

The Role of Platelet Rich Fibrin in Enhancement of Regeneration of Alveolar Bone After Bilateral Extraction

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Abstract

Background: The variable rates of alveolar bone regeneration and bone healing after tooth extraction can be unpredictable, sometimes insufficient or unsatisfactory and may even heal by fibrosis. To address this problem, this study was conducted to assess the role and effectiveness of Platelet Rich Fibrin in the enhancement of alveolar bone regeneration. The main objective of this study was to determine the role of PRF in enhancement of alveolar bone regeneration after bilateral extraction using CBCT for radiographic comparison of the PRF treated and non treated sites, and to note the prevalence of dry socket occurrence.

Design: Single Centered Randomized Clinical Trials. Bilateral extractions were performed on 20 female patients with only the right extraction socket treated with PRF over the period from July 2016 to February 2017. A follow up CBCT was performed in 2 weeks and 4 weeks post operatively and dry socket occurrence was noted.

Results: P-value of 0.00002 for the difference of alveolar bone density between the right PRF treated and left non-treated extraction site on the 2nd week was highly significant and also a p-value of 0.00001 for difference between the right and left sites after 4 weeks was highly significant. 3 patients (15%) presented with dry sockets on the non-treated left sites with no dry sockets occurrence on the contralateral side of the same patients.

Conclusion: The findings of this study showed that the use of PRF vastly accelerates and enhances the regeneration of alveolar bone after extraction and may also have a role in dry socket prevention in prone patients.

Keywords: PRF, platelets rich fibrin, CBCT, centrifuge, bone density, bone healing, bilateral extraction, dry socket.

I. INTRODUCTION

Platelet rich fibrin (PRF) is a fibrin matrix in which platelet cytokines, growth factors, and cells are entrapped and is released after time has elapsed which serves as a resorbable membrane⁽ⁱ⁾. In 2006 Choukroun and his associates were amongst the pioneers for using PRF protocol in oral and maxillofacial surgery to improve bone healing in implant dentistry. Autologous PRF is considered

to be a healing biomaterial, and presently, studies have shown its application in various disciplines of dentistry⁽ⁱⁱ⁾

In Recent years PRF biologically revolted the dental field. PRF contains and releases different growth factors that stimulate bone and soft tissue healing⁽ⁱⁱⁱ⁾. Growth factors participate actively in tissue repair mechanism such as angiogenesis, chemotaxis, cell proliferation, extracellular matrix deposition, and remodeling. Currently, there is available clinical evidence suggesting that healing enhancement rates of surgical sites with PRF is up to two to three times higher than normal untreated surgical sites^(iv).

II. OBJECTIVE

To assess the role of Platelet Rich Fibrin in enhancement of regeneration of alveolar bone after bilateral extractions.

III. PROPOSED METHODOLOGY

This Randomized clinical study was conducted between July 2016 and February 2017. Twenty female patients were conveniently sampled from Khartoum Teaching Dental Hospital between July 2016 and February 2017.

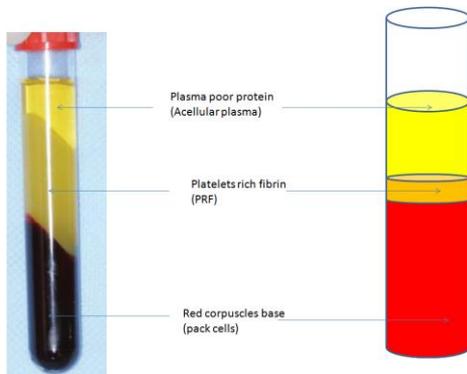
Preparation of PRF:

PRF was prepared in accordance with the protocol developed by Freymiller and Aghaloo^(v).

Intravenous blood (by venipuncturing of the antecubital vein) was collected in a 10 mL sterile tube without anticoagulant and immediately was centrifuged at 3,000 rpm for 10 minutes. Blood centrifugation allowed the formation of a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and a cellular plasma (platelet-poor plasma) at the top. PRF was easily separated from red corpuscles base (preserving a small RBC layer) using sterile tweezers just after removal of PPP (platelet-poor plasma) and then was Placement of PRF in extracted area:

PRF was augmented into the intrabony defect up to the surrounding bone level. A simple interrupted sutures was given using 3-0 non absorbable black silk suture. Post-

operative care was explained to the patient, with instructions to report back after a week for suture removal. Recall examinations after 1, 2 and 4 weeks interval was done to evaluate the healing kinetics of the defect.



image(1): Non heparinized vacutainer after procedure that showed separation of the collected blood



Image (2): PRF hold by Alice forceps



Image (3): placement of PRF in extracted area.

