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ÖZGÜN ARAŞTIRMA / ORIGINAL RESEARCH

Histomorphometric Evaluation of Bone Regeneration in Peri-Implant Osseous Defects Treated With Titanium Prepared Platelet Rich Fibrin: An Experimental Study in a Rabbit Model

Periimplantal Kemik Defektlerinin Titanyum Tüplerde Hazırlanan Trombositten Zengin Fibrin ile Kemik Rejenerasyonunun Histomorfometrik Değerlendirmesi: Tavşan Tibiasinda Deneysel Çalışma

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Öz

Amaç: Bu çalışmanın amacı titanyum tüplerde hazırlanan trombositten zengin fibrinin (T-PRF) deneysel olarak hazırlanmış periimplantal kemik defektlerindeki rejenerasyonu incelemektir. **Gereç ve Yöntem:** Çalışmada 12 adet erkek wistar rat kullanılmış olup her bir tavşanın sağ ve sol tibiasına trephan frez ile 7 mm genişliğinde 4 mm derinliğinde defektler oluşturulmuştur. Daha sonra 3,3X8 mm lik implantlar tibialara yerleştirilmiş olup periimplantal defektler sırasıyla Otojen greft, T-PRF, Bifazik Kalsiyum Fosfat greft (BCP) ile restore edilmiştir. Kontrol grubu boş bırakılıp spontan iyileşmeye bırakılmıştır. 4 hafta sonra tüm tavşanlarötenazi ile öldürülerek implant ile beraber etrafındaki kemik alındı. Kemik-implant kontağı toludin mavisi ile boyanan kesitlerin histomorfometrik incelenmesi ile değerlendirilmiştir. **Bulgular:** Otojen grupta (5267,50±228,95) kemik implant kontağı değeri en yüksek çıkmıştır. Ancak otojen grup ile T-PRF grubu (3932,50±275,50) arasında istatistiksel olarak bir fark gözlenmemiştir. BCP grubu ile kontrol grubunda en düşük BIC değerleri gözlenmiştir. **Sonuçlar:** Peri-implantal defektlerin rejenerasyonunda T-PRF grubunda neredeyse otojen grup kadar BIC değerleri bulunmuştur. Bu yüzden T-PRF gelecekte yönlendirilmiş kemik rejenerasyonunda otojen matriksfonksiyonu görevi görebilir.

Anahtar Kelimeler: Titanyum Trombositten zengin fibrin,periimplantal defekt, kemik-implant kontağı

Abstract

Aim: The present study aims to investigate the healing of artificially created peri-implant osseous defects using Titanium-Platelet Rich Fibrin **Material and Method:** Bone defects (9-mm diameter, 4-mm depth) were created and implant beds (3-mm diameter, 6-mm depth) were prepared in the middle of them in rabbit tibias (24 rabbits) used as the experimental model. Afterwards, dental implants were installed into the left and right tibia (diameter 3.0 mm, length 8 mm). In the experimental groups, the peri-implant defect was filled with Autogenous grafts (AG), Titanium-Platelet Rich Fibrin (T-PRF), Biphasic Calcium Phosphate grafts (BCP). The control group did not receive any filling. The bone-to-implant contact (BIC) was obtained by histomorphometrically

examining the sections taken from the study groups. **Results:** AG group was resulted in a high degree of BIC. No difference of statistical significance was found for the BIC as far as autogenous graft and PRF groups are concerned. **Conclusion:** Peri-implantal defects in regeneration of T-PRF were found to be nearly as affective as the autogenous bone graft. We, therefore, conclude that T-PRF can be used for guided bone regeneration in the function of autogenic matrix in the future.

Keywords: Titanium-prepared-Platelet-Rich-Fibrin, Periimplantal defect, Bone-to-implant contact.

