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Influence of Platelet-rich Plasma on Dental Implants

Osseointegration in well-controlled diabetic patients

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Abstract

The purpose of this study is to evaluate the effect of platelet-rich plasma (PRP) on the osseointegration of dental implants in diabetic patients. A split-mouth design was employed in all 14 patients, with each patient receiving two mini implants. A PRP-coated mini implant was installed in one quadrant as a trial and a plain mini implant was added in the opposite quadrant to serve as a control. Radiographic evaluation was done at 3, 6, and 9 weeks after implant placement. Radiographic density is measured at five points around the implants, repeatedly. Results showed no statistically significant difference between the two groups of implants. The minimally invasive mini implants successfully maintained integration at the end of 9 weeks. There were no cases of implant failure. The results of this study suggest that platelet-rich plasma implant coating has no significant effect in reducing the time for mini implant osseointegration in diabetic patients.

Introduction

Dental implants have been used as a means to successfully restore tooth function in edentulous or partially endentulous patients for decades; traditional dental implants offer patients low failure rates and dramatic quality-of-life improvement. Some studies have shown that implant failure rates in diabetic patients are higher than those in non-diabetic patients. Most studies on diabetes and dental implants have shown no absolute contraindication to dental implant placement in the diabetic patient; however, definitive guidelines with objective criteria are missing when it comes to implant placement in patients with long-standing diabetes, as well as those with type I diabetes. The glycosylated hemoglobin level (hemoglobin A1C) can be used as a marker for diabetic control over a three-month period.[1] Studies of onlay bone-grafting procedures have shown high failure rates in diabetic patients with elevated hemoglobin A1C levels, and the need for a method to increase the success of grafts in this patient population was

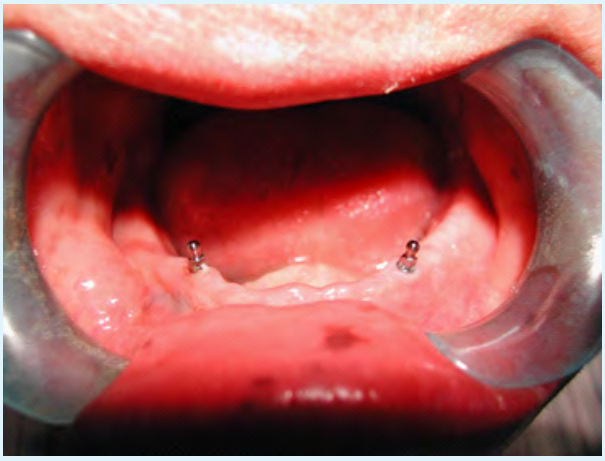

Figure 1: Group 1 and II implants with and without PRP

described.[2] Currently, the only method of controlling diabetes and its effects is metabolic control.

The use of growth factors would provide a means of enhancing bone growth and, thus, could potentially improve the success rates of implants placed in diabetic patients. A natural source of growth factors is platelets. Many growth factors such as platelet-derived growth factor (PDGF-1), keratinocyte growth factor (KGF-2), and human recombinant bone morphogenetic protein (rhBMP-2) have been used in various animal and human experiments, with varied results. However, little is known about the effects of timing, combinations, and concentrations of the individual growth factors in wound healing.[3] PRP has shown promising results in diabetic animal experiments: It normalized the proliferation and early chondrogenesis while improving the mechanical strength of a fracture healing.[4] Its use has been recommended for radiotherapy patients and diabetics as a means to improve osseointegration.[5]

A recent introduction to the field of implantology is the minimally invasive flapless procedure. This procedure requires minimal preparation and placement protocol, with reduced trauma to the blood supply of the cortical bone and minimized removal of bone for implant placement. The results of these studies have shown enhanced osseointegration levels up to 71%, compared to implants placed with flaps.[6] This procedure would have an advantage over conventional placement methods when considering issues such as impaired wound healing of diabetic patients.

