Histologic Examination of Teeth with Necrotic Pulps and Periapical Lesions Treated with 2 Scaffolds: An Animal Investigation

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Abstract

Introduction: Traditional pulp regeneration procedures that use a blood clot as a scaffold have produced histologic evidence of bone, cementum, and connective tissue growth within the root. Platelet-rich plasma (PRP) is a bioactive scaffold containing growth factors that enhance wound healing. **Aim:** The aim of this study was to histologically compare the tissues generated when PRP or a blood clot is placed into teeth with preexisting necrotic pulps and periapical lesions. **Methods:** Twenty-four canine teeth from 6 immature ferrets were used. Two ferrets served as positive controls. Sixteen experimental canine teeth from 4 ferrets were infected, debrided, treated with a triple antibiotic paste, and randomly distributed to the following groups: group 1 (blood clot/Gelfoam), group 2 (PRP), and group 3 (no scaffold). At 3 months, the ferrets were sacrificed, and the tissues were evaluated histologically. Data were analyzed by using the Fisher exact test ($P < .05$). **Results:** In 3 of 6 teeth in the PRP group, 2 of 6 teeth in the blood clot group, and 1 of 4 teeth in the no scaffold group, an ingrowth of hard tissues was observed in the apical third of the roots. When using PRP or a blood clot as a scaffold, we found significantly more apical narrowing and hard tissue deposition in comparison to not using a scaffold ($P < .05$). **Conclusions:** The use of PRP or blood clots as scaffolds results in the ingrowth of bone-like, cementum-like, and connective tissue in the apical third of the roots at inconsistent rates. *(J Endod 2015;41:846–852)*

Key Words

Bone, cementum, dentin, ferret, regeneration, revascularization, revitalization

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An undesired consequence of pulp necrosis in immature teeth is the cessation of root development, rendering endodontic treatment with conventional techniques and materials difficult because of their open and divergent apices (1). In addition, immature teeth are susceptible to fracture after endodontic treatment because of their thin dentinal walls, particularly at the cervical region (1, 2). Treatment strategies include long-term apexification therapy or the application of a mineral trioxide aggregate (MTA) barrier. Apexification procedures are lengthy, can weaken root dentin, and are unpredictable (3). Although MTA apical plugs are quite successful in accounting for the apical seal, they do not address the development of the entire root (3). Therefore, this treatment may not completely reduce the chances of cervical root fracture (1). The ideal outcome for a necrotic, immature tooth would be predictable regeneration of pulp tissue lined with odontoblasts. The objective of pulp regeneration is to restore the physiological functions of the pulp. The pulp has the ability to promote dentin formation, which will increase root thickness and length to prevent fracture and will also result in the development of an apical morphology more appropriate for conventional endodontic therapy if future treatment becomes necessary (1).

Many clinical studies demonstrating pulp regeneration have been published that show good clinical outcomes and radiographic evidence of continued thickening of the dentinal walls, apical closure of roots, and resolution of periapical infections in immature teeth with preexisting necrotic pulps and periapical lesions (2, 4, 5). A few histologic case reports have been published after completion of regeneration procedures. Two reports found evidence of pulp-like loose connective tissue (4, 5), and one reported an ingrowth of cementum, bone, and connective tissue (6). Animal studies examining regeneration procedures have shown that the types of tissues found within the root are not pulp but consist of bone, cementum, and connective tissue (7–11).

The 3 major components of tissue regeneration include stem cells, growth factors/morphogens, and a scaffold that can support cell growth and differentiation (6).