Measurement a degree of bone remodeling by radiograph: A cone beam CT was obtained at 1-, 2 and 4 weeks and comparisons of area where PRF was used and the contralateral area was done and observations were recorded on the degrees of bone growth and maturation of both areas.



Image(4)(A) cone beam CT of a patient shows a bone density in bilateral extracted area at first 2 weeks



Image(4)(B) cone beam CT of a patient in previous image (A) shows a bone density in bilateral extracted area at 4 weeks

Safety measures:

Only sterile equipment were used during all levels of the procedure.

All needles were disposed off in standard sharps box.

All laboratory equipments were sterilized between patients.

Plan for Data:

Data was collected using a form filled out by the investigator.

All the information obtained during the investigation was pooled into a main table and data entered and analyzed using SPSS.

The results obtained were then represented in tables, graphs and diagrams and discussed.

Ethical consideration

A written informed consent was signed or finger printed by the patient and all available knowledge, complications or adverse reactions were explained to the patient.

IV. RESULTS

Statistical analysis was done on a total of 20 female patients ranging from age 18 to 38.

Analysis of differences

To test if there is significant difference between measures on the right and left in 2 weeks and 4 weeks, the appropriate test is Paired Sample t-test, because the observations are related. It is equivalent to one sample t-test for difference.

The first step of t-test analysis is to ensure that the data is normally distributed.

The test for normality is Kolmogorov-Smirnov which tests the null hypothesis stated that data is normally distributed against the alternative hypothesis that data is not normally distributed.

Kolmogorov-Smirnov ^a			
	Statistic	difference	P-value
DiffR2_L2	.176	20	.104
DiffR4_L4	.148	20	.200
DiffR2_R4	.215	20	.016
DiffL2_L4	.348	20	.0001

Table (2): Kolmogorov-Smirnov test.

Kolmogorov-Smirnov test shows that difference between R2_L2 and R4_L4 are normally distributed since p-value > 0.05. But the difference between R2_R4 and L2_L4 are not normally distributed since their p-values are < 0.05, therefore t-test cannot be performed until the data is transformed to normal distribution.

Since data contains negative values we cannot perform ordinary transformation functions such as log and square root. After trying many functions of transformation, we reached that sin gives good results.

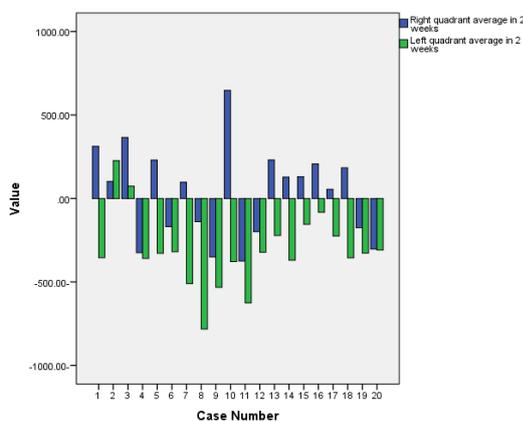
Kolmogorov-Smirnov ^a			
	Statistic	difference	P-value
sinDiffR4_R2	.168	20	.143
sinDiffL4_L2	.172	20	.122

Table(2):shows that the variables are now normally distributed since p-values> 0.05.

		Right quadrant average in 2 weeks	Left quadrant average in 2 weeks	Right quadrant average in 4 weeks	Left quadrant average in 4 weeks
N	Valid	20	20	20	20
	Missing	0	0	0	0
Mean		32.9	-312.8-	274.2	-127.95-
Median		100.0	-327.8-	302.0	-205.25-
Std. Deviation		276.0	223.9	462.8	303.61
Range		1023.0	1009.0	1872.5	1263.0

Table (3): Mean, Standard deviation, Median and Range in all quadrants of extracted area.