Introductıon

Dental implants are generally consider as both effective and popular methods used in restoring missing teeth. Despite highly desirable outcomes and the long-term survival rate, between 5 and 11 percent of dental implants end up with failure.[1,2] Peri-implant bone defects should heal for optimal function and aesthetics. A large number of researches have been carried out with a view towards treating such defects, in search of an optimal material and/or technique. However, no consensus has been reached upon the best one yet.

Various graft materials have been used in restoring the peri-implant bone defects.[3,4] It is autogenous grafts that are considered the gold standard because they are not only osteogenic but osteoinductive and osteoconductive as well. The advantage of autogenous bone is that it maintains bone structures such as minerals and collagen, as well as viable osteoblasts and bone-morphogenic proteins. Notwithstanding the potential osteogenic characteristics that Autogenous Bone Grafts (AG) possess, irregular resorption and morbidity that occur where the graft has been harvested hinders the absolute success of this procedure.[5] Bone-grafting materials like alloplasts, allografts and xenografts are used in instead of autogenous bone. The disadvantage of alloplasts is that they are unpredictable in allowing bone formation[9]; therefore, particles can be encountered within the grafted site when the clinician returns for implant placement. The alloplasts are known as artificial bone replacement material. The advantage of alloplasts is that they do not communicate diseases like HIV and hepatitis, and low cost and unlimited volume of the materials.[6]

Platelet-based preparations from patient's own blood provide an inexpensive alternative to commercially available bioactive materials. Choukroun Leucocyte-Platelet Rich Fibrin (L-PRF) consists of a fibrin matrix with platelets and leukocytes.7 L-PRF has been reported to include a large number of cytokines and many growth factors that have an impact upon how the soft tissue is finally regenerated and how it matures, plus platelet-based growth factor AB (PDGF-AB), transforming growth factor-1 (TGF-1), Insulin-like Growth Factor (IGF), and vascular endothelial growth factor (VEGF). By fostering angiogenesis, mitosis, chemotaxis and proliferation of stem cells, these help increase bone regeneration in the beginning.8 Platelet concentrates split into 4 groups based upon how many leucocytes and fibrins they contain: P-PRP (pure platelet-rich plasma, without leucocytes), L-PRP (PRP with leucocytes included), P-PRF (unmixed platelet-rich fibrin) and L-PRF (leucocytes-containing PRF)9. Unlike other platelet-rich products, L-PRF not requiring any biochemical modification through anticoagulants or bovine thrombin.[7] PRF can continually release growth factors from at least 7 days to 28 days at the most.[8,10,11] An application of L-PRF upon the titanium implant surfaces showed that growth factors covered the implants forming a fibrin layer for platelets to adhere[3,12-14]. Recently studies have shown L-PRF to promote a gradual release of autologous growth factors, resulting in better and more lasting impacts upon how rat osteoblasts grow and differ.[11,12]

Tunalı et al. report that platelet aggregation due to titanium and the clot observed in the titanium tubes did not clinically differ much when compared with glass tubes.[15] They also report that the fibrin carpet created by titanium showed a better network structure[15], plus the period during which the tissue was reabsorbed was a much longer one[16]. One reason why Titanium-Platelet Rich Fibrin (T-PRF) is preferred is to prevent short-term and/or long-term negative impacts dry glass tubes, as well as removing the concerns over silica[17]. Interestingly enough, T-PRF has not yet been reported to be effective in bone healing. Furthermore, none of the studies conducted so far has attempted to achieve a clear assessment of bone healing. The present study seeks to assess how bone gets regenerated in artificially-induced peri-implant osseous defects that were treated with T-PRF.

Materıal And Methods

Experimental protocols were approved by Animal

Ethics Committee of Faculty of Medicine, Cumhuriyet University (B.30.2.CUM.0.01.00.00-50/77). The study animals were put in separate cages in a room (with 12 h day/night cycles) with a temperature of 21°C. They were provided with both libitum water and a pellet diet suitable for laboratory conditions. To adapt to the laboratory, the rabbits were placed in their cages for two weeks prior to the onset of the experiment.

