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A Comparative Evaluation of Secondary Implant Stability with and without The Use of Advanced Platelet Rich Fibrin Using Resonance Frequency Analysis - A Randomized Controlled Clinical Study

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Abstract

Introduction: Addition of biologically active molecules during implant placement have osteoconductive effect, increase osteoblastic differentiation and enhance healing of peri-implant bone regeneration leading to faster osseointegration. Advanced Platelet Rich Fibrin due to lower speed of centrifugation protocols have significantly higher quantity of growth factors as TGF-β, PDGF, VEGF, and chemotactic molecules. Adding PRF might help in accelerated osseointegration during early healing period on implant.

Material and Methods: A total of 24 dental implants were placed into the healed edentulous sites in posterior mandibular region and divided into two equal groups (n=12). Group A Implant placement without Advanced Platelet Rich Fibrin (A-PRF) and Group B Implant placement with Advanced Platelet Rich Fibrin (A-PRF). The clinical evaluation was done by recording Implant Stability Quotient (ISQ) values at baseline, 1 week, 2week, 4week, 6week, 8week and 12week using Penguin RFA^{TM} device. The data was statistically evaluated using SPSS version 16.0 Software.

Results: The mean ISQ values at the baseline was 75.17±1.115 for Group A, which started to reduce with a statistically very highly significant dip at 1 week with ISQ of 72.83±1.115 and continued to dip till week 2 ISQ of 70.50±1.314 which was again a very highly significant difference. But on week 4 a significant increase with ISQ value of 72.83±1.115 to very highly significant increase from week 6, week 8 and highly significant increase at 12 weeks with ISQ values of 75.17±1.115, 78.50±1.567 and 80.00±1.206, respectively was observed.

On Intergroup comparison a very highly significant difference was observed between mean values at week 4

($p<0.01$) i.e. the mean ISQ values of Group A increased appreciably at week 4. However, on further comparison a significant difference in ISQ values ($p<0.05$) was observed at week 12 indicating that use of A-PRF induced better healing as compared to implants placed without A-PRF (Test Group A).

Conclusions: The clinical success of implants placed with and without the use of A-PRF was similar. However, adding A-PRF did not add any valuable significance in early healing period till 4 weeks, but a Significant difference was observed at end of 12 weeks on using A-PRF this indicate that use of A-PRF induced better healing as compared to implants placed without A-PRF.

Keywords: Dental implants, Implant surface, Implant Stability Quotient, Resonance Frequency Analysis, Advanced Platelet-Rich Fibrin,

Introduction

The history of implants goes back to Egyptian civilization in 3000 B.C., to where first copper stud was used in the oral cavity[1]. In 1900s a revolution was seen in dental implants and various materials were used such as porcelain, cobalt-chromium-molybdenum, titanium etc.[1] Since then, efforts have been made to develop a dental replacement that can be implanted into the bone.

P.I Branemark and collaborators during the 1960s bring about the revolutionary changes by introducing osseointegration in dental implant.[2] Osseointegration was defined as a direct contact between living bone and the surface of a load-carrying implant at the histological level.[2] It can also be defined as " A process whereby clinically asymptomatic rigid fixation of alloplastic material is achieved and maintained in bone during functional loading" – "Functional ankylosis"[3] Or "It is the direct anchorage of an implant by the formation of bone directly on the surface of an implant without any intervening layer of fibrous tissue.[4] Osseointegration is accepted as a histological term denoting direct bone apposition on the implant surface with no interposition of soft tissue.[4]

Implant stability is a requisite characteristic of osseointegration. Without it, long-term success cannot be achieved. Continuous monitoring in a quantitative and objective manner is important to determine the status of implant stability[5]. Osseointegration is also a measure of implant stability which can occur in two stages: primary and secondary. Primary stability mostly occurs from mechanical attachment with cortical bone. Secondary stability offers biological stability through bone regeneration and remodeling. Primary stability is affected by bone quality and quantity, surgical technique and implant geometry (length, diameter, surface characteristics). Secondary stability is affected by primary stability.[5]

Various techniques and devices have been introduced to measure the implant stability for e.g. implant tapping, insertion torque measurement, removal torque analysis and the Periotest[6].

