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 ± 1.2 mm), FGG (4.4 ± 0.9 mm) and PRF&FGG (4.9 ± 0.6 mm) were better preserved than the control (3.7 ± 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 ± 30.74) showed a significant greater bone density than FGG (108.59 ± 29.99) and control (91.31 ± 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
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 settled. 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®), Jannsen-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 (Dipyrene® 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. 1A). 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. 1B) 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 scalp knife. 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
through the surrounding gingiva with resorbable suture material (Vicryl® 4-0; Ethicon, Norderstedt, Germany).

2.2.3. Group III: alveoli filled with PRF and sealed with FGG
The alveoli were filled with PRF and covered with the FGG according to the technique mentioned earlier.

2.2.4. Group IV: alveoli exclusively with blood clot (Fig. 1C)
The alveoli were filled with natural blood clots, sutured and allowed to heal spontaneously.

At the end of each designated healing period of 6 and 12 weeks, the animals were sacrificed by induction of deep anesthesia followed by withdrawal of the entire blood volume.

2.3. Clinical observation

After surgery and during the follow up period of 2, 6 and 12 weeks after extraction, the alveoli dimensions were recorded using a periodontal probe (Thin Williams Probe, Hu-Friedy, IL, USA). Mean values and standard deviations were calculated for the mid bucco-lingual width, the concavity of the alveolar ridge, and occluso-gingival concavity of each group pre- and postoperatively (Fig. 3). All measurements were done by one examiner (B.K.).

2.4. Radiographic observation

Immediately after surgery, periapical radiographs of the experimental sites were obtained to evaluate the initial bone height and optical density. Radiographs were taken digitally using a portable X-ray (NOMAD, Aribex Inc., UT, USA) with digital sensor size 0 attaching to digital sensor holders (XCP-DS®, Rinn, Dentsply, IL, USA). An individual bite block was used to control the vertical distance (Fig. 4A). The settings for all exposures were 60 kVp, 2.3 mA and 0.30 s.

Under intravenous anesthesia, the serial radiographs were taken with an occlusal jig to evaluate bone height and optical density of the alveoli at 2, 6, and 12 weeks post operatively.

Radiographic bone height and density of the alveolus area were determined by using the image analysis software (Image Pro Plus 5.0, Media Cybernetics, MD, USA).

To measure the bone height, 5 referenced lines were used (Fig. 4B). Firstly, the horizontal base line was drawn perpendicular to a line located 13 mm below the mesial marginal ridge of the Pm3 or 11 mm below the distal marginal of first permanent molar (M1) for Pm2 and Pm4 socket respectively. Once the horizontal base line was drawn, 5 vertical lines were drawn perpendicular to the base line and ended at the bone level visualized: bone height-1 (H1) was located in the mesial extremity of the alveolus; bone height-2 (H2) was located in the distal extremity of the alveolus; bone height-3 (H3) was located in the center of the alveolus; bone height-4 (H4) was located in the middle between H1 and H3; and bone height-5 (H5) was located in the middle between H2 and H3.

Optical density of the alveolus area was evaluated through densitometry variations of grayscale, which varied from 0 to 255 (transparent to opaque). The measurement area was delimited between the mesial root of the M1 and the distal root of Pm3, including the alveolar area of extraction socket (Fig. 4C).

2.5. Histological and histomorphometric analysis

The maxilla and mandible were harvested and the alveolar ridges were cut in a block of 1.5 cm in height including the extraction socket. Each block contained two experimental sites for histologic preparation. The harvested blocks were fixed in 10% formalin for 2 weeks before decalcified in EDTA, dehydrated in increasing concentrations of ethanol, embedded in paraffin. Decalcified specimen, 5-μm thickness, was prepared in mid coronal plane along the axis of the extraction socket, representing the central portion (in mesial-distal direction) of each site, and stained with hematoxyline and eosin.
Histomorphometrical analysis was done by image captured using a light microscope (Axiostar, Carl Zeiss, Germany) at a magnification of 5×, associated with a camera (Axiocam mRC, Carl Zeiss, Germany). Digital images were evaluated using a software program Image Pro® Plus 5.0 (Media Cybernetic, Silver Springs, MD, USA). The following aspects of the alveoli were evaluated:

(A) The percentage of newly formed bone (%) measuring the area and bone formation at the socket orifice extending not beyond 2 mm vertical from the coronal area of new bone.

\[
\text{Newly formed bone(\%)} = \frac{\text{Mineralized bone area} \times 100}{\text{Total bone defect area}}
\]

(B) The vertical distance (VD) between buccal and lingual bone crests (Fig. 5) measuring the vertical distance between the highest point of the buccal and lingual crest. Firstly, an axis line from the uppermost part of the alveolus soft tissue contour to the center of the socket (C–C) to separate the buccal and lingual compartments was drawn. Subsequently, horizontal lines (L and B) perpendicular to C–C connecting with the most coronal portions of the buccal and lingual bone crest to C–C were drawn. The difference of vertical distance between the buccal and lingual intersections with C–C was measured. (C) The buccal and lingual bone width (Fig. 5) was determined at three different levels: A, B and C, that were located at 1, 2, and 3 mm apically from the crest.

