Evaluating the Implant Stability of Titanium Prepared Platelet Rich Fibrin Treated Peri-implant Osseous Defects with Resonance Frequency Analysis

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ABSTRACT

Objective: In this study, we aimed to examine the efficiency and the effect of Titanium-Platelet-Rich Fibrin (T-PRF), which can be used in guided bone regeneration, and its early period regeneration capacity on implant stability.

Methods: Twelve male New Zealand white rabbits were used for this study. Each of the right and left tibias of rabbits using a trephine bur (diameter 7.0 mm), monocortical defects were prepared (7 mm width, 4 mm depth). Subsequently, dental implants (AL-Technology dental implants sharkimplants) 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 respectively 1-autogenous grafts, 2-T-PRF, 3-synthetic grafts (MIS 4BONE). The control was left in an unfilled state. Implant stability was evaluated with the resonance frequency analysis method (RFA).

Results: The Ostell ISQ for the experimental groups respectively 1-autogenous graft group 76.00±3.89, 2-T-PRF group 75.00±3.84, 3-synthetic graft group 67.00±5.72 while control group 56.33±5.12. The difference between the 2 groups (autogenous graft and prf groups) was statistically not significant while all other differences between groups are statistically significant.

Conclusion: Within the limits of this study, it was found that periimplantal defects in the regeneration of T-PRF is effective as the autogenous bone graft. We believed that T-PRF can be used guided bone regeneration and guided tissue regeneration as autogenic matrix in the future.
INTRODUCTION

Peri-implantitis is a lesion that occurs as the result of a series of inflammatory reactions can be detected on radiography, starting on the marginal bone around the neck region of the implant with the progression of the peri-implant mucositis and then progressing in the form of a groove.

Mombelli et al. defined the clinical symptoms of peri-implantitis as follows:

1) Vertical bone loss can be detected on radiography.
2) Peri-implantal pocket formation.
3) Bleeding on probing (BOP).
4) Edema, hyperemia on the mucosa.
5) No patient complaint as to any pain.1

It was suggested in the former studies that results in surgical treatments proved to be better when compared with those of the conventional treatment in the treatment of peri-implantal bone losses2, 3. In surgical treatments, regenerative materials must be used for a new bone formation in the wake of a well-performed debridement. Of the materials used for such purposes, bone graft is one of the materials with the most common clinical use. Since autogenous grafts contain osteoinductive, osteoconductive and osteogenic effects within themselves, they are the best standard in bone graft practices; yet, they also have disadvantages, such as their unpredictable resorptions and the requirement for a second receiving area. It was indicated in a study conducted on the resorption amount of autogenous grafts that the height of the autogenous block graft had decreased at a rate of 60% in the 10th month following the treatment.4

Alloplastic grafts, when compared with the autogenous ones, has some disadvantages specific to their category, such as being likely to cause a foreign body reaction and not showing a regenerative characteristic like the autogenous graft despite its advantages like an extra area of operation and not requiring an additional duration for anaesthetics as well as being able to be provided at a desired amount.5

PRF (Platelet-rich fibrin) is a second-generation platelet concentration developed by Choukroun et al. in France in 2011, which makes a positive contribution in accelerating soft and hard tissue recovery. The fact that its acquisition and clinical use are easier than those of PRP (Platelet-rich plasma) and that it is economical as no extra material is added from outside are the superior aspects of PRF compared to former platelet concentrations. PRF contains a number of cytokines within itself with respect to immunity, wound recovery and inflammation.6

According to scientific data, it is obvious that the platelet concentrate better than the PRF than PRP.7

In the studies conducted for obtaining satisfactory results in the surgical treatment of bone defects, PRF has gained popularity in recent years. Here, the underlying mechanism is to carry the growth factor in high concentration to the wounded region through PRF and to enhance the probable regeneration there.8

