The Effect of Platelet-Rich Plasma on Osseointegration Period of Dental Implants

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ABSTRACT

Background: The preparation of Platelet-rich plasma (PRP) is minimally invasive way, simple, low cost to obtain natural autologous growth factors and is now being widely used in different fields of medicine for its ability to increase the regeneration potential of tissue. The aim of this study was to investigate the effect of local application of autologous PRP gel on acceleration rate of osseointegration period by clinical assessment accomplished by determining the changes in implant stability during 3 months healing period using resonance frequency analysis (RFA).

Materials and methods: A total of 28 dental implants were inserted in edentulous maxillae or mandibles of 13 patients using a split mouth design, i.e. each patient was received at least two dental implants at the same session, one implant was implanted in association with PRP which was placed locally in one site, to serve as PRP group, and the other implant was placed without PRP, to serve as a control group. Both groups were followed with repeated implant stability measurement by means of resonance frequency analysis at different time intervals (at the time of implant placement, 8th week, and 12th week postoperatively).

Results: There was no obvious statistically significant difference in mean ISQ between PRP and control groups ($P > 0.05$) at baseline, 8 weeks, and 12 weeks postoperatively.

Conclusions: Within the limitations of the present study, no appreciable clinical effect was observed to accelerate the rate of osseointegration of sandblasted acid-etched endosseous dental implants when using topical application of autologous PRP gel into the prepared drill holes.

Key words: platelet rich plasma, osseointegration, stability, dental implant. (J Bagh Coll Dentistry 2015; 27(4):101-106).

INTRODUCTION

The development of dental implants to replace missing teeth with the demand by patients to decrease the osseointegration period following implant placement have led to a major works by researchers to enhance a quality of biomaterials and to develop implant surfaces with improved microscopic and macroscopic structures that allow induction of osseointegration [(1)].

In regard to the classic treatment protocol proposed by Branemark, three months of tissue healing after tooth removal, following three to six months of load-free interval after implant installationt are necessary for bone integration. Due to the great success rate of dental implants, this consuming period may lead to one year of reduced life quality, which can influence the judgment for dental implant rehabilitation [(2)].

Some researchers have attempted to shorten osteogeneration period by employing growth factors. The best source of autologous growth factors in the body is platelets. Currently, they are recognized that platelets have many functions further than their role of hemostasis. Platelets have important growth factors that are responsible for increasing cell mitosis, initiating vascular in-growth, inducing cell differentiation, increasing collagen production, and recruiting additional cells to the area of wound [(3)].

The growth factors are present in the α-granules of platelets and released at the site of wound during activation. These growth factors are: Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor β (TGF-β), Platelet-Derived Epidermal Growth Factor (PDEGF), Vascular Endothelial Growth Factor (VEGF), Insulin-like Growth Factor-1(IGF-1), Platelet factor-4 [(4)].

Clotting cascade can activate platelets, which begin releasing their growth factors immediately. They secrete 70% of their stored growth factors within 10 minutes and close to 100% within the first hour, then they produce additional amounts of growth factors for about 8 days until they are depleted and die [(5,6)]. Among factors which can activate platelets are: ADP, thrombin, thromboxanes, epinephrine, collagen, and shear stress [(7-9)].

Marx et, al., first introduced the technique of autologous platelet concentration in 1998 to create the first platelet-rich plasma (PRP) for application in dental surgery [(1,10)].

PRP is a high concentration of autologous platelets within a small quantity of autologous plasma [(11)]. The clinical application of PRP was expanded to other fields, including cosmetic medicine, cardiac surgery, oral and maxillofacial

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surgery, ophthalmology, sports medicine, orthopedic surgery, and plastic surgery.

It would seem intuitive that a higher platelet count would yield more growth factors and better clinically results, however, this has not yet been determined [12]. Weibrich et, al., in 2004 required plasma preparations to have a platelet concentration of 1000 × $l0^3$/µL to be the considered therapeutic platelet rich plasma [13]. Marx et, al., in 1998 demonstrated an increase in bone density six months after using platelet concentrate with platelet concentrations 595,000–1,100,000 platelets/µl [10]. Graziani et, al., in 2006 showed that optimal results were observed when platelet concentration increased 2.5 times from basic platelet count. Higher concentrations (maximum 5.5 x concentration) reduced proliferation and osteoblast function [14].