2.6. Statistical analysis

All data were presented in mean and standard deviations. One-way analysis of variance and Post hoc test with Scheffé test were applied to detect differences among groups where appropriated. The Paired T-Test was used to analyze the difference between the 2 time intervals. The statistical analysis was performed using SPSS software (version 13.0, SPSS, Chicago, IL, USA). The significance level was <0.05.

3. Results

3.1. Clinical observations (Fig. 6)

All alveoli healed uneventfully. Overt signs of soft tissue inflammation (swelling and redness) were seen during the first week of healing. No infections or wound dehiscence were observed during the study period. After 2 weeks, the socket orifice in the control group was covered with granulation tissue with shallow niche in the central portion of the ridge. In PRF group, the granulation tissue covering the socket orifice was denser and more mature than other groups but was not completely covered by epithelium. After 6 weeks, all sites were completely covered with mature epithelium. Alveolar ridge resorption was clearly observed in the direction of bucco-lingual (B–L) and occluso-gingival (O–G) at 12-week period (Table 1). The B–L concavity was more pronounced on the buccal side than the lingual side and progressed rapidly in all groups except the PRF group.
Table 1
Means and standard deviations of alveolar ridge width comparing between groups at 6 and 12 weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mid bucco-lingual width (mm)</th>
<th>PRF</th>
<th>FGG</th>
<th>PRF&amp;FGG</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concave (bucco-lingual)</td>
<td>5.21 ± 1.22</td>
<td>4.38 ± 0.89</td>
<td>4.87 ± 0.63</td>
<td>3.73 ± 1.11</td>
<td>0.088</td>
<td></td>
</tr>
<tr>
<td>Concave (occluso-gingival)</td>
<td>1.07 ± 0.19</td>
<td>1.14 ± 0.69</td>
<td>1.43 ± 0.61</td>
<td>1.30 ± 1.03</td>
<td>0.742</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>5.10 ± 0.75</td>
<td>4.90 ± 0.71</td>
<td>4.92 ± 0.76</td>
<td>4.41 ± 0.68</td>
<td>0.345</td>
</tr>
<tr>
<td>Concave (bucco-lingual)</td>
<td>1.2 ± 1.09</td>
<td>1.2 ± 0.97</td>
<td>1.08 ± 0.49</td>
<td>1.17 ± 0.75</td>
<td>0.976</td>
<td></td>
</tr>
<tr>
<td>Concave (occluso-gingival)</td>
<td>1.4 ± 0.55</td>
<td>1.2 ± 0.57</td>
<td>1.57 ± 0.52</td>
<td>1.47 ± 0.52</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>1.29 ± 0.49</td>
<td>1.50 ± 0.50</td>
<td>1.21 ± 0.64</td>
<td>1.57 ± 0.67</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Fig. 6. Clinical appearance of healing socket at 2, 6 and 12 weeks post-extraction.

3.2. Radiographic evaluation

3.2.1. Bone height analysis

Fig. 7 showed the results of bone height as measured from 5 locations (H1–H5) immediately after extraction, 2, 6, 12 weeks after extractions. In the control group, bone height was decreased markedly in 6 weeks after extraction then increased gradually until 12 weeks after extraction. The experimental groups represented the same pattern of bone remodeling as the control group, except the FGG group that the bone height had further decreased from 6 to 12 weeks after extraction. Mean bone height change were presented in Table 2. The overall bone height alteration of the control (−1.57 mm), PRF (−1.11 mm), FGG (−1.88 mm), and PRF&FGG (−1.79 mm) were not statistically different.

Concerning to the location of analysis, the H1, H3 and H2 locations were the most susceptible to bone height reduction in all groups at 12 weeks after extraction. Only the PRF group showed a mean bone height alteration less than the control in every location. Only the mean bone height alteration at H1, H3 and H2 in the PRF (−1.81, −0.49, −0.91 mm) were lower than the control group (−2.50, −1.96, −1.7 mm), while in FGG (−2.44, −2.23, −1.46 mm) and PRF&FGG (−2.35, −2.14, −1.67 mm) the mean bone height were comparable to the control. No statistically significant differences were detected among the experimental
groups, location of measurement and time frame at 0, 2, 6 and 12 weeks after extraction ($P > 0.05$).