The evaluation of bone-implant interface has been done by various means, from periotest, resonance frequency analysis, micro CT, digital subtraction radiography, computer assisted densitometric image analysis, etc., most of which have evaluated indirectly the bone-implant interface. Periotest values of mini implants emerged recently in a one-year follow-up study showing +3 to be an integrated implant.[7] The resonance frequency analysis method for the evaluation of implants is not possible, as the method requires the placement of a smart peg into the abutment screw position. As a one-piece implant, the mini implant does not provide this option. Micro CT recently was used to demonstrate bone-to-implant contact in a comparative study of flapless and flap procedures, and yielded a very accurate measurement of the bone-implant interface.[6]

The purpose of this study was to evaluate the effects of platelet-rich plasma on osseointegration in well-controlled type II diabetic patients. The test implants were PRP-coated implants and control implants were those placed without PRP with a minimally invasive mini implant protocol.

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Table 1: Diameter, length, and placement torque values of all the implants in the maxilla and the mandible.

No. of Implants (n)	Diameter: mm		Length: mm			Torque at Placement: Ncm			
	2.0 mm	2.5 mm	10 mm	11.5 mm	13 mm	< 20 Ncm	20 Ncm	30 Ncm	> 35 Ncm
Maxillary implants (n)	-	14	2	4	8	1	-	3	10
Mandibular implants (n)	14	-	2	-	12	-	2	-	12

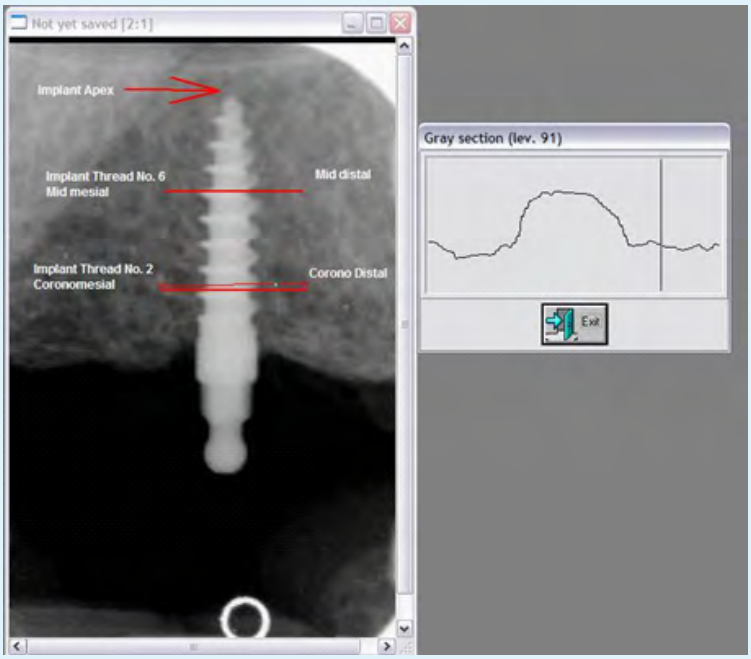


Figure 2: On the left: Five sites where measurements were taken (histogram option). On the right: The implant diagram with Gray section.

Material and Methods

Fourteen patients (nine male and five female; mean age of 61 years; range 57-65 years) were treated. Seven patients received two implants in the mandible and seven patients received two implants in the maxilla.

PRP Production

To obtain platelet-rich plasma, 10 mL of blood was drawn from each patient before surgery and placed into 3.8% sodium citrate, which served as anticoagulant; there was no delay in transferring the blood to avoid coagulation. A specially made container was used to place the blood into the Universal 32 centrifuge. The blood was placed into the centrifuge and counterbalance was centrifuged at 1800 r.p.m. for 10 minutes. This separated the red blood cells from the plasma. A pipette was used to remove the platelet-rich layer of plasma, which was activated with 10% CaCl2 for 20-30 seconds prior to placement into the osteotomy site and coating of the test implants.

