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ORIGINAL ARTICLE

Comparison of Alveolar Ridge Preservation with Autograft Cancellous Bone Transplant and PRF Following Canine Tooth Extraction in Dog

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Abstract

Objective- Due to its specific position, the maxillary canine tooth is more exposed to the injuries, fractures and pulp necrosis. In these cases, the damaged teeth are usually pulled out. The root of this tooth is very large and sometimes extends over the premolar teeth. To extract it, a large amount of alveolar bone tissue should be harvested.

Design- Experimental study

Animals- Five adult male dogs

Procedures- In this research, five dogs were selected and their maxillary canine teeth in right and left (10 teeth in total) were surgically removed. Then the right dental cavity was filled with insulin PRF and the left one filled with autograft cancellous bone chips. Three weeks after the surgery, the cavity was sampled for histopathological examination. Factors such as epithelialization rate, fibroplasia, fibrotic reaction and intensity of inflammation were studied.

Results- The average score obtained from histological studies implies that epithelialization in PRF group is significantly higher than the other group, and there is no significant difference in other parameters.

Conclusion and Clinical Relevance- Since PRF preparation is not painful and risky for the patient and does not require any special care after the surgery. Therefore, it is used preferably.

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1. Introduction

Periodontal diseases are the most commonly diagnosed disease in dogs and cats. Posterior teeth including premolar 4 and molar 1 in the mandible and maxilla are the most teeth affected by periodontal diseases.¹ As dental care is very weak in domestic livestock; the disease is very advanced when the patient is referred to a clinic which leads to tooth extraction. Multi rooted teeth, such as premolar 3 or 4, are extracted by using open or closed techniques. Tooth extraction is very difficult. Choosing one of these two techniques for tooth extraction depends on the shape of the tooth, the present complication and the surgeon's preference. If the tooth cavity is left open, water and food residues accumulated in the cavity and may cause an infection. Also reported that the closed technique produces more pain.²

If the tooth extracted area is washed daily with an antiseptic solution like other open wounds, the chance of a complication before the closure of the wound is decreased. Due to the presence of saliva, high blood supply and warmth in the oral environment, the risk of infection of the oral ulcers would be less than other areas.³ Not closing the dental cavity is an easy method, but there might not be a proper restoration, so there is a greater chance of infection. On the other hand, covering the cavity using of suture can accelerate restoration, but the risk of pressure on the suture line due to the poor mobility of the soft tissues of gum may result in tearing the suture line and restoring to the first point.⁴ The dry socket is a painful, disabling and relatively common problem after tooth extraction.^{5,6} Recently, the dental cavity is filled with a substance called PRF and it prevents the formation of a dry socket.⁷

Due to its specific position, the maxillary canine tooth is more exposed to the injuries, fractures and pulp necrosis.⁸ In these cases, the damaged teeth are usually pulled out. The root of this tooth is very large and sometimes extends over the premolar teeth. To extract it, a large amount of alveolar bone tissue should be harvested. In addition, the

cavity remains very large which results in forming a cavity after repairing and it is very effective in the form of closure of the mouth and lips.⁹

To remove these teeth, a process called "socket preservation" or "alveolar ridge preservation" can be used.¹⁰ Autograft cancellous bone transplantation is commonly used after a tooth extraction. This material includes osteogenic, osteoinductive, osteoconductive properties. But requiring the use of another surgery to remove it from another site on the patient's body is one of its weaknesses.^{11,12} Using PRF is one of the new methods for accelerating the restoration of the bone scaffold. The efficacy of this substance has been proven in repairing of long bones and in osteoarthritis.¹³

In mammals the size and shape of teeth are different referred to heterodonty, making the teeth specialized in different applications. Specialized teeth include incisors (I), canine (C), premolar (P) and molar (M). In spite of the difference in appearances of the teeth, their components such as enamel, dentin, cementum and pulp are identical. In domestic livestock, two types of teeth can be seen. One is simple teeth, the other is compound teeth. The simple teeth (Brachydont teeth) are small in size, after extracting from gum, their growth can be stopped. The compound teeth (Hypsodont teeth) are longer than the simple teeth, continued to grow for some time after leaving the gum.^{14,15} The aim of this study is to compare the adequacy of autograft cancellous bone and PRF in the dental socket after extraction Also in this study we compared the alveolar cavity restoration rate of the canine tooth with these two compounds in dogs which is important due to preventing forming dry sockets.