Study Design

12 adult white, 6-month old male rabbits from New Zealand were used for this study (right and left tibia bone), each weighing 3.0 kg. Four separate groups were formed according to the study design. The rabbits were placed in these groups indiscriminately. 1-AG group (n=6): receiving graft with Autogenous graft; 2-T-PRF group (n=6): receiving only T-PRF; 3- BCP group (n=6): receiving only Biphasic Calcium Phosphate graft (4BoneTM MIS Israel) group; 4-Control group (n=6): receiving no bone graft. A collagen membrane was placed over all rabbits prior to suturing. Their periosteum and skin were closed by means of a 4-0 suture (Vicryl, Ethicon, (ABD).

T-PRF Preparation Method

T-PRF was prepared prior to the surgery for the experimental animals. First, we took 5 mL of blood from the animals through their central auricular artery under anaesthesia in a blood collection titanium tube, which was centrifuged soon afterwards (3,500 rpm, 15 minutes)[16]. The centrifugation resulted in a fibrin clot that was extracted from the tube using forceps under sterile conditions. This T-PRF clot was then cut into small pieces.

Surgical Procedure

All operations were completed with the rabbits under general anaesthesia with 2% of xylazine (Rompun 2%; Bayer, Istanbul, Turkey) and 1% ketamine (Ketalar; Eczacıbaşı-Warner Lambert, Istanbul, Turkey). The site to be experimented with was first shaved and then cleaned using povidone-iodine. Once these sites had been incised, the right and left tibia bones were exposed with subperiosteal dissection. We obtained bony defects with a 7-mm diameter and a 4-mm depth with the help of a trephine drill under saline solution irrigation (Fig 1A,1B). The implant beds with a 3-mm diameter and a 6-mm depth were obtained in the middle of each defect in line with the manual of the implant system.[18]

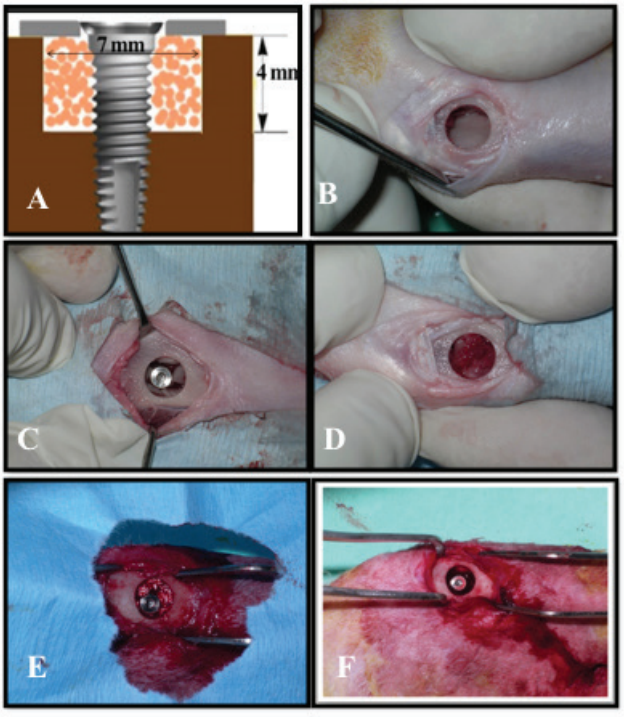

Figure 1: Clinical photographs of surgical procedures. A: Schematic view of defect;13 B: Empty defect; C: Autogenous Graft; D: T-PRF; E: BCP; F: Control

The implant beds were rinsed with saline solution in the aftermath of drilling. The implants (3.3X8 mm AL-Technology dental implants Shark implants, Germany) were inserted in the defects with primary stability (6 mm depth). As for the upper part, it was left free. Afterwards, healing caps were screwed tightly on the implants. A peri-implant defect grafted with AG, T-PRF, BCP and Unfilled are shown in (Fig 1C-1F). The tissues also being tightly sutured in 2 layers via degradable sutures (Pegelak, poly [glycolide-co-lactide]; Dogsan, Trabzon, Turkey).