However , the sensitivity of these methods are poor and their results are not objective, and most of them are not repeatable.[7] Therefore, the need for a user friendly, non-invasive, reliable, and clinically applicable technique to measure implant stability lead to the development of resonance frequency analysis (RFA) by Meridith and co-workers in 1996, which is based on vibration and a principle of structural analysis.[7]Quantitative analysis of implant stability using RFA is reliable and predictable technique for the assessment of success.[8]For this measurement, a transducer was placed on the fixtures. The resonance frequency (RF) transducer consisted of two piezoceramic elements attached to an offset cantilever beam. Stimulation of the elements causes vibration of the beam. The stimulating signal is a sinusoid

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wave with frequency of 5 to 15 Hz and amplitude peak of 1 V. RF values are recorded as Implant Stability Quotient (ISQ) on a scale from 1 to 100.[9] Higher ISQ values are reportedly associated with greater implant stability and osseointegration.[10]

One of the latest innovations in advanced surgical dentistry is the use of platelet concentrates for *in vivo* tissue engineering applications. Two types of such platelet concentrates are available: (1) platelet-rich plasma (PRP) and (2) platelet-rich fibrin (PRF). These are concentrated suspension of growth factors found in platelets that act as bioactive surgical additives when applied locally to induce wound healing.[11]

Platelet- rich plasma (PRP) is a first generation platelet concentrate. It is a platelet- rich fraction of plasma and is clinically used to deliver growth factors in high concentrations to the site of bone defect or a region requiring augmentation.[12] However, use of bovine thrombin as an anticoagulant for PRP preparation has been a major concern because it is associated with development of antibodies to clotting factors V, XI and thrombin, which has occasionally led to life threatening coagulopathies[12]. Also Monov et al and Ergun et al applied PRP during implant surgery and compared the stability of these implants with implants placed with conventional protocols and concluded that PRP application did not enhance the stability of the implants.[13,14.]

Platelet Rich Fibrin (PRF) is a second-generation autologous platelet concentrate and is a fibrin mesh consisting of leukocytes and cytokines. In 2001, it was developed in France by Choukroun et al.[15,16] PRF is a natural Fibrin based biomaterial prepared from an anticoagulant free blood harvest without any artificial biochemical modification (no bovine thrombin is required) that allows obtaining fibrin membranes enriched with platelets and growth factors.[12] Also PRF activates the vascular system and angiogenesis and releases growth factors slowly over a period of 7 or more days like PDGF, insulin like growth factor, vascular endothelial growth factor, and TGF, which are involved in soft and hard tissue healing.[15,16.]

Platelet-rich fibrin improves bone regeneration and also helps in faster titanium implant osseointegration which in turn improves the stability and maintenance of dental implants by increasing Bone to implant proximity.[17,18.] The PRF releases growth factors, favors cell migration and accelerates the process of bone regeneration.[19,20,21] PRF membrane acts as a Scaffold and forms a 3D architecture which is able to integrate with different cells types including circulating stem cells.[12]

Advanced platelet rich fibrin (A-PRF) was first described in 2014 as a new concept of cell-based tissue engineering, by decreasing the rpm and increasing the centrifugation time as compared to standard platelet rich fibrin (PRF). This method provides more platelet concentrate as a part of top layer and hence been termed as A-PRF.[42,43]A-PRF release significantly higher total quantities of growth factors when compared to traditional PRF[42,43].

Therefore, further studies are needed to evaluate the advantage of using A-PRF along with dental implants so as to assess its role in osseointegration and faster healing.

Aims & Objectives

Aims

A comparative evaluation of secondary implant stability with and without the use of advanced platelet rich fibrin using resonance frequency analysis - a randomized controlled clinical study.

Objectives of the Study

- To clinically evaluate secondary implant stability after implant placement in posterior mandible by

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assessing implant stability quotient using Resonance Frequency Analysis at baseline, 1, 2 ,4 ,6, 8, and 12 weeks.

- To clinically evaluate secondary implant stability after implant placement with Advanced Platelet Rich Fibrin (A-PRF) in posterior mandible by assessing implant stability quotient using Resonance Frequency Analysis at baseline, 1, 2 ,4 ,6, 8, and 12 weeks.
- To clinically evaluate and compare secondary implant stability after implant placement with and without use of Advanced Platelet Rich Fibrin (A-PRF) in posterior mandible by assessing implant stability quotient using Resonance Frequency Analysis at baseline, 1, 2 ,4 ,6, 8, and 12 weeks.