Platelet-rich plasma (PRP) is an autologous blood source that has been cited as a potentially ideal scaffold for regenerative endodontic treatment regimens (1). PRP contains an increased concentration of growth factors, stimulates collagen production, recruits other cells to the site of injury, produces anti-inflammatory agents, initiates vascular ingrowth, induces cell differentiation, controls the local inflammatory response, and improves soft and hard tissue wound healing (12–14). Gelfoam, a popular material used in oral surgery, has been used in previous studies as a scaffold for regenerative endodontics (11, 15). More recently, Gelfoam was successfully used in a regenerative study in combination with a blood clot and dental pulp stem cells (15). Gelfoam combined with a blood clot and PRP have been studied separately as scaffolds in regenerative endodontics, but no study has compared the 2 scaffolds directly. In a previous investigation Torabinejad et al (8) histologically examined the types of tissues generated in noninfected ferret teeth by using 2 scaffolds. They reported growth of bone-like material into the root canals of these teeth. The purpose of this study was to histologically compare the tissues generated when PRP or a blood clot combined with Gelfoam is placed into teeth with preexisting necrotic pulps and periapical lesions.

Methods

All animal experiments were performed in accordance with protocols approved by the Loma Linda University Animal Research Committee. Six 70-day-old male ferrets with immature canine teeth were used in this experiment. Two animals provided the positive controls, and the 4 remaining ferrets provided the experimental teeth. A total of 16 experimental and 8 positive control teeth were used in this study. Preoperative radiographs were obtained to confirm the presence of an open apex and the absence of preexisting pathosis in each canine tooth.

All experimental procedures were conducted under a clean protocol with the use of sterile materials and equipment. General anesthesia was induced in the ferrets by using 3% isoflurane gas (Phoenix Pharmaceuticals, Inc, St Joseph, MO). The animals were then intubated with a 2.5-mm endotracheal tube and maintained under 2% isoflurane gas. Endodontic access was prepared in each experimental tooth by decoronating the canines with a sterile diamond bur approximately 3 mm above the gingival crest. The access was refined with a sterile flame-shaped diamond bur. The entire pulp was extirpated with sterile barbed broaches (Dentsply, Tulsa, OK). After the canals were irrigated with saline and left exposed to the oral cavity for 1 week, a cotton pellet was placed in the access opening and then sealed with Cavit (3M ESPE, St Paul, MN) to promote the growth of anaerobic bacteria. Lesion development was allowed to occur for 2 weeks. Periapical radiographs were obtained to verify the presence of periapical radiolucencies.

Each experimental canine tooth was isolated with a dental dam and a modified #212 clamp. The operative field was disinfected with 0.12% chlorhexidine (16). Each tooth was reentered, and the root canals were debrided with 10 mL 1% sodium hypochlorite (NaOCl; Clorox, Oakland, CA) and minimally instrumented with K-files (Dentsply) to avoid further weakening of the root. Max-i-probe needles (Dentsply), 25-gauge, with side vents were placed mid-root to minimize apical extrusion of the irrigant. The canals were then dried with sterile paper points (Dentsply Maillefer, Tulsa, OK) and filled with a triple antibiotics mixture (metronidazole, minocycline, and ciprofloxacin, 166.3 mg of each drug). The triple antibiotics mixture was made into a paste form by the addition of sterile saline (0.9% sodium chloride; Hospira Inc, Lake Forest, IL) and delivered into the canal with a Centrix syringe (Centrix Inc, Shelton, CT) to the level of the cementoenamel junction (CEJ) (2). The access openings of all teeth were sealed with Cavit. After 3 weeks, the teeth were reentered; each canal was irrigated with 10 mL sterile saline and then irrigated with 5 mL 17% EDTA as a final rinse. The canals were then dried with coarse paper points, and a sterile paper point soaked in Emdogain (Straumann USA, Andover, MA) was applied to the root canal walls. Emdogain was added because of its beneficial effects of providing an additional source of exogenous growth factors that may aid in pulp regeneration (17). At this point, all of the experimental canine teeth were randomly assigned to the following treatment groups: blood clot/Gelfoam, PRP, or no scaffold.

In 6 teeth, intracanal bleeding was induced by placing a sterile #15 K-file in the periapical area 1–2 mm past the apex, resulting in the canal filling with blood originating from the periapical tissues. The coagulation of blood was controlled below the CEJ. Preformed Gelfoam cones (Pfizer, New York, NY) were placed to the working length extending 2 mm below the CEJ to aid in coagulation and to serve as an initial matrix to allow a 3-mm layer of gray ProRoot MTA (Dentsply Inc) to be placed over the blood clot (18). A 2-mm layer of GC Fuji IX glass ionomer (GC America Inc, Alsip, IL) was placed over the MTA.