Postoperatively, the rabbits each were intramuscularly given 50 mg/kg of ceftriaxone (Rocephin; Deva, Istanbul, Turkey), and carprofen, 4 mg/kg (Rimadyl; Pfizer, New York) in one dose for three consecutive days.

The animals were sacrificed 8 weeks after the implantation process. Finally, all signs of unusual healing were documented with the bones dissected along with the implants.

Specimen Preparation

The bone, along with the implants, was totally removed and exposed to four percent of neutral buffered formaldehyde before histological assessment could be made. Dehydration with rising percentages of ethanol was observed after it was embedded in a methyl methacrylate-based resin (Technovit 7200 VLC; Kulzer & Co, Wehrheim, Germany). method Using the method developed by Donath and Breuner, undecalcified ground sections were harvested from not only the implants but also from the surrounding bone.19 Sections were derived from every single implant with the same longitudinal plane and reduced to a thickness of 50 mm with diamond grinding. By the end of this procedure, four sections had been derived from the specimens and stained with toluidine blue.
Histomorphometric analysis

The sections obtained were meant to be used for histomorphometric evaluations. We captured images through a light microscope (Olympus BX50; Olympus Optical, Tokyo, Japan) with the aid of an attached digital camera (Olympus DP70) at X4 magnification. All images were then downloaded to a personal computer. Before performing image analysis, a specific region of interest had been determined. Relevant evaluations were made with Bioquant Osteo II image analysis software (Bioquant Image Analysis, Nashville, TN). We measured the length of bone-to-implant contact (BIC) (µm) from implant shoulder to end of the gap (4 mm).

Statistical analysis

Statistical analyses were made, using the SPSS software (version 16.0 for Windows; SPSS, Inc., Chicago, IL, USA). The implants were incorporated as independent values for analysis. Also, we determined mean values and standard deviations for every single group and variable factor. The disparity between the groups was assessed using the variance analysis and Tukey test. The disparity within the groups was assessed through the Student t-test. The disparities determined were accepted to be statistically significant ($p < 0.05$).

Results

We did not observe any complications in the course of surgical operation. The rabbits showed no post-operative infections and made a quick recovery. However, four of them died due to general anaesthesia, which had to be replaced with another four rabbits. The implants were observed to be still in situ when the animals were euthanized. At the very end of the experiment, osseo-integration was clinically achieved for all the groups.

The histomorphometric measurements of the defects showed satisfactory levels of implant osseointegration. The bone tissue surrounding all implants indicated healing (Fig 2A-2D). The newly formed bone showed