Materials and Methods

Source of Data

The study was conducted on 24 patients visiting outpatient department of Periodontology at Inderprastha Dental College & Hospital, Sahibabad. Both male/female patients aged between 18 to 60 years with no relevant medical history with at least one edentulous site in mandibular posterior region, who would benefit from Implant supported tooth restoration and were willing to give informed written consent were enrolled in the study. Ethical committee approval was obtained by the institutional review board of Inderprastha Dental College and Hospital.

Inclusion criteria

- Patients aged between 18 to 60 years with at least one edentulous space in posterior mandible region.
- Extraction socket which was completely healed.
- Adequate bone quantity at the implant site.
- Patients maintaining good oral hygiene and having plaque index score ≤ 1.
- Co-operative patient, willing to participate in study and willing to give written informed consent.
- RFA value of more than 47, immediately after placing the implant, to be included in the study.

Exclusion criteria

- Medically compromised or patients taking any immunosuppressive drugs which may complicate the treatment outcome.
- Subjects younger than 18 years of age.
- Patients with history of any bleeding disorder or on anticoagulant therapy.
- Physically challenged / patients with special needs.
- Smokers.
- History of bruxism / parafunctional habits.
- Pregnant / lactating mother.

Materials Used

Implants

- The Bredent SKY® Implant System – blue SKY will be used in the study.

Abutments: SKY® Aesthetic gingiva former.

Device for A-Prf Preparation

- For A-PRF preparation - REMI R-8C with REMI Swing out Head, R 81 will be used.

Study Design

The study was single blind randomized controlled clinical trial. Subject recruitment was accomplished between November 2019 and July 2021. An informed written consent, confirmed by the Committee on Human Studies Inderprastha Dental College, was obtained from all the subjects enrolled in the study after they fulfilled the selection criteria.

22 Patient were randomly selected with a toss of a coin and assigned to either of the two groups.

- Group A (12 patients) – Implant placement without Advanced Platelet Rich Fibrin (A-PRF). (CONTROL GROUP).

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- Group B (12 patients) – Implant placement with Advanced Platelet Rich Fibrin (A-PRF). (TEST GROUP).

Result

The current study was carried out in the Department of Periodontology, Inderprastha Dental College and Hospital, Sahibabad, for Comparative evaluation of the secondary implant stability with and without the use of advance platelet rich fibrin using resonance frequency analysis. A total of 24 subjects with edentulous site in posterior mandibular region were recruited and randomly distributed equally (N=12) in two groups, Group A (Implant placement without Advanced Platelet Rich Fibrin CONTROL GROUP) and Group B (Implant placement with Advanced Platelet Rich Fibrin TEST GROUP).

The outcome measures of the study were ISQ values, recorded at Day 0 i.e. immediately after implant placement and then at week 1, week 2, week 4, week 6, week 8 and week 12. All Implants, osseointegrated successfully and were well tolerated by all the subjects with no adverse tissue reaction, infection or impaired healing during the study.

The collected data of both the groups (A & B) was entered in MS Excel format, tabulated to form the master chart. Statistical test of significance using SPSS version 16 were applied for descriptive analysis.

Statistical Analysis

All the data collected and entered in MS excel and analyzed using SPSS 16.0 for windows (SPSS Inc, Chicago, IL, USA, 2001). The normality of data was tested by Shapiro Wilks test and data was found to be normally distributed, thus parametric test was used to analyze the observations. Descriptive statistics, including the mean and S.d. S.E.M. were calculated for all measurements.

The test of significance difference of parameter between groups (Inter group comparison) was tested by t- test for two independent groups and within group between two times intervals (Intra group comparison) was done by paired t- test. The 95% C.I. and 5% level of significance was used for analysis of data.

p values were as follows:

Significant $p<0.05$,

Highly significant $p<0.01$

Very Highly significant $p<0.001$

Not significant $p>0.05$

Formula Used for the Analysis

Mean: To obtain the mean, the individual observations were first added together and then divided by the number of observations.

$$\overline{X} = \frac{\Sigma X}{N}$$

Where:

$\overline{X}$ = the data set mean,

$\sum$ = the sum of,

X = the scores in the distribution,

N = the number of scores in the distribution

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Table 1: Distribution of Mean & Standard Deviation of ISQ Values in GROUP A & GROUP B at Different Time Intervals.

