfare Committee of the Direção-Geral de Veterinária of Portugal (no. 0420/2011) and complied with the International Guiding Principles for Biomedical Research Involving Animals (Geneva, 1985).

Based on previous studies (16, 23), 4 male beagle dogs, aged approximately 6 months, had 4 one and 10 two-rooted premolars involved in the study protocol. All experimental teeth were block randomized, and 76 roots were assigned to the test group and 20 to the control groups. All groups were present in each dog and distributed in alternate quadrants in each animal (24).

Tooth Preparation

For the first 3 interventions, dogs were anesthetized with an intravenous administration of 0.2 mg/kg diazepam (Labesfal, Campo de Besteiros, Portugal) and 2 mg/kg propofol (Propofol Lipuro 2%; B Braun Medical, Queluz de Baixo, Portugal) and maintained with inhalation of O_2 and 1%–2% isoflurane (Isoflo; Esteve Farma, Lisboa, Portugal). All animals received a single dose of meloxicam 0.2 mg/kg (Meloxidyl; Ceva Santé Animale, Libourne, France) postoperatively. For radiographic follow-up, dogs were sedated with an intramuscular administration of 0.2 mg/kg butorphanol (Dolorex; MSD Animal Health, Porto Salvo, Portugal) and 0.005 mg/kg dexmedetomidine (Dexdomitor; Orion Pharma, Espoo, Finland).

Before any interventions, teeth were radiographed using custom bite registration and paralleling devices (XCP-DS Fit; Dentsply Rinn, York, PA). At the first intervention, teeth had coronal access performed with a bur mounted in a high-speed handpiece and pulp tissue mechanically disrupted with a 30 K-file. Supragingival plaque was collected from the gingival sulcus, mixed with sterilized water (B Braun Medical), placed into the access cavity, and temporarily sealed with Cavit (ESPE 3M, Seefeld, Germany). After 3 weeks, the development of AP was confirmed radiographically. The second intervention was performed under rubber dam isolation, and disinfection of the operative field was achieved with 10% iodopovidone solution (Betadine; Meda Pharma, Lisboa, Portugal). All teeth in the test groups were reaccessed, irrigated with 10 mL 2.5% sodium hypochlorite, and disinfected with calcium hydroxide in the apexification group or filled with triple antibiotic paste (Coimbra Hospital and University Centre, Coimbra, Portugal) composed of ciprofloxacin, metronidazole, and minocycline (20 mg of each antibiotic per mL) before REP protocols. The paste was applied until the cementoenamel junction using a 27-G needle calibrated 2 mm shorter than the working length. A sterile cotton pellet was placed on top of the medication, and teeth were temporarily sealed with Cavit.

Two weeks later, intracanal medication was removed with copious irrigation using 3 mL 2.5% sodium hypochlorite (only on the pulp chamber) followed by a final flush with 10 mL saline solution. Then, canals were dried with paper points and treated according to the following test groups.

1. Apexification (19 roots): mineral trioxide aggregate (MTA) (White ProRoot MTA; Dentsply Tulsa, Johnson City, TN) was mixed according to the manufacturer and inserted into the canal using a suitable-sized carrier (Map System, Dentsply Tulsa). MTA was packed apically using a suitable plugger (Buchanan Hand Plugger #2; SybronEndo, Orange, CA) to fill the root canal 2–3 mm apical to the cementoenamel junction. A sterile wet cotton pellet was then placed in the pulp chamber and coronal access restored with GIC (Ketac Fil, ESPE 3M).

Groups of REP Procedures

2. BC (19 roots): bleeding into the canal was evoked by gentle overinstrumentation with a sterile size 30 K-file leading to filling of the canal space until the level of the cementoenamel junction and waiting for the formation of a BC. After clot stabilization, the coronal portion of the root canal was double sealed with 2–3 mm white MTA and GIC.
3. HA:CS scaffold (19 roots): preparation of sodium HA:CS scaffolds was performed based on a HA:CS of 2:1 mass ratio according to Coimbra et al (21). Induction of bleeding was performed as in the BC group; then, the lyophilized hydrogel of HA:CS was inserted into the canal using a suitable-sized plugger and double sealed.
4. P:CS scaffold (19 roots): preparation of P:CS polyelectrolyte complex scaffolds was performed based on a P:CS of 2:1 mass ratio according to Coimbra et al (22). Induction of bleeding was performed as described in the BC group; then, the lyophilized hydrogel of P:CS was inserted into the canals using a suitable-sized plugger and double sealed.

Control Groups

5. Negative control pulpal (4 roots): pulps were mechanically disrupted and exposed to oral microbiota. At the second intervention, teeth were extracted for histologic analysis of the pulp content.
6. Negative control periapical (4 roots): same protocol performed as in the negative control pulpal group; however, at the second intervention, teeth were restored with GIC and maintained until the end of the study.
7. Positive control (12 roots): normal teeth of the same type as in the previous groups without any intervention included for histologic reference of the physiologic root development.

Histologic Analysis

At 13 weeks post-treatment, animals were euthanized by anesthetic overdose (pentobarbital at 30 mg/kg intravenously; Butler Company, Columbus, OH) followed by bilateral perfusion with 10% phosphate-buffered formalin. Mandibular and maxillary blocks were dissected

Download English Version:

https://daneshyari.com/en/article/5641098

Download Persian Version:

https://daneshyari.com/article/5641098

Daneshyari.com