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Original research

## Socket preservation using platelet-rich fibrin in conjunction with epithelialized palatal free graft in minipigs

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### ABSTRACT

**Aim:** To evaluate the potential of platelet-rich fibrin (PRF) and epithelialized palatal free graft (FGG) for preserving the alveolar ridge after tooth extraction in minipigs.

**Materials and methods:** Forty-eight alveoli from six minipigs were randomly sealed with PRF, FGG, PRF&FGG, and blood clot. After 2, 6 and 12 weeks of healing, alveolar ridge width and height, as well as, radiographic optical density were measured. The decalcified specimens were processed for histological and histomorphometric analysis.

**Results:** PRF clinically showed early healing of soft tissue covering socket orifices in the first 2 weeks. At the 6th week, the ridge width of PRF ( $5.2 \pm 1.2$  mm), FGG ( $4.4 \pm 0.9$  mm) and PRF&FGG ( $4.9 \pm 0.6$  mm) were better preserved than the control ( $3.7 \pm 1.1$  mm) ( $P > 0.05$ ). Radiographically, the mean bone height/overall height alteration at the 12th week of PRF ( $8.96/-1.11$  mm), FGG ( $7.99/-1.88$  mm), PRF&FGG ( $8.37/-1.79$  mm) and control ( $8.40/-1.57$  mm) were comparable. However, the PRF ( $158.57 \pm 30.74$ ) showed a significant greater bone density than FGG ( $108.59 \pm 29.99$ ) and control ( $91.31 \pm 37.33$ ) ( $P < 0.05$ ). Histomorphometrically, the newly formed bone in PRF group was increased from 2nd to 12th weeks ( $42.31-52.00\%$ ), while the others showed unchanged percentage (FGG,  $42.72-42.00\%$ ; PRF&FGG,  $42.72-42.00\%$ ; control,  $39.65-42.74$ ) ( $P > 0.05$ ).

**Conclusions:** The use of PRF is an effective modality for short-term ridge preservation, while the use of FGG with or without PRF does not demonstrate any effect on early ridge preservation as evidence from clinical, radiographic and histomorphometric analysis.

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### 1. Introduction

Following extraction of the tooth, a dimensional loss of bone height and bone width is a natural occurrence during the healing phase. A recent systematic review evaluating alveolar bone dimension changes of extraction sockets in humans showed a range of width reduction of 2.6–4.6 mm [1,2]. The mean width of the alveolar ridge was reduced by 50% from 12 mm to 6.1 mm in 12 months. Two-thirds of the loss occurred in the first 3 months [3]. It is widely accepted that ridge preservation procedures following tooth extraction result in greater oro-facial dimension of bone than where no ridge preservation was performed. The remodeling of alveolar bone at the extraction site always decreases ridge volume and deforms the ridge configuration, which consequently

impairs placement of dental implants in the ideal positions [2,4] and orthodontic movement of the tooth posteriorly [5].

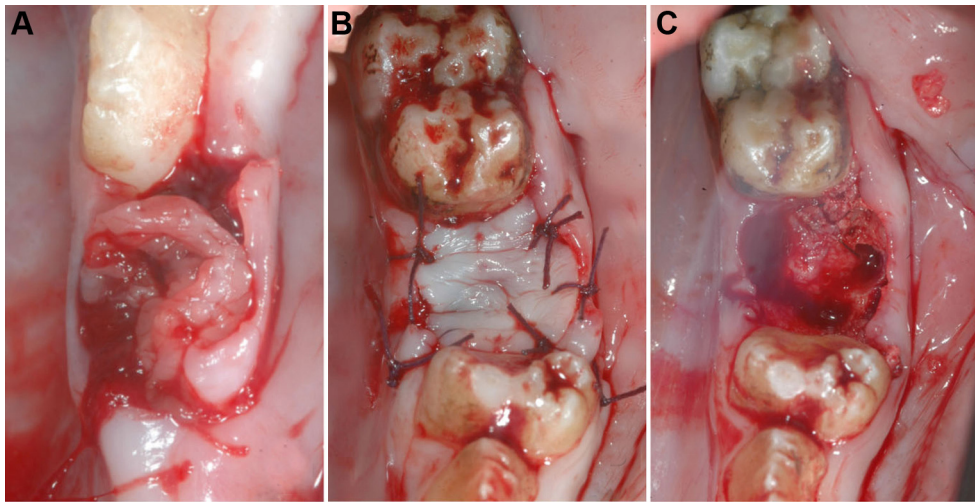
Selection of grafting material in the extraction socket is crucial and dictated by the timing of implantation. Implant placement can be Type 1 immediate placement or Type 2 placement in tissue healed socket, Type 3 placement in partially bone filled socket (4–12 weeks) or Type 4 placement in fully healed socket (after 12 weeks), which influences the stability of implant and esthetic profile particularly in the anterior region [6]. Several studies have proposed various ridge preservation techniques following tooth extractions, aiming to preserve the bone and acquiring healthy soft tissue. Allograft and xenograft with slow degradation have been shown good results in Type 4 implant placement [7–9]. However, in Types 2 and 3, slow degrading material may interfere with implant placement and the normal healing of the extract sockets [7,10]. Implantation at this stage gains advantages from soft tissue maturation, partially bone heal and limited bone resorption.