Accomplished clinical results have been indicated with PRF9-13. Some clinicians reported damage to silica activator when using glass tubes14. O’Connell recipes the inevitable contact. The particles of silica in the tube, in spite of their sufficient intensity for sediment with the red blood cells, are small enough for a fraction to remain suspended colloidal in the buffy coat, fibrin, and platelet-poor layers of plasma.14

(T-PRF) is a new platelet concentrate and its preparation method is based on the hypothesis that titanium tubes may be more effective in activating the platelets than the glass tubes used in Chouckroun’s method.13
This material is used to avoid any adverse effects and speculations in the short or long term about silica. Activation of platelets with titanium compared with activation with silica particles provides the distinctive properties of T-PRF including its increased biocompatibility. The Resonance Frequency Analysis (RFA) used in the evaluation of implant stability was first tried by Meredith in 1996. It was reported that with the help of this method, both the primary stability of implants could be measured and the long-term follow-ups of implant stability could be performed and also the osseointegration could be evaluated by being converted into digital measurement as in-vivo. In this method, a frequency wave proceeding in a stationary state and an inducible transductor are used. This transductor is screwed on the implant, and the stability of the implant within the peripheral tissue is measured after the response received for the frequency sent to the transductor.

Ostell ISQ is a device recently designed for the Resonance Frequency Analysis, along with several advantages such as being more minimized in size, ease-of-use, possessing a Smartpeg within its kit as well as the presence of smartpegs produced by every implant company in compliance with their own implant design. With Data Manager software can the patient information and measurement results be kept in the computer.

Even though the ISQ value varies digitally between 0-100, the clinical measurements range from 40 to 80 to a great extent. In some clinical studies conducted, the acceptable range in the implant stability was reported to be between 55-85 ISQ.

In our study, our aim was to evaluate the osseointegration taking place before and after regenerative application through the method of resonance frequency analysis in peri-implantal defects that had been formed experimentally.

**MATERIAL-METHOD**

Experimental protocols were approved by the Animal Ethics Committee of Cumhuriyet University School of Medicine. The experimental part of the study was performed in the Research Laboratory of Experimental Animals in Cumhuriyet University, School of Medicine. The equipment required for the study as well as the majority of consumables were provided by Cumhuriyet University, Faculty of Dentistry, Department of Periodontology. The process of the preparation of undecalcified histological samples and their histomorphometric evaluation were performed in the Research Laboratory of Erciyes University, Faculty of Dentistry.

**Study Design**

12 male and 5-month-old New Zealander white rabbits that weighed 2.5 kg on the average and that were determined through the veterinary control to be healthy were included in the study. Throughout the experiment, the rabbits were put into standard experimental cages (at 22-24 °C, with 55-70% humidity, 1 atm. in a 12 h day/night cycles,) and were then subjected to a standard laboratory diet. The rabbits were placed within their cages two weeks before the experiment so as to be able to adapt to the laboratory environment and were observed in terms of their health status. The rabbits were separated into 4 groups:

1. The group that received autogenous bone graft
2. T-PRF group
3. The group that received synthetic graft
4. Control group

Within this study, rabbits were distributed into groups so that each group had 3 rabbits, and a total of 24 experimental periimplant defects (width of 7 mm to 4
mm in depth) were created in one of the right and left tibia.

Figure 1: Evaluation of the implant stability through Resonance Frequency Analysis (Ostell ISQ).

T-PRF preparation and Surgical Procedure

T-PRF would be prepared in accordance with the previous studies. Following the general anaesthesia, 3-5 ml blood would be drawn from the central artery in the ear and would be transferred into the titanium tubes. It would, then, be centrifuged at 3500 rpm for 15 minutes, and the T-PRF’s in the mid-section of the blood separated into 3 fractions would be used.