The mean is not suitable in this case, where large variation and outliers, so here it is better to consider median.



Figure(2): Histogram showed bone density in extracted after 2 weeks for both right and left site.

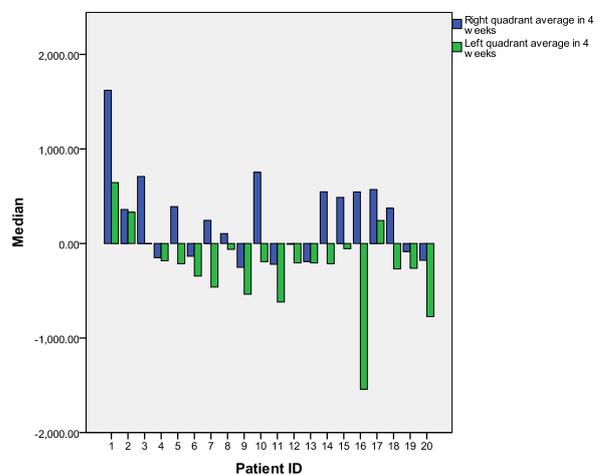


Figure (3): Histogram showed bone density in extracted area after 4 weeks for both right and left

T-test;

This is commonly used to test mean difference for both independent and related or matched samples.

In this study a paired sample t-test is applied to examine difference between the right and left quadrants.

Variable	T	Df	P-value	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Difference between R2_L2	5.598	19	0.00002	345.72500	216.4733	474.9767
Difference between R4_L4	5.802	19	0.00001	402.15000	257.0896	547.2104
Sin(difference between R2_R4	-0.689	19	0.499	-0.11642	-0.4700	0.2372
Sin (difference between L2_L4	-0.485	19	0.633	-0.07211	-0.3857	0.2392

Table (4): t-Test for difference in means between all extracted area right and left after 2 and 4 weeks

According to Bonferroni criteria when applying multiple tests, the level of significance should be adjusted by dividing level of significance by k (k=number of tests). In this case the level of significance should be $\alpha^* = 0.05/4 = 0.0125$. So if p-value < 0.0125 the difference is considered significant otherwise no evidence for significant difference.

Table (4) shows t-test with relevant statistics as means of difference and 95% confidence of interval of mean difference. It is noticed that p-value for difference between right quadrant in 2 weeks and left quadrant in 2 weeks is p-value 0.00002, which is less than 0.0125, indicating highly significant difference between readings after 2 weeks. The p-value for the difference between right quadrant and left quadrant in 4 weeks, shows highly significant difference of 0.00001. There is no significant difference between right after 2 weeks and 4 weeks and left after 2 weeks and 4 weeks, since p-value is higher than 0.0125 respectively.

** . Correlation is significant at the 0.01 level (2-tailed).

No significant correlation between age and bone density for right at 2 weeks and 4 weeks.

V. DISCUSSION

This study was done as a single centered randomized clinical trial to compare radiographically, the effectiveness of PRF in enhancement of bone healing as a post-extraction remedy. The right extraction site was treated by PRF, and left side was left untreated in all 20 female patients in the study. All were given thorough post operative instructions. The study group had uneventful uncomplicated healing except for 3, patients number 6,7

and 12 developed dry Sockets on the left sides(untreated) on day 3. These patients were treated by irrigation and antibiotic coverage coupled with mouth wash use. This episode gave us an opportunity for a soft tissue examination and noticed that, not only was the treated extraction site dry socket-free but the soft tissue healing was vastly accelerated, virtually completely healed. This goes in accordance with the work of Eshghpour M. et al., and Donald R.Hoaglin and Gary K.Lines(2014) , where the latter who occupied the use of PRF as a preventative method against localized osteitis (LO), with 1% incidence of LO in treated group and a staggering 9.5% in the untreated group¹⁸ and the study of Eshghpour M. et al. also confirmed the same observations¹⁹.