### 3.2.2. Optical density analysis

Table 3 and Fig. 8 showed the mean bone optical density among the groups at 0, 2, 6, and 12 weeks after the extractions. The control group, representing the normal remodeling, revealed a gradual decrease in optical density from immediate after extraction (105.49 ± 47.34) until the end of the observation period at 12 weeks (91.31 ± 37.33). At 12 weeks, the PRF group (158.58 ± 30.74) showed significant increased in mean optical density when compared to the control (91.31 ± 37.33) and FGG (108.59 ± 29.99) groups ($P < 0.05$).

### 3.3. Histology observation

#### 3.3.1. Six-week of healing (Fig. 9)

After 6 weeks of healing, all groups represented the same feature of complete keratinized mucosa, incompletely closure of the socket orifice with hard tissue bridge but not the PRF group that represented nearly complete hard tissue bridge.

The coronal portion of all groups were comprised of woven bone extending from lateral socket walls and penetrating along with provisional matrix (PM) which contained densely packed mesenchymal cells present in a collagen-rich connective tissue matrix. However, the PRF group showed denser woven bone than the other groups. At the central and apical portions, only the PRF presented of woven bone extending from the sockets wall toward the center of socket, while the other groups presented with bone marrow. In all groups, the thin buccal and thick lingual bone walls were observed and the buccal crest was consistently “apical” to its lingual counterpart.

#### 3.3.2. Twelve-week of healing (Fig. 10)

After 12 weeks of healing, all experimental groups showed histologically similar to the control group. The alveolus was characterized by the presence of a hard tissue bridge sealing the coronal portion of the socket. The hard tissue bridge was continuously connected the buccal and lingual bone wall. This marginal bone bridge was mainly composed of woven bone in all groups except the PRF.

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**Fig. 7.** Bone height comparison between the groups, location at 0, 2, 6, 12 weeks post-extraction.

**Fig. 8.** Graph describing the mean bone density at 0 weeks, 2 weeks, 6 weeks and 12 weeks post-extraction between the groups.

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**Table 2**

<table>
<thead>
<tr>
<th>Time</th>
<th>Change in bone height</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(difference of bone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>height between time</td>
<td></td>
</tr>
<tr>
<td></td>
<td>intervals (mm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRF</td>
<td>FGG</td>
</tr>
<tr>
<td>0 week</td>
<td>10.07 ± 1.9</td>
<td>9.86 ± 2.51</td>
</tr>
<tr>
<td>2 weeks</td>
<td>9.27 ± 1.93/−0.80</td>
<td>8.94 ± 2.44/−0.92</td>
</tr>
<tr>
<td>6 weeks</td>
<td>7.78 ± 1.79/−1.49</td>
<td>8.21 ± 1.00/−0.73</td>
</tr>
<tr>
<td>12 weeks</td>
<td>8.96 ± 1.81/−1.18</td>
<td>7.99 ± 2.04/−0.22</td>
</tr>
<tr>
<td>Overall bone change</td>
<td>−1.11</td>
<td>−1.88</td>
</tr>
</tbody>
</table>
group that it was replaced with lamellar bone in some area. The apical portion of socket was dominated by bone marrow except the PRF group which comprising of woven bone and small lamellar bone. The crestal region of buccal bone wall was resorbed apical to its lingual bone wall.

3.4. Histomorphometric analysis

3.4.1. The percentage of newly formed bone

The percentage of newly formed bone was shown in Fig. 11. The percentage of newly formed bone in PRF group at 12 weeks was about 1.07 and 1.24 time to the control group. No statistically significant difference between groups was found in either of the observed parameters ($P = 0.181$).

3.4.2. The vertical distance (VD) between buccal and lingual bone crests (Fig. 12)

At 6-week interval, the buccal bone crest was located $2.04 \pm 1.48$ mm apical to the lingual crest in control group. The VD between buccal and lingual bone crests in PRF ($1.55 \pm 0.65$ mm), FGG ($1.89 \pm 0.99$ mm), and PRF&FGG ($1.46 \pm 0.64$ mm) were apical to the lingual crest and less than the control group.