2. Materials and Methods

Animals

In this study, five adult male dogs over one year old were used. Dogs were kept in individual cages for two weeks, adapting to the environment and obtaining basic

information. Before the study, animals were evaluated for oral and dental health. During the study, animals had free access to water and food. The protocol of this study was conducted according to ethical principles approved by the research committee of the Veterinary Faculty of Islamic Azad University.



Figure 1. PRF preparation from the blood (above) and putting PRF in the dental cavity (bottom).

PRF Preparation

Ten milliliter of blood sample of cephalic vein was taken. The blood collected in a tube without anticoagulant agents like EDTA. After two minutes of resting, the tube centrifuged at 30000 rpm for 10 minutes. The white jelly part above the clot, which is PRF, was isolated (Figure 1).

Bone Chip Preparation

After anesthetizing the animal to gain cancellous bone sample, the protuberant part of the Ilium crest was

determined, shaved, and the surface scrubbed with an aseptic method. Until the bone appears, dissecting the site was continued. Then the bone was trimmed by an electric saw and the desired chip was gained.¹⁶ It was put on a completely bloody sterilized gas and transferred to the remained cavity of the tooth (Figure 2). Absorbable suture was used for the muscular layer and non-absorbable suture (nylon) was used for the skin.¹⁷



Figure 2. Putting the bone chip on the tampon (above). Inserting the bone chip in alveolar cavity (bottom).

Surgical Procedure

Before surgery, the animals were kept off feed for 10 hours. For pre-anesthesia, acepromazine (0.1 mg/kg, IM) was used. After catheter administration in cephalic vein, anesthesia was induced with a combination of ketamine hydrochloride (10 mg/kg) and diazepam (0.5 mg/kg).¹⁸ Oral cavity was prepared using chlorhexidine (0.2%) solution. First, a mucosal flap was provided from the

lingual or maxillary surface. Using surgical micromotor and round milling, the alveolar bone was harvested semicircular to the root of the tooth. Using a dental elevator, the periodontal ligament of the tooth was loosened and the tooth pulled out with dental forceps (Figure 3). Five dental alveolar cavities were filled with bone graft and the other five cavities were filled with PRF. Then, the mucosal flap was sutured using a simple interrupted suture pattern by synthetic absorbable suture (Figure 4). After the treatment period, a biopsy was taken for histopathologic examination and comparison of restoration condition and bone matrix formation.



Figure 3. Removing the canine tooth until the root is visible.



Figure 4. Stitching the site at the end of the surgery.

Histopathologic Evaluations

The samples were sent to the histopathology laboratory after being fixed in 10% formalin buffered solution.

Paraffin blocks were prepared and 5-micron sections were provided by the microtome. Hematoxylin-eosin (H&E) method was used for staining the sections. After preparation, sections were studied under light microscope. Wound healing process was evaluated by comparing epithelization, fibroplasia, fibrotic response, and inflammation rate. The histopathological parameters were carried out as described by Gal *et al.*¹⁹

Statistical Analyses

The histopathologic results were analyzed using SPSS-22 software package and t-test. The significance level was considered as $p < 0.05$.

3. Results

The results of the histopathological parameters (Figures 5-6) including the amount of epithelization, the rate of fibroplasia, the response of the fibrotic and the intensity of inflammation after the statistical analysis are presented in Table 1.

4. Discussion

In this study, all the dogs had canine teeth removal surgery with the aim of repairing the remaining cavity of the teeth. During the study, there were no complications such as inflammation and infection in the surgical site and rupture or necrosis of the flap edge. The cavities of the teeth were filled with cancellous bone chips and platelet-rich fibrin (PRF). Three weeks after surgery, a histopathological examination was carried out.