In 6 teeth, PRP was used as a scaffold. To harvest the blood in preparation of PRP, the same procedure in a previous study was used (8). MTA was gently placed on top of the PRP, followed by glass ionomer as described above.

The no scaffold group (negative controls) consisted of 4 teeth. The infected and debrided root canals in this group were simply sealed with MTA and glass ionomer in the access cavity as described earlier. No scaffold was placed, and the canals were left empty.

The positive controls consisted of 8 teeth that did not receive any intervention. As positive controls, they were used to define the normal growth in length, dentin thickness, and apical closure in comparison with the experimental groups.

After 3 months, each ferret was sacrificed and perfused with 10% formalin, and block sections of the maxilla and mandible were removed and stored in formalin-containing jars in the same manner as a previous study (8). The block sections were decalcified in 10% EDTA (Sigma-Aldrich, St Louis, MO), followed by 5% formic acid (Sigma-Aldrich). After radiographic confirmation of decalcification, the samples were rinsed under running tap water for 5 hours, followed by dehydration with ascending concentrations of alcohol. The specimens were processed and embedded in tissue prep paraffin (Fischer Scientific, Fair Lawn, NJ) on a Tissue-Tek VIP 5 unit (Sakura Finetek USA, Inc, Torrance, CA). Step longitudinal serial sections of 5-μm thickness were prepared, and the sections were stained by using hematoxylin-eosin. Samples were evaluated with a light microscope by 5 examiners (4 endodontists and 1 professor of anatomy). The reviewers evaluated the slides together and came to a mutual agreement during a group review. The outcome measures consisted of the morphologic identification of pulp, odontoblasts, bone, dentin, cementum, and inflammation (intracanal and periapical). Dentinal thickening was measured by the degree of hard tissue deposition and was categorized into 3 groups: light (hard tissue deposition occupying less than 25% of the apex), medium (hard tissue deposition occupying 25%–50% of the apex), and heavy (hard tissue deposition occupying more than 50% of the apex). Data were statistically analyzed with the Fisher exact test ($P < .05$).

Results

Clinical evaluation revealed that the teeth in all groups had no evidence of swelling, mobility, or sinus tracts, and all restorations were intact.

In all of the positive control teeth, normal development was noted. The canals were filled with normal pulp tissue lined with odontoblasts subjacent to the predentin (Fig. 1*A*). Normal physiological dentinal thickening of the root and apical closure were observed along with the presence of multiple apical deltas (normal apex anatomy in ferret teeth). The periodontal ligament surrounding the tooth was normal, consisting of dense vascular connective tissue. The alveolar bone contained normal trabecular architecture.

In 3 of 6 teeth in the PRP group, 2 of 6 teeth in the blood clot/Gelfoam group, and 1 of 4 teeth in the no scaffold group, an ingrowth of hard tissues similar to the morphology of bone or cementum mixed with connective tissues was observed in the apical third of the roots to varying degrees (Figs. 1*B–D* and 2*A–C*) (Tables 1–3). The resolution of a periapical lesion was found in all samples with hard tissue ingrowth except for 2 samples that showed a light deposition of hard tissue despite the presence of periapical inflammation (1 from the no scaffold group and 1 from the PRP group) (Figs. 1*D* and 2*C*). The surface of the bone-like tissue was covered with cells similar to the appearance of osteoblasts and osteoclasts as identified by high-magnification light microscopy. The connective tissue contained numerous blood vessels and fibroblasts. Coronal to the ingrowth of hard tissue and connective tissue, granulation tissue and inflammatory cells were present. The thickening of the root walls in the apex appears to be attributed to the ingrowth of bone-like and cementum-like tissue lining the inside of the canal walls. Root lengthening was not observed in