Platelet-Rich Fibrin Membrane Combined With Biphasic Calcium Phosphate Bone Graft in the Treatment of Intrabony Defect: A Three-Year Case Report
Metin Çalişır, Aysun Akpinar, Aysan Lektemür Alpan

Abstract
The ultimate goal of periodontal therapy is to regenerate the lost periodontal tissues caused by periodontitis. The most positive outcome of periodontal regenerative procedures in intra bony defect has been achieved with bone grafts. Growth factors such as platelet-derived growth factors indicate potent effect on the regeneration of periodontium. Biphasic calcium phosphate bone graft materials have potent osteoconductive effects on the alveolar bone. The purpose of this case report is to add knowledge to the existing literature about the combined use of biphasic graft material and membrane shape platelet-rich fibrin in the treatment of combined lesion. This study has three years follow-up. We used latelet-rich fibrin as a membrane with combination Hydroxyapatite with β-tricalcium phosphate bone graft material.

Keywords: B-Tricalcium Phosphate; Hydroxyapatite; Periodontal Regeneration; Platelet-Rich Fibrin.

Introduction
The goal of regenerative periodontal therapy is to completely restore the tooth’s supporting apparatus that has been lost due to inflammatory periodontal disease or injury. It is characterized by formation of new cementum with inserting collagen fibres, new periodontal ligament, and new alveolar bone. Indeed conventional, nonsurgical, and surgical periodontal therapy usually result in clinical improvements evidenced by probing depth reduction and clinical attachment gain, but the healing occurs predominantly through formation of a long Junctional epithelium and no or only unpredictable periodontal regeneration. Therefore, there is an on-going search for new materials and improved surgical techniques, with the aim of predictably promoting periodontal wound healing / regeneration and improving the clinical outcome.1

Various synthetic bone substitutes have been developed to reconstruct the bony defects that clinicians often encounter during surgical procedures. Among various synthetic bone substitutes, calcium phosphate (Ca-P) ceramics have been investigated because their composition and structure are similar to those of human bone.2 These materials have osteoconductive properties but some authors mentioned their osteoinductive properties in their studies.3,4 Hydroxyapatite (HA) is belong to calcium phosphate family and has a similar composition and structure to natural bone mineral.5 HA bone grafts have perfect bone conductive properties which permit outgrowth of osteogenic cells from existing bone surface into the adjacent bone material.6 Furthermore there are no organic components contained in HA on account of this material does not induce any allergic reaction and is clinically well tolerated.7

The use of tricalcium phosphate (TCP) as a bone substitute has been growing in recent years. Although α and β phases of TCP have perfect resorbability, β-tricalcium phosphate (β-TCP) has been shown to exhibit good biocompatibility and osteoconductivity in both animal and clinical studies. In latest years, the researches with highly purified β-TCP have revealed β-TCP’s osteoconductive activity and biodegradable nature in human bone.5,8

Biphasic calcium phosphate (BCP) is two calcium phosphate-based bone substitutes with different resorption patterns, widely used in the management of periodontal and peri-implant bone defects as well as of bone augmentation procedures. The combination of HA+β-TCP might provide a better carcass for predictable bone-volume gain than either HA or β-TCP alone.5 Platelet-rich fibrin
(PRF) is described by Choukroun et al., is a second-generation platelet concentrate which allows one to obtain fibrin membranes enriched with platelets and growth factors. PRF acts like a fibrin network and leads more efficient cell migration, proliferation and cicatrization. PRF is an autologous fibrin matrix used to enhance new bone formation. Gassling et al. demonstrated in their study that PRF membranes are suitable for cultivation of periosteal cells for bone tissue engineering. In a study by Pradeep, et al., concluded that when HA is combined with PRF, it increases the regenerative effects observed with PRF in the treatment of human three wall intrabony defects. The purpose of this case report is to add knowledge to the existing literature about the combined use of biphasic graft material and membrane shape PRF in the treatment of combined lesion. This study has three years follow-up. We used PRF as a membrane with combination HA+β-TCP bone graft material.

Case Report

A 47 year-old male patient was referred to our clinic with localized severe periodontal destruction at the left mandibular posterior region between first and second molar teeth. The patient was systemically healthy and there was no contraindication to the periodontal therapy. First molar tooth had a prosthesis restoration. On periodontal examination and radiographic evaluation, the patient presented with an intrabony defect extending up to apical of the distal root of left mandibular first molar (#36) with a probing depth of 14 mm using William's periodontal probe. Gingival index scores, plaque index scores, clinical attachment level and periapical radiographs were recorded at baseline, three months and three years after flap surgery (Table 1). Diagnosis for #36 tooth was combined periodontal-endodontic lesion. Informed consent was obtained from patient. Initial therapy consisted of oral hygiene instructions, which were repeated until the treatment is over. Scaling and root planning were performed. Patient was referred to Department of endodontics for root canal therapy in relation to #36 tooth. At four weeks following phase 1 therapy, a periodontal re-evaluation was performed to confirm the suitability of #36 tooth for this periodontal surgical procedure.