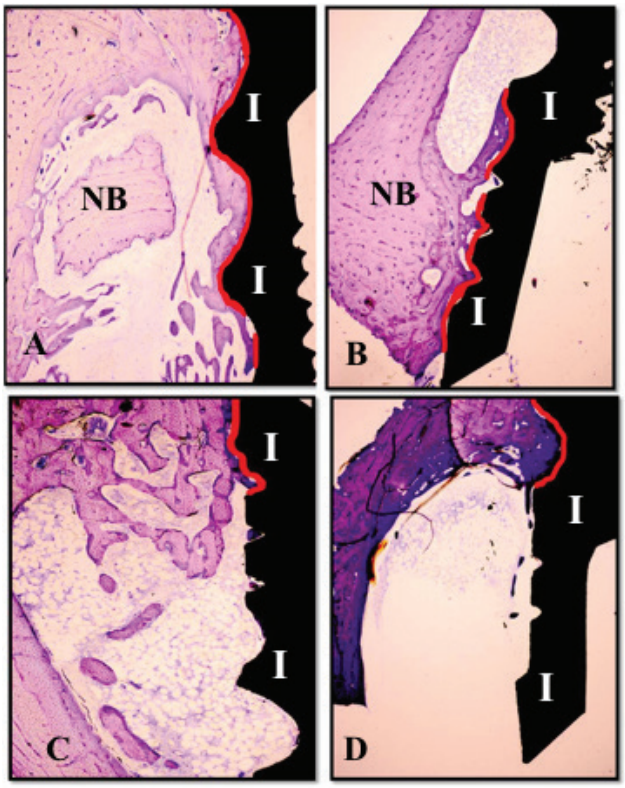

Figure 2: Photographs of the histological sections seen by light microscopy at 8th weeks view of the image in all groups. Length of bone to implant contact in all groups was shown. Sections were stained with toluidine blue. Original magnification, 4x. A: AG group; B: T-PRF group; C: BCP group; D: Control group; NB: New Bone

a direct connection with the implant surface in the groups. BIC, notably in AG and T-PRF groups, resulted in a high degree of BIC.

Comparison of the groups revealed that morphometric measurements of BIC were of statistical significance. There was a rapid increase in not only new bone formation but also in the BIC of the test group (Table 1). AG (5267,50±228,95) and T-PRF (3992,50±275,50) group were resulted in a high degree of BIC. The difference between the AG group with the BCP (2904,50±312,82) and Control (1925,16±294,79) was of statistical significance (p <0,05) but no difference of statistical significance was found for the BIC as far as AG and T-PRF groups are concerned (p>0,05). There was no evidence for fibrotic tissue layer formation in any groups.

Table 1 Length (µm) of BIC at all groups.

GROUPS	BIC (X±S)
AG (n=6)	5267,50±228,95a
T-PRF (n=6)	3992,50±275,50b
BCP (n=6)	2904,50±312,82
C (n=6)	1925,16±294,79
P value	p=0,001

Data are presented as mean (Standard deviation).
Abbreviations: BIC, bone-to-implant contact; AG, Autogenous Graft; T-PRF, Titanium-prepared Platelet Rich fibrin; BCP, Biphasic Calcium Phosphate grafts; Control; Unfilled defect
a Statistically significant difference (p < .05) between AG versus BCP and Control
b Statistically significant difference (p < .05) between T-PRF versus BCP and Control

Discussion

The present study seeks to determine the potential impacts of T-PRF upon periimplantal bone defects healing. Histomorphometric results showed BIC were enhanced in the T-PRF treated peri-implant bone defect in comparison with the AG, BCP and, control groups after the implant placement.

Bone fill in the opening between implant and peripheral bone is important to provide an osseointegrated fixture. Although periimplant bone defect healing can be obtained in gaps less than 2 mm by maintenance of a blood clot, dimensional contraction of the alveolar bone and accompanying resorption of buccal bone after bone defects can lead to esthetic problems. Thus it has been suggested that periimplant bone defects should be grafted in periimplantal bone defect cases to enhance osseointegration.[20]

Many studies have assessed reconstruction of peri-implant bone defects with graft materials. These study found enhanced BIC values in peri-implant defects that were fixed with autogenous bone[21,22]. In a dog study by Kim et al[23]., it was reported the effects of biphasic calcium phosphates upon how peri-implant bone defects heal and concluded that biphasic calcium phosphate bone substitute helped with defect resolution.