All the surgical procedures were performed under sterile surgical conditions by taking into consideration asepsis, antisepsis and sterilization principles. In order to provide a general anaesthesia, injections with Xylazine hydrochloride (Rompuns; Bayer, Leverkusen, Germany) (10 mg/kg) and Ketamine Hydrochloride (Ketasol %10 50 mg/kg) were performed intramuscularly on all the experimental animals, and following anaesthesia, the tibial bones of the rabbits were cleared of off their furs and were disinfected with povidone-iodine. Full-thickness flap was removed, and the bone surface was reached, after which cylindrical defects of 4 mm depth and 7 mm diameter were formed in right and left each tibial bone and a marking process starting right from the mid-point of the formed defect was performed by means of a drill operating at 800 rpm.

Afterwards, implant wells were prepared starting from the marked area for 3x8 mm-implants (AL-Technology dental implants sharkimplants), hence, a peripheral peri-implantal defect was formed in the 4 mm-coronal section.

In the control group, the defect area was closed with only a resorbable membrane (OsteoBiol by Tecnoss), whereas in the T-PRF group, T-PRF obtained from the rabbit’s blood and a membrane which could get resorbed upon it were placed in. In the group with autogenous bone graft, the autogenous bones obtained while the defect was being formed were split into small pieces, and after they were placed into the defect area, a resorbable membrane was placed upon it. In the synthetic bone graft group, on the other hand, after the defect was filled with synthetic bone graft (MİS 4BONE), a resorbable membrane would be used to cover its surface. And then the periosteum would be approximated as much as possible, and the cutaneous and subcutaneous tissues would be sutured with a 3/0 polyglactin (Vicryl, Ethicon, ABD). To ensure the post-surgical analgesia, 4 mg/kg Carprofen (Rimadyl, Pfizer, New York, NY) would be administered subcutaneously every 24 hours for 3 days.

In all the groups, the rabbits were sacrificed by means of a high dose of Pentothal Sodium Injection (Pentothal; Abbott Diagnostic Division, Abbott Park, IL) 200 mg/kg i.v.) at the end of the 8th week. Both at the moment when implants were placed into the rabbits, and after the euthanasia in the 8th week, the implant osseointegration rate was evaluated through RFA method before the tibiae of the rabbits were removed.
RESULTS

The experimental stage of the study along with the laboratory studies were performed without having any complications. No problem was experienced during the operations, and the postoperative healing period was completed without any complication.

The data obtained from our study were evaluated after having been loaded onto SPSS 14.0 program. When the parametric test hypotheses were fulfilled in the evaluation of the data, the Variance analysis, Tukey test, Paired-Samples “t” test were all performed, and the level of significance was calculated as 0.05. The Osstell values in each subject which were measured during the initial and conclusion periods were given as shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Osstell measurement initial (ISQ)</th>
<th>Osstell measurement last (ISQ)</th>
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<tr>
<td>1</td>
<td>23</td>
<td>77</td>
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<tr>
<td>Autogenous</td>
<td>25</td>
<td>70</td>
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<tr>
<td>Graft</td>
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<td>81</td>
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<td>35</td>
<td>75</td>
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<td></td>
<td>Mean : 26,16±4,79</td>
<td>Mean : 76,00±3,89</td>
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<tr>
<td>2</td>
<td>22</td>
<td>72</td>
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<tr>
<td>T-PRF</td>
<td>31</td>
<td>69</td>
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<tr>
<td></td>
<td>33</td>
<td>78</td>
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<td>Mean:27,16±4,35</td>
<td>Mean:75,00±3,84</td>
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<tr>
<td>3</td>
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<td>Synthetic</td>
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</tr>
<tr>
<td>Graft</td>
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<td></td>
<td>Mean:25,33±3,66</td>
<td>Mean:56,33±5,12</td>
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</tbody>
</table>

Table 1: The representation of Osstell Initial and last measurements in the form of a table.
The difference between Osstell initial values among the groups was found to be insignificant. While the statistical inter group difference in peer-to-peer matchings was significant when the groups were compared in terms of Osstell, the difference only between group-1 and group-2 was found to be insignificant. The difference between Osstell initial and Osstell final values was found to be statistically significant in all the groups.