At 12-week interval, the buccal bone crest was consistently located apical to its lingual counterpart. The VD between buccal and lingual bone crests in control group ($2.48 \pm 1.38$ mm) was higher than at 6-week and also than the PRF ($0.85 \pm 0.42$ mm), FGG ($0.86 \pm 0.41$ mm), and PRF&FGG ($1.02 \pm 0.96$ mm) significantly.

3.5. The buccal and lingual bone width

The width of the buccal and lingual bone walls at levels A, B and C were described in Table 4. The marginal portion of the lingual bone wall of the alveolus was slightly wider than the corresponding portion of the buccal wall in both 6 and 12 weeks. The marginal portion of the original buccal bone wall was located apical to its lingual counterpart. Buccal plate configuration exhibited a concave appearance with invagination toward the alveolus.

3.5.1. Six-week of healing

The width of buccal bone wall at level A of all experimental groups was significantly wider than control group ($P < 0.05$). The
Table 4
Mean and standard deviations of buccal and lingual bone width at the different level.

<table>
<thead>
<tr>
<th>Level</th>
<th>Buccal (mm)</th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>P</th>
<th>Level</th>
<th>Lingual (mm)</th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.15 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94 ± 0.26</td>
<td>0.33</td>
<td>A</td>
<td>1.88 ± 0.98</td>
<td>1.64 ± 0.48</td>
<td>0.587</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.50 ± 0.56</td>
<td>1.09 ± 0.31</td>
<td>0.12</td>
<td>C</td>
<td>2.13 ± 0.76</td>
<td>1.82 ± 0.81</td>
<td>0.499</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.61 ± 0.75</td>
<td>1.14 ± 0.45</td>
<td>0.219</td>
<td>C</td>
<td>2.28 ± 1.25</td>
<td>2.45 ± 0.97</td>
<td>0.785</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.08 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89 ± 0.33</td>
<td>0.277</td>
<td>A</td>
<td>1.19 ± 0.41</td>
<td>1.33 ± 0.49</td>
<td>0.602</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.13 ± 0.41</td>
<td>1.26 ± 0.60</td>
<td>0.664</td>
<td>B</td>
<td>1.49 ± 0.47</td>
<td>1.78 ± 0.53</td>
<td>0.343</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.30 ± 0.60</td>
<td>1.47 ± 0.81</td>
<td>0.689</td>
<td>C</td>
<td>1.68 ± 0.30</td>
<td>2.46 ± 1.00</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRF&amp;FGG</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.05 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66 ± 0.25</td>
<td>0.051</td>
<td>A</td>
<td>1.19 ± 0.41</td>
<td>1.50 ± 0.63</td>
<td>0.333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.47 ± 0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.75 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.002</td>
<td>B</td>
<td>1.80 ± 0.51</td>
<td>1.86 ± 0.66</td>
<td>0.852</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.18 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.88 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0</td>
<td>C</td>
<td>2.18 ± 0.45</td>
<td>0.53 ± 0.93</td>
<td>0.489</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.48 ± 0.13&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.03 ± 0.48</td>
<td>0.02</td>
<td>A</td>
<td>1.08 ± 0.43</td>
<td>1.14 ± 0.41</td>
<td>0.813</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.94 ± 0.35</td>
<td>1.53 ± 0.74</td>
<td>0.103</td>
<td>B</td>
<td>1.51 ± 0.46</td>
<td>1.50 ± 0.55</td>
<td>0.993</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.06 ± 0.43</td>
<td>1.39 ± 0.70</td>
<td>0.358</td>
<td>C</td>
<td>1.71 ± 0.65</td>
<td>1.87 ± 0.58</td>
<td>0.67</td>
<td></td>
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<sup>a</sup> Significantly different from control at 6 weeks (P<0.05).
<sup>b</sup> Significantly different between 6 weeks and 12 weeks (P<0.05).

PRF group demonstrated the widest width in every level comparing with other groups.

3.5.1.2. Twelve-week of healing

The horizontal dimensions at the three levels of the lingual side demonstrated no significant differences among the treatment groups.

4. Discussion

The effects of PRF and FGG were evaluated sequentially in clinical, radiographical and histological dimensions after tooth extraction.

Platelet-rich fibrin (PRF) was a platelet concentrate, introduced by Dohan et al. [18] for specific use in oral and maxillofacial surgery. PRF was reported to have better effects than PRP in improvements of tissue healing and bone formation [22–24]. PRF has been reported to gradually release autologous growth factors and expressed stronger and more durable effect on the cellular proliferation and differentiation than PRP in vitro [19,25].