An epithelialized palatal free graft (FGG) harvested from the palatal area can generally supply adequate donor sites for soft tissue alveolar ridge augmentation [11,12]. Its advantages include predictability [13], color matching [14], and minimal adverse palatal

\* AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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**Fig. 1.** Clinical appearance of immediate extraction alveoli filled with epithelialized palatal free graft; platelet-rich fibrin; PRF (A), FGG (B); empty socket; control (C).

postoperative sequelae [15–17]. Although it is good for soft tissue, its effect to bone is still not known.

Platelet-rich fibrin (PRF), a rich source of autogenous cytokines and growth factors can be considered as a healing biomaterial for Type 2 and Type 3 sockets [18–20]. PRF has important properties of healing such as angiogenesis, immune control, harnessing the circulating stem cells, and wound protection by epithelial cover [21]. The properties of PRF are considered for promoting both soft tissue and bone regeneration and suitable for ridge preservation particularly in Type 2 to Type 3 implant placement.

The prime time for implantation is dictated by well maturation of soft tissue and much bone filled before remodeling occurs. The methods to enhance soft tissue and hard tissue regeneration are crucial and still not settle. The enhancement of soft tissue healing and promote bone healing in the extraction socket by means of using epithelialized palatal free graft and autologous growth factors from PRF were investigated. The aim of this study was to evaluate the potential of PRF and FGG to preserve the alveolar ridge after tooth extraction in minipigs.

## 2. Materials and methods

### 2.1. Animals

The study protocol was approved by the Committee for Animal Research, Prince of Songkla University (Ref no. 10/53). Six 15 month-old male minipigs weighing 45–60 kg were used. The animals were kept and fed pig food with a daily amount equivalent to 2% of the animal's weight and water ad libitum. The alveoli created in the minipig jaws were randomly assigned into 4 groups: Group I – alveoli filled with PRF; Group II – alveoli sealed with FGG; Group III – alveoli filled with PRF and sealed with FGG; and Group IV – alveoli filled with a blood clot and allowed to heal spontaneously (control group).

### 2.2. Surgical procedures

During the examinations and surgical procedures the animals were sedated by intramuscular injection with Azaperone (Stresnil®, Janssen-Cilag, Neuss, Germany, 2 mg/kg weight). Anesthesia was induced with an intravenous bolus of Tiletamine hydrochloride and zolazepam hydrochloride (Zoletil 100®, Virbac, Milperra, Australia, 2.5 mg/kg weight). In the area exposed to surgery, 1.8–3.6 ml of local anesthesia 4% articaine hydrochloride (Ubistesin® 1:200,000; 3M ESPE, Platz, Seefeld, Germany) was

injected. Before surgery and until 3 days following surgery, the animals were given 1 g of prophylactic amoxicillin (Vetrimoxin®, Ceva Sante Animale, Libourne, France, 15 mg/kg weight) intramuscularly. The single dose of metamizol (Dipyrone® 5 mg/kg weight) was administered intramuscularly for postoperative analgesic. The animal was checked daily for the first operative week for signs of infection and fed a standard diet and water ad libitum until the date of sacrifice.

The incisions were made in the crevice region of the 2nd (Pm2) and 4th (Pm4) permanent premolar. The papillae remained entirely intact and were still attached to the cementum of the adjacent teeth. The alveoli in the maxillary and the mandibular jaws of each minipig were created by removing the Pm2 and Pm4 from each quadrant of the jaw. The four alveoli of each jaw were sealed or filled with either method (1) PRF; (2) FGG; (3) PRF&FGG; or (4) control group (blood clot) and allowed to heal spontaneously. Each group of material comprised of four socket sites, which were assigned alternately to each site by rotating in a cycle permutation of the listed order above. In this way, each method represented every alveoli locations of the jaw. All surgical interventions were performed by one operator (L.N.).

#### 2.2.1. Group I: alveoli filled with PRF (Fig. 1A)

While extraction sites were prepared, 10-ml of autologous whole blood was collected from the femoral vein (from forelimb) by needle gauge No. 20 connected with a 10-ml sterile syringe without anticoagulant. Then the whole blood was transferred into a 10-ml glass tube which was immediately centrifuged using Hettich Zentrifugen centrifuge EBA 20 (Andreas Hettich GmbH & Co. KG, Germany) for 10 min at 3000 rpm. A fibrin clot was then obtained in the middle of the tube just between the red corpuscles at the bottom and acellular plasma at the top (Fig. 2A). The fibrin clot was collected with straight non-toothed forceps and was cut from the red corpuscles with scissors, then a fibrin was separated into two vertical halves of PRF (Fig. 2B) for two extraction sockets in the mandible or maxilla. The PRF was held in place by one figure of eight suture across the socket entrance with resorbable suture material (Vicryl® 4-0; Ethicon, Norderstedt, Germany).

#### 2.2.2. Group II: alveoli sealed with FGG (Fig. 1B)

Partial thickness epithelialized palatal free grafts 2–3-mm thick were obtained from the palate using a 15C scalpel blade. Each graft was slightly larger in diameter than the socket orifice. The FGG grafts were held in place by five to six simple sutures passing

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