Surgical Procedure

After the standard preoperative examination and bone width estimation, 28 Mini Drive Lock (Intra-lock® International, Inc. FL. USA) implants were placed in 14 patients. Each patient received two implants of the same length on opposite sides of the same jaw (split mouth design) with and without platelet-rich plasma (see Figure 1). In the maxilla, 2.5 mm implants were placed, and in the mandible, 2.0 mm implants were placed, with lengths ranging from 10-13 mm. All the implants were placed according to the standard surgical placement procedure provided by the manufacturer. Placement torque of all the implants was noted, and this information was used when considering immediate loading of the implants as recommended by the manufacturer (Table 1).

The PRP previously obtained from each patient's blood was injected into the osteotomy site of the respective patient with a sterile insulin syringe, and a test implant was coated with PRP prior to placement of the implant in one of the quadrants. On the contra-lateral side, the fixtures were placed without any coating or injection of PRP. Loading of the implants was done on the appropriate visit.

Clinical Evaluation

Clinical evaluation of the implants was done at 1, 3, 6, and 9 weeks postoperatively. Each implant and implant site was evaluated, at each visit, for the following: implant mobility, presence of pain, peri-implantitis, suppuration, and infection.

Table 2: Marginal mean values of PRP and non-PRP implants at preoperative 3-, 6-, and 9-week intervals.

	Means of implants with PRP (95% C.I.) n = 14	Means of implants without PRP (95% C.I.) n = 14	F-statistic (*df*)	*p* –value
Pre-operative	134.7 (119.0, 150.4)	127.1 (112.9, 141.4)	2.74 (1, 12)	$p = 0.125$
After 3 weeks	139.3 (121.0, 157.6)	128.5 (112.8, 144.1)		
After 6 weeks	139.5 (122.9, 156.0)	132.0 (116.9, 147.1)		
After 9 weeks	146.6 (130.0, 163.3)	133.0 (111.6, 154.4)		

Radiographic Examination

A pre-operative orthopantomogram was taken with Gendex (Orthoralix 9200 series, 60-84kV, 3-15 mA) and scanned in a Den Optix Scanner (Gendex Dental Systems, Hatfield, PA, USA). The radiographic evaluation of the implants was done at 3, 6, and 9 weeks postoperatively. The radiographic density of the peri-implant bone was evaluated with software called VIXWIN 2000 (Gendex Dental Systems, Hatfield, PA, USA). Peri-apical radiographs were taken on size 3 photosensitive phosphor plate periapicals (Gendex). A single-use barrier membrane was used for protection against cross contamination. The radiographic images were subjected to magnification correction in the software. After magnification, five points of evaluation were determined around the implants with the thread numbers 2 and 6 taken as reference points for future measurements. Once the digitized image of the implant was obtained, the histogram option in the VIXWIN software was initiated. This software allowed cross sections of the implants to be evaluated in grayscale values. Along the section a red line appears and values can be accurately noted, as in Figure 2. Values of these five points were noted and the process was repeated at 3, 6, and 9 weeks postoperatively.

Statistical Analysis

Data were described in terms of means and standard deviations. A repeated measures Anova was used to model the evaluation of radiographic density and to test the comparison between PRP and non-PRP groups and effects over time. All *p* values are one-sided, and $p < 0.05$ was considered significant. All analysis was done with SPSS version 12.0 (SPSS, Inc. Chicago IL. USA).