The present study shows that PRF enhanced early healing of soft tissue for covering socket orifices in the first 2 weeks as seen by mature mucosa covering the orifice, and maintained the alveolar ridge dimension at 6 and 12 weeks better than the control. The use of FGG with or without PRF did not show neither faster soft tissue healing, nor better alveolar ridge dimension than the control group, especially at 6 weeks after extraction. The marked collapse of buccal bone walls were demonstrated at 6 weeks and progressed until 12 weeks. These findings are consistent with data published by Oghli and Steveling [26] that free soft tissue graft cannot prevent alveolar bone loss, however, it can improve only the esthetic outcome. This current experiment is in agreement with Araujo and Lindhe [27] that the use of a free gingival graft had no beneficial effect on contour alteration of the ridge, particularly at the buccal aspect, whereas, Landsberg and Bichacho [28] stated that free gingival

Fig. 11. Histogram describing the mean percentage of newly formed bone at 6 and 12 weeks between the groups.

Fig. 12. Histogram describing the vertical distance between the buccal and lingual bone crests. All the investigated stages the buccal crest was consistently “apical” of its lingual counterpart (*significantly different from control P<0.05).
graft could prevent soft tissue collapse. However several clinical trials supported that the additional use of a free gingival graft to seal the orifice of the extraction socket gave successful treatment outcomes with secondary wound healing over alien materials placed in the extraction socket.

The present data showed that the utilized autologous PRF in extracted sockets allows acceleration of bone healing, as indicated by thicker buccal bone wall, greater bone optical density and less marginal bone resorption. It is postulated that PRF fibrin contains various kinds of bone healing cytokines [18-29,30] that can protect growth factors from proteolysis [31], prolong the release of growth factors and delay the peak of releasing until day 14 [25]. These finding along with the data that the ingrowth, proliferation and differentiation of osteoblasts occur during the initial 14 days [32,33], it is the reasonable explanation that PRF could stimulate faster bone regeneration in situ without waiting for normal response of the body.

After tooth extraction, the amount of vertical bone loss was more pronounced on the buccal than on the lingual bone wall, this observation was in agreement with previous clinical studies [3,27,34–37]. Araujo and Lindhe [27] demonstrated that 1 week after tooth extraction the crest of the buccal bone plate was situated coronally to the lingual bone plate. They also demonstrated that osteoclasts were present in the exposed area of the outer alveolar ridge at 1st and 2nd week after tooth extraction [27]. It was assumed that the osteoclastic activities at the internal and external sides of the alveolar bone were synergized and led to a more pronounced loss of the buccal bone plate. Consequently, when the buccal bone plate was resorbed, the soft tissue complex could not be stabilized and then be collapsed into the previously bone space. As the buccal soft tissue occupies the bone resorbing space then there was no room for bone regeneration and led to bucco-oral shrinkage and marginal bone resorption.

In this study, the notable marginal bone resorption was reported. The FGG group presented bone height loss consistently to 12 weeks, whereas the other groups showed that the height was recovered after 6 weeks. In the FGG and PRF&FGG groups, the periosteum of the marginal gingiva was detached to facilitate the placement of free soft tissue graft. By elevating the periosteum, the blood supply of the exposed bone surface was compromised, leading to osteoclastic activity and bone resorption [38,39]. In the present study, only PRF (~1.11 mm) demonstrated the least overall bone alteration. To reduce the vertical bone loss to be less than 1 mm seem very little but generates more effect where dental implantation is needed especially in an esthetic area.

From a histological standpoint, only the PRF group showed lamellar bone at 12 weeks while other groups showed a few bone with large marrow space at 6 and 12 weeks. Previous reports [40–42] explained that the healing of nonload area such as the extraction socket formed only the cortical bridge without demand for mineralized tissue in the space. PRF group acted differently in promoting bone formation to be woven bone at 6 weeks in the tooth socket and bone maturation with small lamellar bone after 12 weeks.

5. Conclusion

In conclusion, platelet rich fibrin (PRF) has the positive effect on both soft and hard tissue of extraction socket in an early phase of healing by promoting faster healing of soft tissue covering the socket orifice in the first 2 weeks, enhancing bone healing, and preserving the marginal bone height and width as an evidence from the radiographic optical density and histomorphometric analysis at 12 weeks. The used of epithelialized palatal free graft (FGG) with or without PRF did not demonstrate any effect on early ridge preservation.

Within limitations of this study, the used of PRF for ridge preservation seemed to be able to limit the resorption processes to a certain context. PRF is recommended to be used for short-term ridge maintenance, as in type II implant placement.

Acknowledgment

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References


