Full Length Research Paper

Evaluation of histological validity of platelet-rich fibrin on alveolar socket healing

Barış Altuğ AYDİL¹*, Levent PIKDOKEN², Berkem ATALAY¹, Emre BARİŞ³, Lutfiye YAZAR¹ and Aysenur UZUN¹

¹Department of Oral and Maxillofacial Surgery, Istanbul University, Istanbul, Turkey.
²Department of Oral and Maxillofacial Surgery, Gülhane Military Medical Faculty, Haydarpaşa Academic Hospital, Istanbul, Turkey.
³Department of Oral Pathology, Gazi University, Ankara, Turkey.

*Corresponding author. E-mail: baris.aydil@istanbul.edu.tr, Tel: +90 532 295 7221

Accepted 11 July, 2017

The aim of this study was to evaluate the effect of platelet-rich fibrin (PRF) on the early stage of bone healing in extraction sockets. Ten patients in whom the extraction of at least two neighbouring teeth was necessary for dental implant placement prior to prosthetic therapy were included in the study. In a total of twenty neighboring extraction sockets in nine patients, ten were treated by PRF and ten left without further treatment. Four weeks after extractions, biopsy samples were obtained for histological and histomorphometric analysis to evaluate and compare the volume and area of regenerated bone in the PRF-treated sockets to non-PRF-treated sockets. Although platelet-rich fibrin demonstrated the enhancement of the bone regeneration after the fourth week following tooth extraction, histologic and histomorphometric analyses showed no significant differences between the two groups. The present study indicates that the use of PRF for bone formation on alveolar socket preservation has no significant effect on the early healing.

Key words: Alveolar bone, extraction socket, platelet rich fibrin, bone regeneration, dental implant.

INTRODUCTION

Preserving the main structure and volume of the alveolar bone is providing esthetic and functional prosthetic restoration after implant therapy. The residual alveolar ridge height and width is almost always reduced after dental extraction. Bone loss after tooth extraction usually occurs within the first six months, but continues resorption lifelong slowly (Hauser et al., 2013; Oghli and Steveling, 2010; Horowitz et al., 2012).

Guided bone regeneration (GBR) is an accepted surgical method in implant dentistry to increase the bone quantity and quality in areas of localized alveolar defects (Barry et al., 2011). Currently, GBR methods use various bone promoting molecules such as enamel matrix derivative, recombinant growth and differentiation factors, and autologous platelet concentrates that are either bioresorbable or nonresorbable (Vignoletti et al., 2012; Baslarli et al., 2015; Del Fabbro et al., 2011). One of the adjunctive measures in alveolar socket preservation is platelet-rich fibrin (PRF) (Choukroun et al., 2006; Zhang et al., 2012).

PRF is an autologous fibrin product belonging to a second generation of platelet concentrates. It is a slowly and naturally polymerizing fibrin matrix in which growth factors (PDGF-ββ, TGF β-1, VEGF, and insulin-like growth factor-1), leukocytic cells, and their cytokines (interleukin [IL]-1β, IL-6, IL-4, and tumor necrosis factor-α) are enmeshed. Almost all the platelet and leucocyte content of the collected blood are concentrated in PRF. As evidenced by increased alkaline phosphatase activity, PRF can lead to in vitro osteoblastic proliferation and differentiation of human bone mesenchymal stem cells dose-dependently (Dohan et al., 2006; Choukroun et al.,
2016).

Some applications of PRF have been performed in oral and maxillofacial surgery, in dental implantology, and plastic surgery all of which yielded satisfactory clinical healing results (Simonpieri et al., 2012).

The aim of this study was to investigate whether PRF was capable to enhance bone healing in PRF-treated extraction sockets compared to non-PRF-treated sockets four weeks after tooth extraction with histologic and histomorphometric analyses.

MATERIALS AND METHODS

Study design

This research study was conducted in accordance with the requirements of Helsinki Declaration of 1975 as revised in 2008.