Results

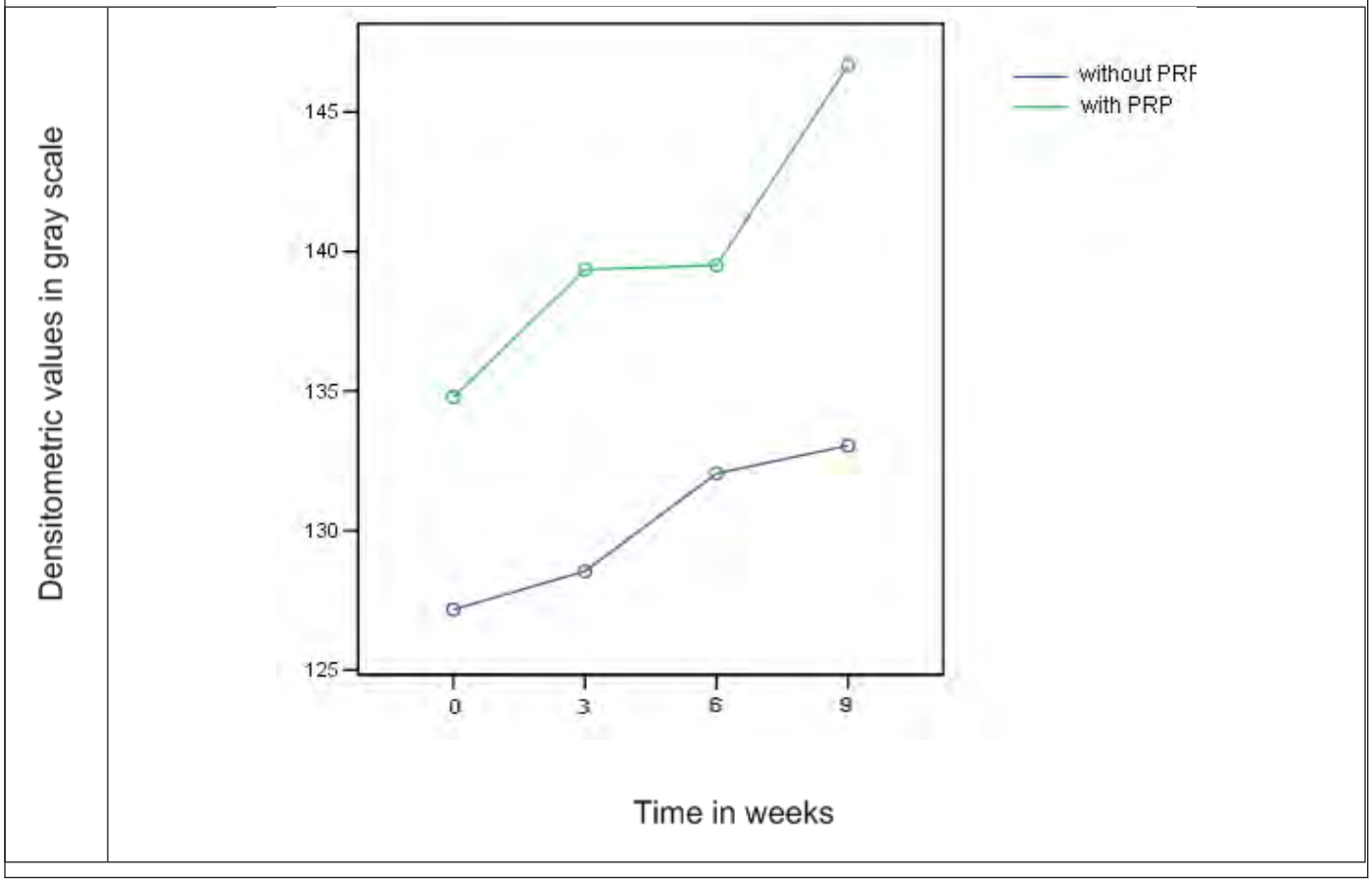
Clinical Findings

Table 1 shows the distribution of inserted implants with length, diameter, and torque at the time of placement of the implants. All the implants showed no mobility, dehiscence, or suppuration at the time evaluation at 3, 6, and 9 weeks of review. Patients did not report pain at this time either. One implant, however, failed to attain primary stability at time of placement, which was noticed by the torque value below 20 Ncm, and was thus removed. The same patient developed an infection at the site of the implant due to non-compliance with antibiotic medication. When the patient took the medication, the infection resolved, and no effects were noted on the integration of the implants.