Many studies have found that PRP induces and accelerates soft tissue regeneration and bone repair, the preparation of PRP can be applied to metal of dental implant surface and could create a new dynamic surface that might potentially show biologic reaction. This protein stratum consists of a fibrin mesh and growth factors that covers the implant surface and makes the initial interactions of surrounding tissues with implant surface. It also enhances cellular proliferation, attachment, differentiation, and bone matrix deposition [15].

Anitua showed that osseointegration of implants was improved by covering the implant surface with PRP, rich in growth factors, before installation into the bone [16]. Similarly, Nikolidakis and co-workers, found a significant result of bone formation around titanium implants bioactivated with PRP solution [16, 17], but Garcia et, al., (2010), observed that PRP did not enhance bone apposition to acid etched implants [5, 18].

The aim of the present study was to investigate the effect of local application of autologous PRP gel on acceleration rate of osseointegration period by clinical assessment accomplished by determining the changes in implant stability during 3 months healing period using resonance frequency analysis (RFA).The initial euphoria about the use of PRP for improving bone formation and subsequent controversial results suggest the need for more studies on this topic.

MATERIALS AND METHODS

Subjects:

Thirteen patients, 7 women and 6 men with the mean age of 42 years (22-60) were opted in this study whom fulfilled the inclusion and exclusion criteria. Each patient was received at least two dental implants at the same session, one implant was placed in association with PRP in one site (to serve as PRP group) and the other implant was placed without PRP (to serve as a control group) in the same edentulous region or in a bilaterally symmetric to the median line (split-mouth design) provided by the two implant beds have the same range of bone quality (density).

Implant Systems:

A total of 28 titanium screw-shaped implants (Implantium® / Dentium® / Seoul / Korea) and (Maxicell® / Nucleoss®/ Turkey) with a surface modified by TiO_2-large grit sandblasting and acid etching surface were utilized in the study. The diameter of Ø3.4 mm, Ø3.8 mm Ø4.3 mm or Ø4.8 mm and a length of 8 mm, 10 mm, 12 mm or 14 mm were placed in the patients′ jaws, who are selected in the present study.

Pre-Surgical Examination:

The patients were pre-surgically evaluated for any conditions that are implicated for dental implant therapy and platelet function.

Preoprative standardized interactive CT scans were taken for registering bone density at the implant sites and also estimation of proper implants length and diameter according to bone width and length measurements and evaluation of proximity to the vital structures.

Preparation of PRP:

Platelet separation and concentration starts before the implantation procedure with an aseptic and minimally traumatic phlebotomy technique.

Nine milliliters of venous blood was collected from the veins of antecubital fossa in EDTA (K3) vacuum tube using 10 ml syringe.

Eight times gently rock the tube back and forth to incorporate the whole blood and anticoagulant, at this stage we can take blood sample for complete blood count test. Using low speed fixed rotor laboratory centrifuge (80-1, China), the blood tube was placed in the centrifuge hole and another tube was placed opposite to the first one (with the same weight) containing normal saline for balancing centrifugation.

First spin (Separation spin) was begun at 1200 rpm (160 g) for 10 minutes, which separates the red blood cells (red lower fraction) from the rest of the whole blood, i.e. white blood cells, platelets, and plasma (upper straw-yellow turbid fraction) (figure 1).

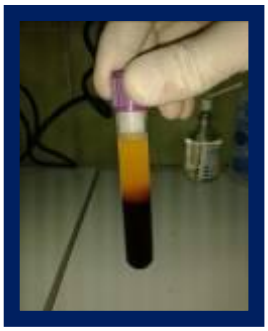

Figure (1): The Result after the 1st Spin.

The whole upper fraction was pipetted by 100-1000 µl micro-pipette and transferred to a glass plain tube. The remaining red blood cells fraction was discarded.

Now, the aspirated fluid was submitted to a second spin (concentration spin) of about 3200 rpm (1200 g) for 10 minutes. The second spin separates and compacts the platelets, white blood cells, and a small number of residual red blood cells (precipitate at the bottom of the tube) from the plasma, which is clear straw yellow color (platelet poor plasma (PPP)) (figure. 2).

By using the same micro-pipette, PPP was drawn off and discarded, leaving approximately 1 ml of the fluid (figure. 3).

Figure (2): The result after the 2nd Spin.

Figure (3): The Remaining of PPP and the Precipitated Blood Cells before Resuspension.

After that, the remaining fluid was resuspended before clinical use. This is the PRP. Now the PRP was pipetted and transferred into Eppendorf tube and stored at room temperature until implant procedure start (figure 4). Platelets were counted using a Cell Dyn Emerald hematology analyzer and ranged between 800,000 to > 1500,000/ µl.

