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Treatment of experimental furcation perforations with mineral trioxide aggregate, platelet rich plasma or platelet rich fibrin in dogs' teeth



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ABSTRACT

This work evaluates the effect of mineral trioxide aggregate (MTA), platelet rich plasma (PRP) or platelet rich fibrin (PRF) on healing of non-contaminated and contaminated furcation perforations. A total of 192 teeth of 12 dogs was divided into three equal groups according to evaluation period. Each group was further subdivided into MTA, PRP, PRF, negative and positive control subgroups. Each experimental subgroup was further subdivided according to perforation status into non-contaminated and contaminated subdivisions. Root canal therapy was carried out and furcation perforation was made in all teeth except in negative control subgroup. The furcation perforation was repaired immediately in subdivision (1) and after 4 weeks in subdivision (2). The change in vertical bone loss was measured by radiography. Inflammatory cell count, cemental deposition, new bone formation, bone resorption and epithelial proliferation were assessed. Both PRP and PRF demonstrated statistically significant reduction in vertical bone loss and inflammatory cell count than MTA. No significant difference was found between MTA, PRP and PRF in cemental deposition, new bone formation, bone resorption and epithelial proliferation. The non-contaminated teeth demonstrated better treatment outcomes than the contaminated teeth. In conclusion, PRP and PRF are successful treatment options for repairing of furcation perforation in both non-contaminated and contaminated teeth in dogs with superior outcomes in non contaminated teeth.

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1. Introduction

Root perforation is a mechanical or pathological communication between the supporting periodontal apparatus and the root canal system. This communication compromises the health of the periradicular tissues and threatens the viability of the tooth (Duggins et al., 1994). Once an infection has established itself at the perforation site, prognosis for treatment is precarious and the complication may prompt extraction of the affected tooth (Gorni and Gagliani, 2004).

Furcation perforation usually occurs during a search for a canal orifice. Therefore, it is usually accessible. Numerous materials have been used for furcation and root perforation repair but none was an ideal biomaterial (Samiee et al., 2010).

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In several studies, mineral trioxide aggregate (MTA) has proved to be superior than most of the endodontic materials for repairing of furcation perforations due to its predictable periodontal ligament regeneration and cemental deposition (Holland et al., 2001; Hashem and Hassanien, 2008). However, extended setting time, poor handling and relatively high price are main disadvantages (Lysaght and Reyes, 2001; Ferris and Baumgartner, 2004; Tawfik et al., 2013; Nagy et al., 2014).

Platelet-rich plasma (PRP) is a composite of multiple endogenous growth factors which are able to enhance cell proliferation and differentiation (Anitua et al., 2007). It stimulates osteoblastic cells and the proliferation of periodontal ligament and inhibites epithelial cell proliferation (Okuda et al., 2003).

Platelet-rich fibrin (PRF) is described as a second generation platelet concentrate which has fibrin enriched with platelets and growth factors (Choukroun et al., 2006). Slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycanic chains in the fibrin meshes (Dohan et al., 2009).

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The aim of the present study was to evaluate the effect of MTA, PRP or PRF on healing of non-contaminated and contaminated furcation perforations in dogs' teeth by using radiographic and histologic examinations.

2. Materials and methods

This study was approved by the Ethical Committee at Faculty of Dentistry, Ain Shams University and Animal Use and Care Committee at Faculty of Veterinary Medicine, Cairo University, Egypt. All efforts were made to minimize animal suffering and to reduce the number of used animals. Three premolars and first molar teeth in each quadrant of 12 healthy mature mongrel dogs were used. These teeth were divided into 3 equal groups (64 teeth each) according to post-treatment evaluation time including; group I (one week), group II (one month) and group III (3 months).

Each group was subdivided into 3 experimental and two control subgroups according to the treatment protocol. These subgroups included; a (MTA), b (PRP), c (PRF), d (negative control) and e (positive control). Each experimental subgroup was further subdivided according to the perforation status into subdivision 1 (contaminated) and subdivision 2 (non-contaminated).