Radiographic Results

The radiographic results were obtained from radiographic density measurements at five points around the implants. The densitometric profile data of the implants are given in Table 2 showing mean values of PRP and non-PRP implants preoperatively and at 3-, 6-, and 9-week intervals. Graphic representation of the marginal means of the implants with and without PRP preoperatively and at weeks 3, 6, and 9 are shown in Figure 3.

Figure 3: Marginal means of implants with and without PRP preoperatively at 3-, 6-, and 9-week intervals



Discussion

Several features of this study design make it a unique and relevant contribution to the literature on the use of PRP in implant patients, including operational technique of the study (a split-mouth design), flap design (flapless), and patient population (diabetic). Incorporating these three features into one study design offers advantages and disadvantages; namely, comparison to studies that examine one feature of this patient population (be it flap design or presence of diabetes) may offer some likenesses, but may not be fully reflective and, therefore, only relative comparisons can be made. That said, reflection on existing data may shed light on, and enhance, the pertinent outcomes from this study.

All previous prospective studies that included a test population of diabetic patients have been randomized, controlled clinical trials, with test groups and control groups, employing a two-stage implant protocol. A split-mouth design was used for this study because it would eliminate issues with age- and sex-matching of the patients.[8] The bone density was matched, as two implants were placed on either side of the mandible or maxilla of every patient in the same tooth position. The location of the implant was the same on each side of the jaw so that the bone density of the implants was comparable.

Another benefit to this study lies in the technique of implant insertion (flapless implants with immediate loading). In all prior prospective studies, a two-stage implant placement procedure was followed with discrete implant uncovering and prosthetic loading time periods.[9,10,11] In this study, flapless implants were used, which allowed for a single-stage technique and afforded the ability of immediate loading. A temporary prosthesis was provided for all the implants, and the loading of the implants was done when there was adequate documentation of maintained radiographic density over subsequent visits. All the implants were functionally loaded with a definitive prosthesis when no loss of density was noted.

The glycosylated (A1C) hemoglobin levels in our study were monitored, as recommended by earlier researchers.[1] One study by Shernoff and Olson in which the hemoglobin A1C levels were measured demonstrated higher implant failure rates (survival of 91% at 5 years follow-up) than in the general population, but an absence of correlation of hemoglobin A1C or fasting glucose with failure rates. Discussion in these studies suggests that not all the participants maintained their blood glucose levels as well controlled as possible, but that did not necessarily change the outcome in terms of failure versus success.[9,12] Our study also had some patients with poorly controlled blood sugar levels — two patients did not maintain

their glycosylated hemoglobin level within the requested range; however, at the 9-week postoperative review, no loss of integration was reported, which is consistent with prior research.[12] A long-term follow-up, such as that carried out by the authors in the Olson study, is recommended so that the relationship of glycosylated hemoglobin and implant survival can continue to be thoroughly reviewed, especially in the context of PRP administration, which should, theoretically, improve wound healing in those with poorer immune systems (as all diabetics are somewhat predisposed to be). Further, it should be noted that Olson's study did find a correlation with duration of diabetes, which may be a surrogate marker for the degree to which the immune system has been compromised by the chronic condition.

In the design of the study, the use of the flapless technique was selected to serve a specific purpose in this cohort of patients. The blood supply of the cortical bone is provided only by the periosteal blood vessels, compared to trabecular bone, which is supplied by the bone marrow vessels. When a flap is raised, the blood supply of the cortical bone is lost and, as such, there is loss of vascular supply and bone resorption in the initial healing phases after implant placement.[6] For this reason, the flapless procedure was used in this study to maintain as much vascularity as possible in diabetic patients already predisposed to vascular issues and poor wound healing.