Figure (4): PRP Solution in Eppendorf Tube.

During implant surgery, PRP gelation was induced by adding 10% calcium gluconate to the PRP with volume ratio of 1:10 using 10-100 µl micropipette. This requires agitation of the tube every one minute and waiting between 5-15 minutes (figure 5).

The role of calcium is to nullify the effect of anticoagulant and to restore the physiological function of coagulation process lead to the formation of thrombin, which causes activation of platelets.

Figure (5): Activated PRP Gel.

Implant Surgical Procedure:

The surgery was started with locally anesthetizing the area to be implanted. An extensive flap design was raised. The implant sites were exposed and prepared using conventional drilling procedure in sequence until reaching a desired size.

Before dental implants placement into their drill holes, PRP gel was gently introduced into one site (PRP implant) and the other site left without PRP (control implant site) (figure 6).

After the implants had been inserted in their beds, primary implant stability for both implants (PRP and control implants) by Osstell ISQ through screwing the smart peg into the body of

implant and two readings of the ISQ values were recorded; in a bucco-lingual and in mesio-distal directions.

Figure (6): Application of PRP into the One Implant Site.

2nd Stage Surgery and Follow up Visits:

The first visit of follow up started 8 weeks postoperatively. Local anesthesia was given using an infiltration technique. With the use of tissue punch drill, both PRP implants and control implants were uncovered and implants secondary stability were measured in the same manner of primary stability measurement.

Healing abutments were placed according to the size of implants and gingival thickness to gain access for subsequent 12th week measurement.

Statistical Analysis:

Data were translated into a computerized database structure. Statistical analyses were done using SPSS version 21 computer software. Because we had two ISQ measurements for each implant at each time point (mesio-distal and bucco-lingual measurement), average of the two ISQ measurements was used in this results. The independent samples t-test was used to test the statistical significance of difference in mean between the two groups.

RESULTS

All the implants were successfully healed over the three months follow-up period with a survival rate of 100%. One patient reported pain associated with the peri-implant suppuration on probing around one control implant but the lesion treated and resolved without further incidence.

Resonance Frequency Analysis Difference between the Two Groups:

There was no obvious or statistically significant difference in mean ISQ between the two groups at surgery (P = 0.35), the mean and standard deviation values of RFA at placement (primary stability) were 70.46 ± 7.32 for the control implants and 73.21 ± 8.13 for the PRP implants.

Eight weeks postoperatively, the mean and standard deviation values of ISQ were 69.57 ± 10.69 at the control sites and 73.81 ± 5.90 at the PRP sites. The difference in mean ISQ was greater in the PRP group by 3.61 ISQ units compared to control group this is considered as non significant (P = 0.27) and evaluated as a moderate effect (Cohen's D= 0.42).

At 12 months follow-up period, the mean and standard deviation values of RFA were 71.96 ± 8.51 for control implants and 74.32 ± 5.44 for PRP implants. The difference in mean ISQ units after 12 weeks of surgery was also non significant between the PRP group compared to control group (P = 0.39) and the treatment effect was evaluated as a moderate effect (Cohen's D = 0.33) (figure 7).

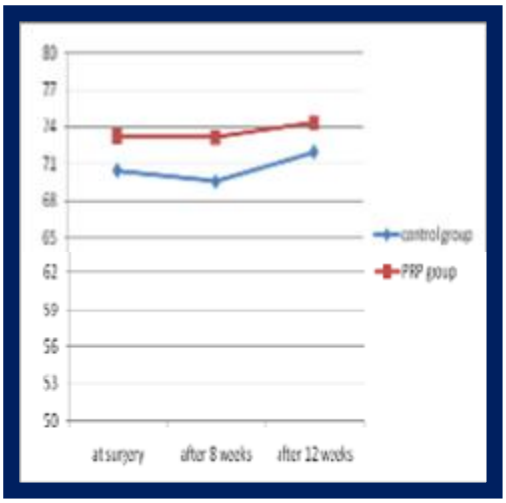

Figure (7): The Mean ISQ of PRP and Control Implants within the Three Successive Periods.

DISCUSSION

PRP gel is a normal autogenous blood clot that contains a highly concentrated number of platelets and native concentration of fibrin. It is minimally invasive way, simple, low cost to obtain natural autologous growth factors. Because it is the patient's own blood, it is free of transmissible diseases and cannot cause hypersensitivity reactions [(4)].