2.1. Anesthesia of the dogs

All dogs were premedicated with subcutaneous injection of atropine sulphate 0.05 mg kg^{-1} body weight (Atropine Sulphate; Misr Co., Cairo, Egypt) and intramuscular Xylazine HCl 1.1 mg kg⁻¹ body weight (Xylaject; ADWIA Co., Cairo, Egypt). The anesthesia was induced by intravenous Ketamine HCl 5 mg kg⁻¹ body weight (Ketamine hydrochloride; Rotexmedca Co., Trittau, Germany). The anesthesia was maintained by 25 mg kg⁻¹ intravenous incremental doses of 2.5% solution of thiopental sodium (Thiopental sodium; Sandoz, Kundl, Austria).

2.2. Tooth instrumentation

Preoperative radiographs were taken and endodontic access opening was done in all experimental and positive control teeth. Extirpation of the pulp tissue, instrumentation and irrigation of root canals with 2.5% sodium hypochlorite were performed. The canals were obturated with gutta-percha and Endofill sealer (Dentsply Hero, Petrópolis, Rio de Janeiro, Brazil).

2.3. Perforation creation

In subdivision 1 (non-contaminated), the access cavity was sealed with a temporary filling (Coltosol F: Coltosol Whaledent, Altstatten, Switzerland) without creation of furcation perforation.

In subdivision 2 (contaminated), furcation perforation was carried out in experimental and positive control teeth using a # 4 round bur. The perforation length was limited to 1 by rubber stopper. The access cavity was left open for 4 weeks to induce infection. Then, postoperative radiographs were taken.

For pain and infection control, the dogs were given intramuscular cefotaxime sodium at a dose of 10 mg kg^{-1} and diclofenac sodium at a dose of 1.1 mg kg^{-1} once/day for 5 days after surgery (Abu-Seida, 2012).

2.4. Treatment modalities

In subdivision (2) radiographs were taken to confirm bone resorption. Both access cavity and perforation were cleaned, curetted, irrigated with 2.5% sodium hypochlorite and then normal saline.

In subdivision (1), removal of the temporary filling and creation of furcation perforation were done.

In subgroup (a), ProRoot MTA powder (Dentsply Tulsa Dental, OK, and USA) was mixed, dispensed into the perforation canal with an amalgam carrier and condensed with small plugger.

In subgroups (b) and (c), pieces of PRP and PRF, respectively were used to repair furcation perforation. Mineral trioxide aggregate was used as a base over the pulp chamber floor and the access cavity was sealed with glass ionomer (Medifill: Promedica, Germany).

The teeth were left untouched in subgroup (d) and the access cavity was left open without repair in subgroup (e).

2.5. Radiographic evaluation

The radiographs were digitized using transparency scanner. Digital image file was converted into 32-bitt TIFF files using Image J software (Image J. 1.47. NIH, USA). TurboReg plug-in was used to transform non-standardized radiograph into standardized images (Thevenaz et al., 1998). From the base line and follow up radiographs, the change in vertical bone loss was calculated and expressed in percentage according to the following equation:

percentage of bone loss change

$$=\frac{follow \ up \ bone \ loss - base \ line \ bone \ loss}{base \ line \ bone \ loss} \times 100$$

2.6. Histological evaluation

According to the groups, the dogs were sacrificed by over dose of thiopental sodium. Each tooth with its surrounding bone was separated. Samples were fixed in 10% buffered formalin solution and decalcified for 8 weeks using formic acid-sodium citrate. Specimens were processed by conventional methods, sectioned at $4-6\,\mu$ m, stained with hematoxyline and eosin and examined for the followings:

2.6.1. Inflammatory cell count

The average of inflammatory cell count of three representative microscopic fields were measured using image analysis software (Image J 1.47)(Holland et al., 2007).

2.6.2. Cemental deposition

It was measured according to (Alhadainy et al., 1998) as follow: Score 0, 1, 2 and 3: absence, deposition of newly formed cementum on lateral walls of perforation or close to it, partial and complete newly formed cementum barrier respectively

2.6.3. New bone formation

It was measured as followings (Alhadainy et al., 1998):Score 0: no osteoblasts or osteoid.

- Score 1: slight osteoblastic rimming with no osteoid.
- Score 2: moderate osteoblastic rimming with some osteoid.
- Score 3: heavy osteoblastic rimming with abundant osteoid.

2.6.4. Bone resorption

It was measured as followings (Alhadainy et al., 1998): Score 0, 1, 2 and 3: no, few, moderate and many osteoclasts, respectively.

2.6.5. Epithelial proliferation

It was evaluated as followings (Alhadainy et al., 1998):

Score 0 and 1: absence and presence of epithelial proliferation, respectively.

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