**DISCUSSION**

Bone losses that occurred due to peri-implantitis were classified by Schwarz et al., and it was observed that mostly the
peri-implantal loss occurred in the form of a peripheral defect in both experimental animals and in humans. We have created an experimental model of periimplantitis as peripheral defect in this study.

According to Simonpieri et al., said that the use of PRF in the course of bone grafting provides 4 advantages: First, PRF plays a biologically integrative role among blood clot, membrane and bone particles. Secondly, thrombocyte/platelet-derived cytokines (PDGF,TGF-B,IGF-1) are gradually released from PRF; thus, a permanent recovery takes place. Thirdly, this fibrin network stimulates neoangiogenesis, vascularization and the migration of endothelial cells in particular. Fourthly the leukocytes and cytokines within the fibrin network play a major role in the regulation of infectious and inflammation taking place within the region where PRF is applied.

In a study where the regenerative effect of PRF was investigated, a defect had been formed around the rabbit’s tibia after an implant was placed into it, and PRF was used for the regeneration of the defect. During the histomorphometric evaluations the bone implant contact was determined as 39,43% in PRF group, whereas it was 17,11% in the control group. New bone formation, on the other hand, proved to be 29.30% in the PRF group and 11,06% on the average in the control group. As the result of this study, it was observed that the use of PRF increased new bone formation and bone implant contact ratio.

In a study of Zhang Y. et al., PRF material was used as the graft material alone in the sinus lifting process, the amount of new bone tissue that occurred in the PRF group at the end of the 4th week was 49,84%, whereas in the control group in which only Beta tricalcium phosphate (β-TCP) was used, the amount of new bone formation was found to be 24,17% on the average. According to this study, the use of PRF alone increased the amount of new bone formation during the early period to a statistically significant extent. In the 12th week-findings of the study, however, the amount of new bone formation in PRF group proved to be 30,80% on the average, whereas in the group where β-TCP was used, it was determined as 33,52%. As the result of this study, PRF is thought to be likely to be used as the graft material alone. In the histological evaluation, it was determined that there was more new bone formation than that in the control group during the early period, while during the late period, there was no statistical difference between the new bone formation and the control group.

In a study conducted by Choukroun et al., PRF and bone graft were used together in the course of the sinus lifting process, and according to the results of the study, when bone graft and PRF combination were compared with the control group in the 8th month, the results were found to be equal to one another.
In a study conducted by Pripatnanont et al., the combined use of PRF and grafts were investigated, and while the control group, autogenous graft, xenograft and the composite group in which both of them were used in combination were used along with PRF in the study group, only bone graft was used in the control group. The sections were examined through optical density and histomorphometric analysis methods. As a result, including PRF in control group and in autogenous graft groups showed a positive effect; yet, this interaction was not observed within xenograft and autogenous graft groups.27

Huang et al. stated that RFA values proved to be a sort of a useful method in the evaluation of implant stability. In these studies, they also pointed out the fact that the Osstell device could be used during the periodic controls/check-ups performed to determine the stability of the dental implants during the healing period.28, 29

In a study conducted by Nedir et al. for the purpose of evaluating the efficiency of Osstell device in establishing a diagnosis, it was noted that Osstell measurements were not applicable methods of diagnosis for being able to determine mobile implants. Nevertheless, they stated that in order for the ISQ implant stability to be reliable, no loading/imposition of implants with the threshold ISQ value, 47, and with an ISQ value lower than 50 should be performed throughout the 3-month, and that the threshold ISQ value for an immediate loading, however, must be 54.30

In the studies conducted through the RFA measurement in the literature, it is seen that the ISQ values range between 52-90 and that a greater part of the measurement results range between 62-70. It was reported in the former studies that the implants with the ISQ value greater than 50 had the adequate amount of stability to be able to perform a functional loading.31.