Although ISQ values for the PRP implants were consistently greater than the control implants at all time points, the values were not statistically significant (P value > 0.05).

The results are in agreement with Nikolidakis et, al., who investigated the effect of local application of autologous PRP on bone healing in combination with titanium implants placed into tibial cortical bone of goats. They applied PRP fraction either via gel preparation and subsequent installation in the implant site (like in this study),

or via dipping of the implant in PRP liquid before its insertion. The PRP gel was obtained by activation of the PRP fraction, while PRP liquid was used pure without addition of any agent. They failed to show any statistically significant effect of PRP-gel on the bone–implant contact percentage. On the other hand, 'PRP liquid' (without any activation) observed a significant tendency to favor the bone–implant contact [17]. These results corroborate with his previous study Nikolidakis et, al., in which a similar study design was used but where the implants were placed in trabecular bone (femoral condyle) [16].

The results are also in agreement with studies of other researchers. For example Garcia et, al., analyzed the influence of PRP gel on bone regeneration around dental implants placed in canine mandibles. After 55 days of healing period, no advantageous effect on the bone–implant contact rate was seen [5]. Also, El-marssafy et, al., who failed to show any statistically significant effect of PRP gel to accelerate the rate of osseointegration or decrease the crestal bone resorption through first 3 months period in immediately loaded dental implant placed in posterior maxillary area [19].

An explanation for the difference in result between PRP liquid and PRP gel, PRP gel may be squeezed during placement of implant and its solution could be extracted from it and may be displaced apically as a condensed solid fibrin making it without any beneficial effect.

The second reason for not having significant difference could be attributed to the activation of PRP, Zimmermann et, al., found that when thrombin and calcium were added to platelets preparation, the concentration of TGF-β1 decreased by approximately 60% to 65%. This study found that if not activating the platelets, TGF-β1 concentrations did not significantly change over 6 hours [20].

According to Marx, any harm to platelet membrane during PRP extraction will resulting in releasing of growth factors in a non bioactive situation, which would result to adverse clinical outcomes. To guarantee platelets quality, it is essential to consider the velocity force, the time used in centrifugation, type of collection tube, and the anticoagulant among other factors [11].

Concerning to centrifugation force, the procedure described by Mazzucco et, al. was followed in this study and thus the separation spin of 1200 rpm (160 g) for 10 minutes (1600 g.min) followed by a concentration spin of 3200 rpm (1200 g) for 10 min (12000 g.min) with a total of about 13600 g.min [21]. This force application about half the value which is allowed by Marx and Garg in order not to disrupt platelet cell membrane and growth factors loss within supernatant plasma (≤ 30000 g.min) [4].

In regard to the type of collection tubes which were used in the second spin were made from glass (red topped 10 ml plain vacuum tube, AFCO/ Jordan). This type of the tube may not agree with Trindade-Suedam et, al., who suggested for using plastic tube after the second centrifugation especially when the second speed of centrifugation is more than 600 g. He stated "It was easier to release the platelet pellet (precipitated platelet cells) using a plastic tube than a glass tube. This may be attributable to the fact that the glass can damage the platelets causing their aggregation and activation [22].

During aspiration of plasma fraction after the first spin in this study, we aspirated the whole plasma fraction and leaving RBCs fraction without aspiration. Eby recommended for aspiration a small amount 15% (about 1mm) of the very top of the RBCs in the mixture of plasma fraction aspiration. The resultant PRP will become pink to red [23]. The top 10% of the RBCs usually holds a large percentage of immature platelets. The younger platelets, which contain more growth factors and they are larger and therefore centrifuge out in the upper layer of the red blood cell fraction [4].

Araki and his co-worker in 2011 recommended of using EDTA as anticoagulant in preparation of PRP [24]. Efeoglu et al. also used EDTA during their experimental study [25].

Platelet viability can be measured by the P-selectin test. P-selectin is a protein found in the membrane of the platelet alpha granules. The test measures the P-selectin both prior to and following activation with adenosine diphosphate (ADP). The P-selectin values of freshly prepared PRP are about 10% to 20% and increase to 40% to 60% following ADP activation. P-selectin values that do not increase with the addition of ADP indicate damaged platelets [4].

Within the limitations of the present study, No appreciable clinical effect was observed to accelerate the rate of osseointegration of sandblasted acid-etched endosseous dental implants when using topical application of autologous platelet-rich plasma gel into the prepared drill holes immediately before implant placement compared with the control group. The beneficial effect of PRP is not dependent on platelet concentration only, platelets viability and activities after processing are more important.

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