In recent years, flapless implant surgery has emerged as a preferred technique, with many providers advocating for increased use of these procedures. Scientific data have emerged recently that suggest flap and flapless implant procedures are comparable in the animal model. The conclusion was that flapless implants had greater osseointegration and crestal bone height compared to implants placed by traditional flap procedures. A three-dimensional micro CT was used to evaluate osseointegration.[6] When comparing conventional implants and mini implants, research suggests that the raising of a flap and the large osteotomy resultant are disadvantages of conventional implants.

Implant size was also considered in this study, and is relevant in the debate on flapless versus flap technique. The smallest diameter conventional implant is 3.25 mm, with the implant site prepared to the size of the implant. On the other hand, the mini implant pilot drill is 1.2 mm and 1.8 mm diameter for 2.0 mm and 2.5 mm implants, respectively. The result is a smaller size wound and, thus, a shorter healing period. As reported previously, the reduced trauma, decreased postoperative discomfort, and shorter healing time are all advantages of the mini implants over conventional implants.[13] Another aspect of the mini implant is that it is self-tapping, implying that the implant advances to make its own path, unlike conventional implants in which the size of the implant is the size of the osteotomy created when bone tap is required. This results in a longer time for osseointegration of the conventional implant, as there is a larger diameter of bone to fill.

To our knowledge, flapless mini implant procedures have not been previously documented in diabetic patients, and since diabetic patients are known to have compromised wound and bone healing, this procedure may be optimal precisely because it reduces the size of the wound and decreases healing time. The preserved blood supply with this technique is another salient feature that has important consequences in the diabetic patient. A final advantage of the flapless procedure, especially as it pertains to use with PRP or other growth factors, is that the PRP will not "spill" or be lost in the flap while being instilled into the osteotomy. This is in contrast to what the operator may encounter in a full flap technique, as studied previously.[8]

The success of the 27 mini implants in our patient group was excellent. One implant was removed at the time of placement due to lack of bone width. The implant did not attain primary stability at implant placement and, as such, this was not considered an implant failure. Twenty-seven implants placed achieved osseointegration at the end of the 9-week postoperative review.

Noteworthy in our study, especially when compared to other studies of diabetic patients, is the fact that glycemic control was monitored and kept under good control, and this may be one of the reasons why our study did not yield a significant difference between the PRP and the non-PRP group. Ethical guidelines do not allow us to conduct a clinical trial on non-controlled or under-controlled diabetic patients. As discussed in prior studies, it is postulated that PRP normalizes early cellular migration and proliferation and partially restores the mechanical properties of the fracture callus.[4] The platelet count in whole blood can be used as a rough estimate of the platelet count that will be produced in PRP.[14] For this reason, platelet count or complete blood count of all the patients was obtained and noted prior to initiation of treatment to make sure the values were normal.

Yet, a study by Weibrich et al found that the level of growth factor produced and the number of platelets do not have a strong correlation.[14] Weibrich's 2002 study in the *Journal of Craniomaxillofacial Surgery* demonstrated that the growth factor levels did not correlate with the whole blood platelet concentration or the platelet concentration in PRP, and that the platelet count provided no means to gauge the levels of platelet-derived growth factor (PDGF), transforming growth factor (TGF), and insulin-like growth factor (IGF).

In our study, the risk of immunological reactions and disease transmission due to the use of bovine thrombin were avoided by the use of CaCl2 alone and was as effective in activation of the platelets.[7] More than one method of activation has been mentioned in the literature as both being valid and documented, but comparative studies between the two have not been performed.

It should be mentioned here that the platelets of the diabetic patients do not show normal behavior with regard to activity or hyperaggregability. In one study, it was found that the platelets were hyperactive compared to platelets of normal patients, and they were also found to be hyperaggregable,[15] which may carry some effect when used as a therapeutic intervention as with PRP. Weibrich et al discussed platelet concentrations and their effects on the osseointegration of implants placed in the femur of New Zealand white rabbits. They found that the intermediate concentration of PRP had the most beneficial effects contrary to other investigators who found no correlation between the concentration of PRP and its effects.[14] The extent to which this bears weight in the current study is uncertain.