In our study mean BIC in the 8th weeks was the AG group (5267,50±228,95) and T-PRF group (3992,50±275,50). The findings of this study agree with those of Anitua et al[13]. Another investigation by these authors14 showed that a 51% BIC occurs as long as implants are coated with Platelet-Derived Growth factor compared to the 22% BIC observed in the control group following eight weeks of healing. Our results also confirm the data presented by Lee et al[24]. who have reported that the mean BIC was 39% in experimental group and 17% in the control one following eight weeks of healing. Using a similar approach, our results support the findings by Fontana et al25. Fuerst et al[26]. reported a 55% BIC after the implants had been coated with PRGF unlike 39% of control group following four 4 weeks of healing. Wu et al. described, histomorphometric results showed that four weeks after healing, integration of the titanium mini-screws and of the bones was remarkably better. The authors stated that four weeks is a vital time point for the integration of titanium mini-screws and of the bones27. Şimşek et al[28]. reported the DFDBA plus L-PRF group showed significantly greater increases in BIC and compared with DFDBA plus saline solution. Thus, adding L-PRF to DFDBA may enhance how bone is formed in peri-implant bone defects because of its enhanced osteoinductive properties.

In a study by Simonpieri et al[13]., it was revealed that new bone formation was 29.30% in the experimental group and 11.06% in the control group. As to the study

group, BIC was 17.11% in the control and 39.43% in the study group. In this study, L-PRF applications not increased not only new bone formation but also BIC in significant levels.

There are known to be studies that have used only L-PRF and others that have used L-PRF combination with other graft materials. Studies, which use L-PRF as the only filling material while sinus is being augmented and the implant installed simultaneously, have reported that the implant stabilizes with a high amount of regenerated bone at 6 month after the operation.[29] While the sinus floor is being augmented, a freeze-dried bone allograft (FDBA) and a L-PRF are used, which reduces the time in which healing occurs from six months to nearly four.[30]

T-PRF is acknowledged to be a new platelet concentrate. Its preparation is based upon the assumption that titanium tubes could be more successful in activating platelets than the glass tubes preferred for the Chouckroun's method. This stuff is meant to prevent possible side-effects of dry glass as well as glass-coated plastic tubes. It is also meant to remove speculations as regards silica in the short or long terms, or both.[20,21]

In a recent study by Tunalı et al., which investigated the impacts of T-PRF upon connective tissue recovery in rabbits, it was found that T-PRF was still present on the 10th day but was reabsorbed on the 15th day. T-PRF was found to have very good regenerative potentials for both bone and connective tissues. Also, it is the powerful fibrin architecture of the T-PRF that permits an intense slow release. This release is further reinforced by the fact that new growth factors are produced by the leukocytes present in T-PRF membrane. When T-PRF applied to the periimplantal bone defect, regeneration potential of T-PRF may stimulate implant healing over the surrounding bone with the platelet rich layer.

In obtaining T-PRF, the most suitable centrifugation for rabbit experiments was found to be 3500 rpm 15 min[22] . We also followed this protocol for our study. The same study by Tunalı et al. showed the re-absorption period of T-PRF in tissue to be long. It also reported that T-PRF might cause much longer impacts for rabbits with a faster metabolism than PRF. PRF is reputed to possess a re-absorption time that varies from seven to eleven days in mankind.[16] Furthermore, osteoconductive property of T-PRF was good, and T-PRF membrane remains were observed in the first month controls when used as a membrane alone.

Tunalı et al. centrifuged venous blood of 18 ml cultivated from humans and transferred half of this blood into a Grade IV titanium tube of 9 ml and the other half into a glass tube of 9 ml. It was observed that T-PRF cultivated in the Grade IV titanium tube was better than that in the glass one in terms of having a better cellular structure and a fibrin network with a better organization, tight-knit, and narrower intervals.[15]

How bone regeneration gets stimulated is crucial, and superficial applied growth factors can increase bone formation. However, growth factors obtained are expensive and involve several steps. In this study, T-PRF upregulated the growth factors necessary for enhancing bone formation.

Conclusıon

Considering the limitations of our study, we assume that T-PRF application during peri-implant bone defects healing may increases the rate and especially in sites where peri-implant bone defects healing may be required. Furthermore, these results support the hypothesis that grafting peri-implant bone defects of T-PRF may enhance bone formation, thus promoting BIC. Clinical studies are needed to demonstrate efficacy in humans.

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