<table>
<thead>
<tr>
<th>Plaque Index Score</th>
<th>Gingival Index Score</th>
<th>Gingival Recession</th>
<th>Probing Pocket Depth</th>
<th>Clinical Attachment Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline 2</td>
<td>2</td>
<td>0</td>
<td>14mm</td>
<td>14mm</td>
</tr>
<tr>
<td>3 months 0</td>
<td>0</td>
<td>0</td>
<td>6mm</td>
<td>8mm</td>
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<tr>
<td>3 years 0</td>
<td>0</td>
<td>2mm</td>
<td>2mm</td>
<td>4mm</td>
</tr>
</tbody>
</table>

Table 1: Pre-operative and postoperative clinical assessment

Patient's blood was collected in a 10 ml test tube without an anticoagulant and centrifuged immediately at 2700 rpm for 12 min. The end blood product consists three layers following that: top most layers consisting of cellular platelet-poor plasma (PPP), PRF clot in the center, and red blood cells (RBCs) at the bottom. The PRF clot was then squeezed with gauze to form a membrane (Figure 1).

With an intracrevicular incision, full thickness mucoperiosteal flap was raised. The inner surface of the flap was curetted to remove the granulation tissue. Root surfaces were thoroughly planned using hand instruments and ultrasonic scalers. The left mandibular first molar demonstrated distal intrabony defect. After removing granulation tissue thoroughly, necrotic cementum was removed by using curettes. The surgical area rinsed with saline. HA +β-TCP graft was mixed with PPP and placed into the defect area. PRF membrane was placed on the graft and the flap were repositioned to their pre-surgical levels and sutured with silk utilizing an interrupted technique. Periodontal dressing was applied to operation area.

After the operation, the patient was prescribed systemic antibiotics (amoxicillin 500 mg twice a day, for five days), non-steroidal anti-inflammatory drug (ibuprofen twice a day, for five days) and 0.12% chlorhexidine rinse (twice a day for two weeks). Sutures were removed after seven days. Clinical healing was normal with neither infectious episodes nor untoward clinical symptoms. The patient was seen at 1st week, 4th week, 3rd month, 1st year and 3rd year. Periapical intraoral radiographs were obtained from the periodontal defect site at baseline, three months and three years after surgery.
Results
In this case report, the greater reduction in pocket depth and gain in clinical attachment were found after three years of the follow-up. 10 mm CAL gain was measured on the distal side of the first molar. PD decreased 14 mm to 2 mm on the distal side of the first molar tooth (Figure 2).

Discussion
The use of PRF is a recent and promising innovation in periodontal regenerative therapy. PRF is a concentrated suspension of the growth factors found in platelets. These growth factors are involved in wound healing and are postulated as promoters of tissue regeneration. Clinical trials suggest that the combination of bone graft along with the growth factors in the PRF may be suitable to enhance the bone density. PRF is a rich source of Platelet-derived growth factor, Transforming growth factor and Insulin-like growth factor, etc. PRF could improve the periodontal osseous defect healing, as PRF can up regulate phosphorylated extracellular signal regulated protein kinase expression and suppress the osteoclastogenesis by promoting secretion of osteoprotegerin (OPG) in osteoblasts cultures. PRF also demonstrates to stimulate osteogenic differentiation of human dental pulp cells by up regulating OPG and alkaline phosphatase (ALP) expression. In this case report, we decided to utilize PRF as a membrane in combination with alloplast.

The intended role of the membrane shape PRF in the intrabony defect was to deliver the growth factors in the early phase of healing. In a split mouth design study Bharadwaj et al. used HA+β-TCP and PRP combination and results showed statistically significant bone gain compared to baseline in both groups. In another study, PRF in combination in with bone mineral had the ability in increasing the regenerative effects in intrabony defects. Similarly in a case report Pavitra et al. used HA and PRF as a membrane which resulted significant difference was noted in the pocket depth and attachment level values at baseline and five months after treatment. In a case report, the reduction in pocket depth and gain in clinical attachment were found after 6 months follow-up using PRF with alloplast. Radiographs revealed significant bone fill in the intrabony defect compared to measurements at baseline.

PRF application is a cheap and quick applicable method on patients with no additional expense. It is suggested that no need thrombin to produce PRF and it can protect growth factors from the proteolysis thanks to the unique fibrin network. It also provides the relaxed formation of blood vessels and stability of the graft materials due to particulate composition. And it accelerates the healing of soft tissue preventing the collapse of the soft tissue. Use of PRF in the bone defects is quite personal so it can’t be used for another person because of the risk of infection. PRF is a natural clot and it's seems that it can lead significant improvements in bone regeneration. According to results of this study, treatment of one wall intrabony defect with HA+β-TCP under a membrane made of PRF may lead significant PD reduction and CAL gain which can be measured in 3 years.

Summary and Conclusion
This case resulted in a rapid and complete healing with the decrease in the pocket.
depth and a gain in the clinical attachment level. Rapid regeneration of the periodontium can be achieved with bone substitutes by incorporating the various growth factors from autogenous blood. The use of autologous platelet preparations like PRF allows the clinician to optimize tissue remodelling, wound healing and angiogenesis by the local delivery of growth factors and proteins although HA and TCP provide the required area for the formation of bone due to the nature of the space. According to the results obtained in this case report, it could be concluded that the positive clinical impact of additional application of PRF with biphasic graft material in treatment of periodontal intrabony defect. However, long-term, multicenter randomized, controlled clinical trial will be required to know its clinical and radiographic effect over bone regeneration.

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