Platelet regenerative potential was identified in the 1970s when scientists observed the platelets with growth factors being responsible for increasing collagen production, cell mitosis, growth of blood vessels, recruiting other cells to migrate to damaged area and induction of cell differentiation.²⁰ One of the latest innovations in surgeries focuses on the use of condensed platelets in tissue

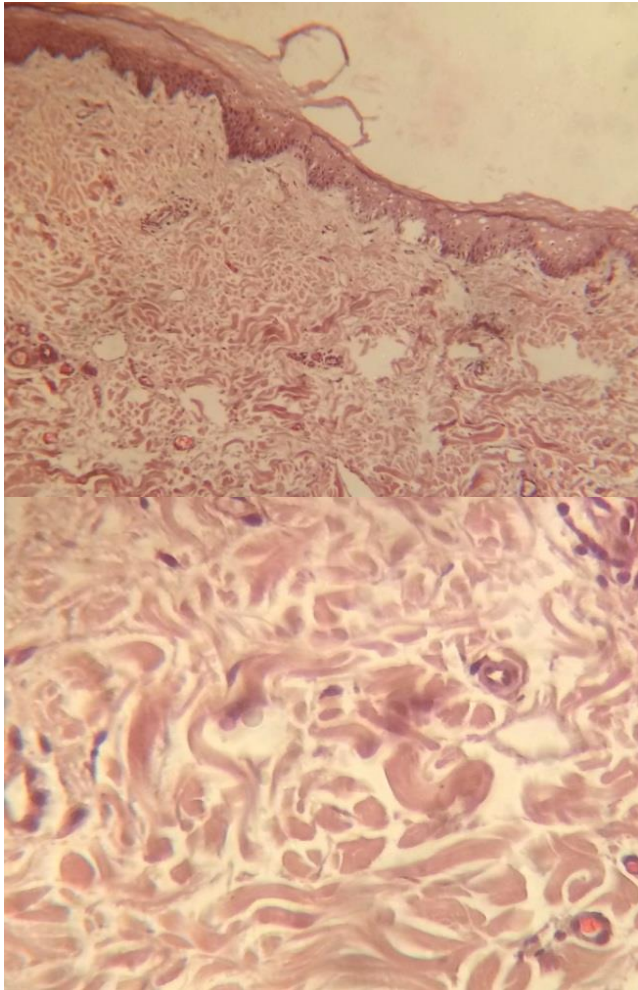


Figure 5. Microscopic images from recovery phase in PRF group 3 weeks after surgery. Above: The overall coverage of the wound by the epithelium (200×). Bottom: Compression and order in collagen fibers (400×).

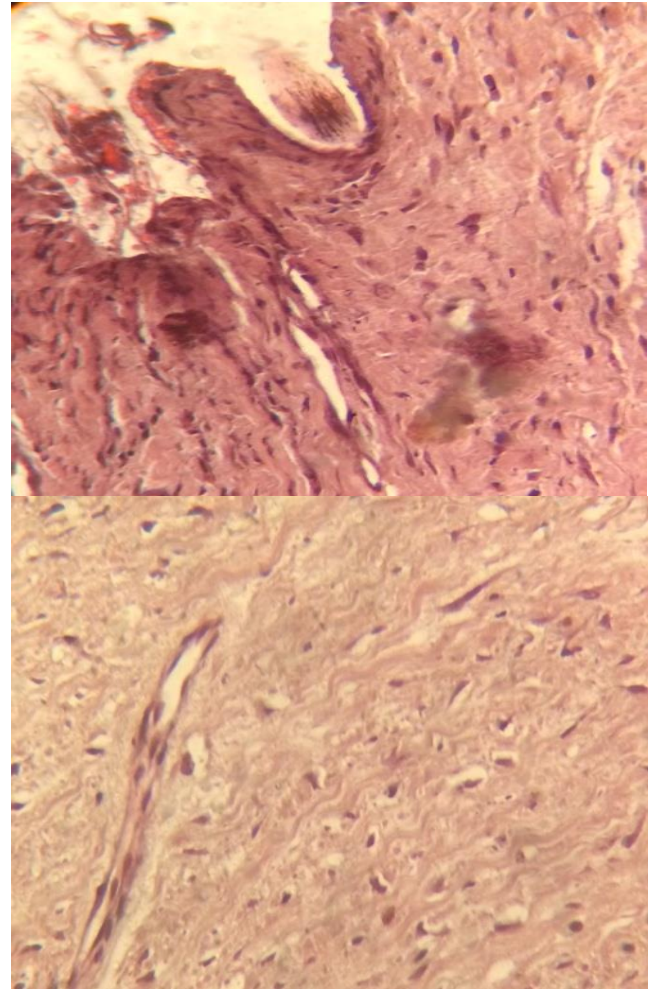


Figure 6. Microscopic images from recovery phase in the bone chip group 3 weeks after surgery. Above: More than half of the wound is covered (400×), Bottom: Collagen fiber expansion (400×).