Ten patients who are systemically healthy (5 males and 5 females, age range 25-35, all non-smokers), in whom the extraction of non-neighbouring two premolars or molars were required for dental implant placement prior to prosthetic therapy, were included in the study. The patients who have poor oral hygiene, systemic disease (such as diabetes, immunosuppression, renal or hepatic failure), and patients undergoing bisphosphonate therapy, pregnant patients were excluded from the study. The extraction defects were divided into two groups. The control group (Group I) consisted of the defects which were left empty. The tooth extraction defects which were filled with PRF alone formed the Group II.

PRF Preparation

For patients in the PRF group, PRF membrane preparation was performed immediately before the extraction procedure. A venous blood sample was taken in 9 mL tubes without anticoagulant or bovine thrombin or any other gelling agent. They were immediately centrifuged at 2700 rpm for 12 min. Blood centrifugation immediately after collection permitted the composition of a fibrin clot between the red corpuscles and a cellular plasma. After centrifugation, PRF clot was separated from the portion of red blood cells (red thrombus), obtaining a fibrin clot with a red small portion in order to include the “buffy” coat richer in large leucocytes. This was a simple procedure without biochemical blood handling and was contamination free (Hauser et al., 2013; Choukroun et al., 2006; Gaetano et al., 2015; Dohan et al., 2006).

Surgical procedure

A simple extraction procedure was retained: local anesthesia (4% articaine with epinephrine 1:100,000; Ubistesin; 3M ESPE AG, Seefeld, Germany); use of dental elevators, extraction with forceps, curettage was done. The teeth were extracted in a non traumatic manner without elevation of full-thickness flaps and preserving the buccal and lingual walls of the alveolar sockets in order to minimize the possible trauma. Socket filling with PRF membranes; placement of the PRF membranes over the alveolar crest for the PRF group and sutured with 3/0 silk (Doğsan, Trabzon, Turkey). Follow-up was performed at Week 1 with suture removal.

Histologic and histomorphometric analyses

Four weeks after tooth extractions, at the time of dental implant placement, biopsy samples were obtained from the PRF-treated and non-PRF-treated sockets by a trephine bur (Meisinger LLC, Centennial, CO) with an internal diameter of approximately 2 mm. In order to facilitate the biopsy sampling, buccal and lingual flaps were gently and carefully raised to the level of alveolar bone crest. Coronal part of each specimen was marked with indelible chine ink. The specimens were immediately stored in 10% formalin-buffered solution and decalcified in 10% formic acid solution and then longitudinally embedded in paraffin blocks. The serial sections in the sagittal plane were cut in 4-6 µm of thickness and stained with hematoxylin-eosin dye. The light (Nikon Eclipse E600; Nikon, Tokyo, Japan) and polarized light (Leica DM-4000B; Leica Microsystems, Wetzlar, Germany) microscopic examinations were performed to evaluate the structure of bone trabeculae and the amount of mineralization, respectively. In the histomorphometric analyses, the images of the areas of newly formed bone and soft tissue from 3 different parts of each section were obtained under 100 magnification by means of a motorized light microscope (Leica DM-4000B; Leica Microsystems, Wetzlar, Germany). The areas of graft material and new bone and soft tissue were quantified in square micrometers by an image analysis program (Leica Q-Win Plus V3; Leica Microsystems, Germany). All histologic and histomorphometric assessments were carried out by the same pathologist who was not informed about the treatment modalities. Since the data on interventions were recorded by a separate clinician, the study was carried out in a double-blind manner.