The cascade of bone regeneration produces bone of good quality and strength. During the process, this valuable time is crucial in many dental procedures that depend on hastened availability of good quality bone, for example dental implants. Thus the popularity of use of PRF has increased in the past few years, producing satisfactory results in bone and soft tissue regeneration and therefore an overall increase in the success rate of many procedures. The use of PRF in Perio-endo regenerative therapy is a recent and promising innovation, the endodontic part of etiology has predictability in its results but the periodontal etiology is unpredictable, therefore the addition of PRF and its positive impact on bone healing could be attributed to the angiogenic, proliferative and differentiating effects on osteoblasts of tissue growth factor β and platelet derived growth factor (TGF- β and PDGF) that are present in PRF in high concentrations^{vi}.

In this study the analysis of data showed that the mean for bone density averages have many variations and outliers in

our results(table 4), due to variations in the initial bone densities of the patients, therefore the median was used as a more normalized data set. It is clear from the readings that an increase of 202 points in the median has occurred on the treated (right) quadrant extraction sites between the second week and the fourth week attributed to the release of the PRF components into the wound and enhancing the healing process. This goes in concurrence with Joy R Das et al, where they concluded that the use of PRF in post extraction site of impacted 3rd molars had a beneficial effect on the bone density and also the reduction of pain. Whereas only an increase of 129.3 points occurred on the untreated (left) sites.

It is also worth mentioning that not only was the final bone formation far more increased on the treated side but also the initial readings of bone density at the 2 weeks point was still negative on the untreated side (-327.8) and the median on the treated side was already (100). This difference of 427.8 points was the direct result of the PRF treatment applied.

After the 4th week the reading's median difference between the right and the left was 500.5 points, which shows even further the superiority of the PRF application on the enhancement of bone healing both initially; at 2 weeks(median difference 427.8) and finally; at 4 weeks(median difference 500.5).

In figures (2) & (3), the histograms reflected the sense of the bone density differences visually, where the right side readings were always higher than the left side (except in one instance patient ID 2) due to the use of PRF on the right extraction site and its absence in the left site.

A further correlation test done on the age of the patient and the average bone density reading at 2 weeks and 4 weeks (table 6), showed that there was no significant relation between the age of the patient and the resultant bone density after PRF application into extraction site. Therefore it was safe to say that the treatment shows an effect on bones enhancement regardless of the age of the patient (within this study's sample age, between 18-38 years old) .

On correlating the differences between the right extraction site at 2 and 4 weeks and the left site at 2 and 4 weeks. Both sites showed no significant difference(right p value 0.499, left p value 0.633), meaning that the majority of bone healing enhancement has already occurred in the first 2 weeks post-operatively. On a similar basis a research done by Lucarelli E et al (2010), found that growth factors released from PRFM was greater at day 1 compared to day 2, day 3 and day 7. These results were consistent with an abundant release of the growth factors within the first day, and a gradual decrease of the release of growth factors thereafter^{vii}. But when taking a closer look, it was found

that the median increase in bone density on the right sites from the 2nd week to the 4th was 202 points and the left sites was 122.55 points, which showed a vast difference of 79.45 points in the rate of bone healing enhancement between the right and left sites.

Finally the t-test gave a highly significant correlation of p value 0.00002 between the treated(right) and untreated(left) sites at 2 weeks, which meant that this difference in bone density is due to the PRF treatment applied to the area; which enhanced the bone regenerative abilities of the body. Also, the difference between the right and left side at 4 weeks was even more significant, p-value 0.00001; further implicating the role of the PRF in this successful result.

VI. CONCLUSION

A newly founded interest in The role of Platelet Rich Fibrin in enhancement of regeneration of alveolar bone has been increasing in the past decade. The increase in the bone's regenerative ability due to PRF application was predictable in this study showing a highly significant difference in the healing of bone on the treated sites detected by CBCT. It's easy preparation, application and low cost makes it an ideal material for routine use in extraction sites especially complicated procedures with large bony defects . Furthermore the autogenous nature of this biologic material avoids the introduction of foreign materials.

VII. FUTURE SCOPES

- The scope of the research deserves a larger sample size with the inclusion of the male gender.
- The follow up CBCT can be done on the span of a longer period up to a year.
- The combination of PRF with other synthetic materials or bone grafts will be more valuable.
- Examination of the soft tissue healing at follow ups need to be considered, future wise.

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