Table 1. The mean and standard deviation of the histopathological indexes.

Groups	Histological parameters			
	Epithelization	Fibroplasia	Fibrotic reaction	Inflammation
PRF	5.5 ± 0.7*	5 ± 1.4	4.5 ± 0.7	6 ± 2.8
Bone chip	3.5 ± 0.7	5	4	8

* Indicates a significant difference

engineering applications inside the body, including platelet-rich plasma (PRP) and platelet-rich fibrin. Condensed platelet or platelet concentrate contains concentrated suspension of growth factors in platelets, which is used as a topical surgical bioavailable supplement to accelerate the healing process.^{21,22}

Platelet-rich fibrin containing cytokines, glycol chains, and structural glycoproteins slowly integrated into the fibrin

network. The beneficial effects of platelet-rich fibrin have been observed in various procedures such as maxillofacial plastic surgery and skin grafts.^{22,23}

Platelet-rich fibrin with its growth factors and cytokines produces healing potential on hard and soft tissues.^{24,25}

Chang *et al.* found that PRF produces the phosphorylated extracellular signal-regulated protein kinase (P-ERK) and stimulates the production of osteoprotegerin (OPG), which

causes the production and differentiation of osteoblast cells.²⁶ *In vitro* studies have shown that this autologous matrix has a great potential for increasing cellular attachment and stimulating the proliferation and differentiation of osteoblast cells.²⁷

Dohan *et al.* stated that PRF with immunological and antibacterial properties may lead to leukocyte degranulation and including some cytokines which can induce angiogenesis and pro-inflammatory or anti-inflammatory responses. In the process of producing PRF, leukocytes can also produce cytokines in response to hemostatic and inflammatory phenomena and play a key role in immunity regulation and inflammation conditions. This concept gives a reason for the reduction of post-operative infections when PRF is used as a surgical additive.²⁸

Chang *et al.* also claimed PRF can be used as an absorbable membrane for bone directed regeneration.²⁹ Simonpieri *et al.* rebuilt natural bone and gum tissue through the use of PRF membranes. In their study, the authors reported satisfactory clinical results on the overall deformation of the alveolar bone and the repair of the gum and bone volume around the implant, achieving a sufficient mechanical and cosmetic qualities.³⁰

In the process of producing PRF by centrifugation, platelets are activated, cytokine releasing. The slow fibrin polymerization during PRF processing leads to the combination of platelet cytokines and glycine chains in fibrin networks. Therefore, PRF in contrast to other platelet concentrates can rapidly release cytokines during regeneration of fibrin matrix which indicates the healing properties of PRF in the treatment process.³¹

In 2010, Huang *et al.* reported that PRF stimulates osteogenesis in human pulp cells by regulating the production of OPG and alkaline phosphatase.³² In 2008, Diss and colleagues used PRF graft for the reconstruction of the sinus floor while they found out that fibrin matrix of PRF directly stimulates angiogenesis. It has been shown that PRF contains osteogenesis induction and

osteoconduction properties.³³ Sun *et al.* stated that PRF promoted cell proliferation and increased expression of ALP, collagen type I and OPG significantly.³⁴ Sharma *et al.* used PRF for the treatment of patients with chronic oral diseases and stated that healing was performed more rapidly in groups that used platelet-rich fibrin therapy.³⁵ Pradeep *et al.* compared the effects of using PRF with PRP to treat bone loss in the mouth. The bone marrow regeneration in the group treated with PRF was greater than that of the PRP group.³⁶

In the present study, based on histological evaluations, there was more epithelialization in the PRF group than the other group. Accordingly, there is no significant difference in the other factors. This means that the PRF causes better healing than the self-bone chip. Therefore, it is preferable because it makes PRF be more comfortable than the chip, and separate surgery on the animal is not needed.

Conflict of Interests

None.

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