Statistical analysis

Statistical analysis was performed with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA). Data were presented as means and standard deviation (SD) values. Statistical analysis was performed using the Mann-Whitney U test to compare the two groups, and statistical significance was assumed at a level of p<0.05.
Table 1. Histomorphometric values of the Group I and II.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (Control)</th>
<th>Group II</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SS</td>
<td>8527589±3616749</td>
<td></td>
</tr>
<tr>
<td>Bone area</td>
<td>Median 6583034 (4426360-8436626)</td>
<td>8134060 (5581099-11256158)</td>
<td>0.214</td>
</tr>
<tr>
<td>Bone+Graft (Total hard tissue)</td>
<td>Mean±SS 6341047±2128243</td>
<td>10642173±5684860</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>Median 6583034 (4426360-8436626)</td>
<td>9103544 (5758661-17444550)</td>
<td></td>
</tr>
<tr>
<td>Soft tissue area</td>
<td>Mean±SS 4902050±2763428</td>
<td>5963928±3401289</td>
<td>0.594</td>
</tr>
<tr>
<td></td>
<td>Median 3770587 (3007059-7931469)</td>
<td>6118349 (2766609-7931761)</td>
<td></td>
</tr>
<tr>
<td>Total area</td>
<td>Mean±SS 11243097±3701987</td>
<td>24913802±27651954</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td>Median 11705015 (8293876-14919658)</td>
<td>15991714 (11341192-25927406)</td>
<td></td>
</tr>
<tr>
<td>Total hard tissue / Total area (%)</td>
<td>Mean±SS 57.63±12.64</td>
<td>64.39±15.12</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td>Median 58.4 (45.82-70.4975)</td>
<td>70.74 (57.17-72.61)</td>
<td></td>
</tr>
<tr>
<td>Total soft tissue / Total area (%)</td>
<td>Mean±SS 42.37±12.64</td>
<td>35.61±15.12</td>
<td>0.243</td>
</tr>
<tr>
<td></td>
<td>Median 41.6 (29.5-54.1822)</td>
<td>29.26 (27.39-42.83)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. A photomicrograph from group I after a healing period of four weeks revealing osteoblastic rimming. Lamellar bone margins (arrows) and fibrous connective tissue (stars). Haematoxylin and eosin staining 100x.

RESULTS

Twenty biopsy samples which were obtained from the PRF-treated and non-PRF-treated were included into the study.

Mean and SD values of the bone, total hard tissue (bone+graft), soft tissue and total area, ratio of the total hard tissue to total area and the total soft tissue to total area levels are presented in Table 1. When Group I were compared with Group II, no significant difference was determined.

In general, all samples demonstrated an ongoing bone tissue formation with lamellar and woven bone lines (Figure 1). The bone formation seemed to be more active...
Figure 2. A photomicrograph from group II after a healing period of four weeks. Graft material areas (star) and osteoblastic activity areas around the graft materials (arrows). Haematoxylin and eosin staining 40x.

Figure 3. Median values of the ratio of the total hard tissue to total area. *No statistically significant differences with p < 0.05.

(larger areas of immature bone/osteoid) in the bone biopsies from the PRF treated site (Figure 2).

According to the histomorphometric results, at the time of implantation, although platelet-rich fibrin demonstrated the enhancement of the bone regeneration after the fourth week following tooth extraction, there was no statistically significant difference between the two groups; both showed the statistically significant lowest mean values (Figures 3 and 4).
DISCUSSION

This study was conducted to evaluate the potential benefits of using PRF for alveolar socket preservation and earlier implant placement.

Tooth extraction is one of the most widely performed procedures in dentistry and this procedure may induce significant dimensional changes of the alveolar ridge. Maintaining the original volume and architecture of the alveolar bone is essential to obtain esthetic, phonetic and functional prosthetic reconstruction. The dilemma that clinicians face is whether to use adjunct treatments to protect or maximize ridge dimensions for the future placement of a dental implant versus no additional treatments following the extraction of tooth.

Systematic reviews indicated that the alveolar ridge undergoes a mean horizontal reduction in width of 3.80 mm and a mean vertical reduction in height of 1.24 mm within 6 months after tooth extraction without preservation therapies (Hammerle et al., 2012; Lang et al., 2012).

Socket preservation procedures main principles are maintenance of the guided tissue regeneration, minimally traumatic tooth removal, and the protection of the blood clot.

Trends indicate that with alveolar ridge preservation procedures, horizontal resorption of just over 1 mm will still occur with relative preservation of vertical height, whereas a lack of alveolar ridge preservation will generally result in more than 3 mm of horizontal loss and at least 1 mm or more of vertical loss (Vignoletti et al., 2012; Morjaria et al., 2014; Ten Heggeler et al., 2011).

Several studies have evaluated whether primary closure, using graft materials, autogenous bone grafts, bone substitutes, guided bone regeneration with resorbable or non-resorbable barriers, immediate implant placement and various bone promoting molecules such as enamel matrix derivative, platelet-rich plasma (PRP), platelet-rich fibrin (PRF) growth factors and bone morphogenetic proteins (BMPs) provide benefits for socket preservation. The scientific evidence from all of these studies seems to suggest that ridge preservation techniques have some benefits but it did not provide clear guidelines with regard to the type of biomaterial or surgical procedure to best achieve ridge preservation (Oghli and Steveling, 2010; Horowitz et al., 2012).