In this study, the implants were not all immediately loaded at the same time, but at an appropriate time of 3, 6, and 9 weeks postoperatively. In addition, the type of prosthesis given to the patients was different. Fixed prostheses over implants and overdentures were provided to the patients. One key study on PRP that measured the effects of PRP on non-diabetic patients over a period of 40 days[8] showed no difference between the PRP group and the non-PRP group beyond four days; the authors concluded that there was no additional benefit to adding PRP. Regarding time-course to follow-up, the effects of PRP have been observed radiographically to be at maximum density on bone at two months.[16] Hence, the time period for assessment was set at two months (or 9 weeks postoperatively in our study). Further, it should be noted that the total lifespan of the platelet is one week, which means that the platelets will produce growth factors for a week.[16] The effects of the platelets are hypothesized to be limited to the time period discussed, when healing of bone is most relevant and important.

The DSR method has been employed by many researchers for the assessment of serial radiographs to evaluate changes in the same region over time. Before an image can be digitally subtracted, it has to be standardized for geometry and contrast. The first- and second-generation methods employ the mechanical standardization, which is too burdensome and expensive, whereas the third-generation methods are not comparable nor routinely available.[18] Literature on this method has emerged, stating that the angle difference between the two images must not be more than 2 degrees, which is quite difficult to obtain.[19] Researchers have used bite blocks to try to maintain image geometry on serial images, which has again been questioned by others. Due to these specific drawbacks, this method was not used in our study.

The present study is not without limitations. First, few patients were studied. A larger prospective study would yield more information on outcomes and further validate this study. Second, specific variables beyond hemoglobin A1C were not quantified in this study, and may contribute to the patients' outcomes — factors like length of time with diabetes, presence of other inflammatory conditions, and degree of burden of oral disease — all possible confounders. Further work on the topic with a larger study and more specific inclusion and exclusion criteria would offer evidence as to the validity of the conclusion of this study.

Conclusion

No significant difference was seen in a cohort of diabetic patients who underwent mini implant placement by flapless technique in terms of osseointegration when comparing PRP-treated sites and non-PRP sites. Further study of this patient population is required to yield a more powerful study. It is theoretically beneficial to reduce the healing time in the diabetic patient in whom baseline wound healing is impaired. For this reason, the flapless technique may be an optimal approach to implant placement.

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1. The study is which of the following?
   a. randomized, controlled trial
   b. clinical trial
   c. prospective study
   d. observational study
   e. all of the above

2. *True/False?* Use of the split-mouth technique offers direct comparison of implant outcomes that have been placed in the same microflora and in bone with comparable bone density.
   a. True
   b. False

3. *True/False?* The purpose of the study was to determine if there was any benefit in terms of implant success rates given the use of platelet-rich plasma in diabetic patients.
   a. True
   b. False

4. *True/False?* Patients in this study had poorly controlled or under-controlled diabetes.
   a. True
   b. False

5. The diabetic marker used to follow patients' glucose levels in this study is:
   a. hemoglobin A1C
   b. fasting glucose
   c. two-hour glucose tolerance test result
   d. non-fasting glucose

6. *True/False?* One of the benefits of this study is that it is directly comparable to prior research on the topic.
   a. True
   b. False

7. *True/False?* The authors conclude that there was no statistical difference between implants placed with PRP-coated implants and regular, non-PRP-coated implants.
   a. True
   b. False

8. *True/False?* The time course selected by the author is that reflected in other studies as the maximal time in which platelet-derived growth factors would be functional.
   a. True
   b. False

9. *True/False?* The authors offer that the use of the flapless technique (mini-implant) is less traumatic and less disruptive to vascularity and, therefore, it would be optimal in diabetic patients with a substrate of poorer wound healing.
   a. True
   b. False

10. *True/False?* One of the limitations of this study is that the number of patients (n) is low, which makes it a less powerful study.
    a. True
    b. False

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