In a study where the effect of PRF on the implant stability in the same patient was measured through RFA method, one of the implants to be applied would be placed within the implant slot, and that the serum emanating in the course of PRF acquisition, on the other hand, was injected onto the PRF implant surface. Other implants, however, were placed in accordance with the normally.32

The study of Tunali et al. on T-PRF used alone as a membrane When T-PRF was used alone as a membrane, it generated new bone with connective tissue in the model of connective tissue wound healing in which regeneration was not expected. The T-PRF membrane started to resorb in rabbit tissue on the fifth day, and was able to remain in the tissues for at least 10 days, which was enough time for the initiation of formation of a new bone. Also, this study showed that to obtain the T-PRF, the most suitable centrifugation was 3500 rpm 15 min. In our study, we have applied this protocol.33

The same study of Tunali et al. reported that the resorption period of the T-PRF in tissue was long, and showed that the T-PRF could have longer effects on rabbits that had faster metabolism than PRF33, which has a resorption time of 7-11 days in humans34. The mechanisms underlying this longer resorption is the titanium having a firmer network structure than glass or glass-coated tubes.35

In the other studies of Tunali et al. the T-PRF is tried in bone healing model using the accurate protocol for rabbits. According to the results of this study, the osteoconductive property of the T-PRF was good, and the T-PRF membrane remains were observed in the first month controls when used as a membrane alone.36

The 1st week and 1st month-ISQ values of the implants within the groups in question proved to be statistically higher on a
significant level as for the PRF group when compared with the other group.37

Friberg et al. used RFA method during the implant follow-up and remarked that if the initial ISQ value was greater than 70, the implants became absolutely stable later on even though they may show lower values temporarily. In the same study, it was emphasized that the implants, the ISQ value of which ranged between 60-65 at the outset, were capable of remaining stable or showing a slight decline without any need for a tough follow-up, and that the implants could show an increase in time if their ISQ value was less than 60.38

Glauser et al. and Balslui et al. stated that the implants yielding an ISQ value above 60 were suitable for an immediate loading/imposition. It was also pointed out that the implants showing values below 60 as the ISQ value had gained 97% osseointegration.39-41

Sennerby and Meredith reported that the implants showing an ISQ value between the range of 60-65 were suitable for loading/imposition, whereas those showing an ISQ value, 40 and below had a high probability of failure in clinically.41

Nedir et al., in their study where there was a comparison made between the ISQ measurements and the cases on whom immediate and conventional loading procedures were performed, ISQ measurements were performed for 3 months. In the study in question, they noted that all the ISQ measurements proved to be between 42-72, and that as the result of the study, the limit value for a successful osseointegration was 54 in immediate loading and 49 in conventional loading procedure.42

In a number of studies, it is put forward that RFA method is more sensitive/susceptible than the other conventional methods in evaluating the implant stability and that although it is said to have been in compliance with torque values, there are also studies claiming just the other way round; separately, these researches have shown that the use of RFA value was not convenient for comparing different implant stabilities and did not produce compliance results to the placement torque values.43

In our study, the ISQ values measured after the 8th-week-recovery period proved to be 56.33±5.12 in the control group, whereas in the study groups, they were 67.00±5.72, 75.00±3.84, 76.00±3.89, respectively. Even though the statistical difference among groups was significant, it was observed that the stability of implants would be sufficient in the wake of the regenerative procedures, accordance with other studies mentioned previously.

One of the limitations of this study is not same infected periimplant wound regeneration with surgical wound regeneration.

RESULT

Within the boundaries of the study, although it is concluded that T-PRF has a regenerative potential almost as much as the autogenous graft when considered in terms of Osstell values and that it could be used as the graft material alone, we are of the opinion that prospective studies are needed considering that the study group is rather small and that it is carried out in terms of only one parameter.

REFERENCES


32. Öncü, E. Trombositten zengin fibrinin implant stabilitesi üzerindeki etkisinin rezonans frekans analizi ile incelenmesi, Sağlık bilimleri enstitüsü periodontoloji anabilim dalı. 2013, Başkent Üniversitesi, ANKARA