Platelet concentrates are easy to apply in clinical practice and offer potential benefits including rapid wound healing and bone regeneration, and can therefore be considered to be new therapeutic adjuvants. Platelet-rich fibrin (PRF) is a second generation platelet concentrate and a rich source of autogenous cytokines and growth factors, can be considered as a healing biomaterial. The properties of PRF are considered to promote both soft-tissue and bone regeneration and are suitable for ridge preservation. The effects of the PRF concept have been extensively evaluated in different models. A lot of clarifying scientific work has been published since the introduction of PRF in reconstructive surgery. However, there is further need for experimental and clinical studies on the interactions of PRF in tissue healing since no histomorphometric evaluation was made (Zhang et al., 2012; Ozdemir et al., 2012; Pradeep et al., 2012).

It has been reported that PRF alone is successful in forming new bone when used alone in sinus augmentation, periimplant defect repair and guided bone
regeneration (Hauser et al., 2013). In the present study, histologic and histomorphometric analyses after tooth extraction were conducted to determine the usefulness of PRF as alveolar socket preservation method.

The current study showed that PRF is beneficial to increase the osteoblastic activity 4 weeks after its placement into the extraction sockets but it is not statistically effective on early healing of the extraction socket in the first 4 weeks.

Simsek et al. (2016) created peri-implant defects in a rabbit model filled with demineralized freeze-dried bone allograft (DFDBA) plus saline solution, DFDBA plus platelet-rich fibrin (PRF), or DFDBA plus rifamycin. After 4 weeks, as a result of analysis performed histomorphometrically that The DFDBA-PRF group showed the highest percentages of new bone formation.

Kim et al. (2014) concluded that addition of PRP, PRF, and concentrated growth factor had significantly increased bone formation at the 6th week.

In another study, Baslarli et al. (2015) extracted bilateral mandibular third molars, after which they applied PRF to one and only one extraction sockets. Bone scintigrams were used to assess bone mineralization and osteoblastic activity in the extraction sockets. It was observed that bone healing did not differ significantly between PRF- treated and non-PRF- treated sockets 30 and 90 days postoperatively.

Some studies with histological analysis showed that filling the socket with grafting material could provide controversial results with no improvement or enhanced bone healing (Kutkut et al., 2011; De Coster et al., 2011).

It is important to point out that according to the authors knowledge, studies on alveolar ridge preservation procedures have only focused on bone geometry and the knowledge, studies on alveolar ridge preservation bone healing (Kutkut et al., 2011; De Coster et al., 2011).

Some studies with histological analysis showed that filling the socket with grafting material could provide controversial results with no improvement or enhanced bone healing (Kutkut et al., 2011; De Coster et al., 2011).

It is important to point out that according to the authors knowledge, studies on alveolar ridge preservation procedures have only focused on bone geometry and the width of the residual alveolar crest after tooth extraction (Lekovic et al., 1997), but without giving any information on bone histomorphometry. In the present study, at the time of implant insertion, all groups revealed the presence of newly formed irregular osteoid bone trabeculae rimmed by osteoblasts, in addition to osteocytes entrapment. These findings emphasize the potentiating effect of proteins secreted by the activated platelets in bone formation. PRF has played a role as space making for bone regeneration and would be stimulatory to bone formation. PRF group which include a large amount of platelets, increased the numbers of osteogenic cells, has a more rate of bone maturation but it is not statistically significant.

The limitation of this study was that because of bone structure differences, to eliminate the differences of the effects of PRF, in the same segment, it has led to difficulties in finding two neighboring tooth extraction and implant cases of necessity.

**Conclusion**

Enrichment with PRF does not significantly improve bone density or morphometric value at 4 weeks post grafting but additional controlled and comparative studies are needed to confirm these findings.

**REFERENCES**


Lekovic V, Kenney Eb, Weinländer M, Hahn T, Klockevoeld P, Nedic M, Orsini M (1997). A bone regenerative approach to alveolar ridge...