	Group	N	Mean	Std. Deviation	Std. Error Mean
Baseline	Group A	12	75.17	1.115	.322
	Group B	12	73.92	2.021	.583
Week 1	Group A	12	72.83	1.115	.322
	Group B	12	74.00	2.296	.663
Week2	Group A	12	70.50	1.314	.379
	Group B	12	70.17	2.623	.757
Week 4	Group A	12	72.83	1.115	.322
	Group B	12	70.17	2.552	.737
Week 6	Group A	12	75.17	1.115	.322
	Group B	12	74.67	1.923	.555
Week 8	Group A	12	78.50	1.567	.452
	Group B	12	78.33	2.103	.607
Week 12	Group A	12	80.00	1.206	.348
	Group B	12	81.50	1.567	.452

Table 2: Inter group Comparison of mean of ISQ values among two groups at different time intervals by independent t-test.

^{NS} Not significant p>0.05, * Significant p<0.05, ** Highly significant p<0.01, ***Very highly significant p<.001

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	P value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Baseline	7.326	.013	1.876	22	.074^{NS}	1.250	.666	-.132	2.632
Week 1	2.768	.110	-1.583	22	.128 ^{NS}	-1.167	.737	-2.695	.361
Week2	2.617	.120	.394	22	.698 ^{NS}	.333	.847	-1.423	2.090
Week 4	7.144	.014	3.317	22	.003**	2.667	.804	.999	4.334
Week 6	2.056	.166	.779	22	.444 ^{NS}	.500	.642	-.831	1.831
Week 8	1.716	.204	.220	22	.828 ^{NS}	.167	.757	-1.404	1.737
Week 12	1.517	.231	-2.628	22	.015*	-1.500	.571	-2.684	-.316

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Table 3: Distribution of Mean & Standard Deviation of change in ISQ values between two groups at different intervals.

	Group	N	Mean	Std. Deviation	Std. Error Mean
Change Baseline to 1Week	Group A	12	2.3333	.49237	.14213
	Group B	12	-.0833	2.39159	.69039
Change Baseline to 2Week	Group A	12	4.6667	.77850	.22473
	Group B	12	3.7500	3.27872	.94648
Change Baseline to 4Week	Group A	12	2.3333	1.15470	.33333
	Group B	12	3.7500	2.95804	.85391
Change Baseline to 8Week	Group A	12	-3.3333	1.15470	.33333
	Group B	12	-4.4167	2.39159	.69039
Change Baseline to 6Week	Group A	12	.0000	1.20605	.34816
	Group B	12	-.7500	2.37888	.68672
Change Baseline to 12Week	Group A	12	-4.8333	1.40346	.40514
	Group B	12	-7.5833	2.31432	.66809
Change 1Week to 2Week	Group A	12	2.3333	1.15470	.33333
	Group B	12	3.8333	2.51661	.72648
Change 2Week to 4Week	Group A	12	-2.3333	1.66969	.48200
	Group B	12	.0000	2.41209	.69631
Change 4Week to 6Week	Group A	12	-2.3333	.77850	.22473
	Group B	12	-4.5000	1.08711	.31382
Change 6Week to 8Week	Group A	12	-3.3333	1.15470	.33333
	Group B	12	-3.6667	1.07309	.30977
Change 8Week to 12Week	Group A	12	-1.5000	1.16775	.33710
	Group B	12	-3.1667	1.40346	.40514

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Table 4: Inter group comparison of mean of change in ISQ values between two time intervals among two groups by independent t- test.

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	P value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Change Baseline to 1Week	3.600	.071	3.429	22	.002**	2.41667	.70487	.95485	3.87848
Change Baseline to 2Week	11.033	.003	.942	22	.356 NS	.91667	.97280	-1.10080	2.93413
Change Baseline to 4Week	4.771	.040	-1.545	22	.137 NS	-1.41667	.91667	-3.31772	.48438
Change Baseline to 8Week	2.575	.123	1.413	22	.172 NS	1.08333	.76665	-.50660	2.67327
Change Baseline to 6Week	3.780	.065	.974	22	.341 NS	.75000	.76994	-.84675	2.34675
Change Baseline to 12Week	4.350	.049	3.520	22	.002**	2.75000	.78133	1.12962	4.37038
Change 1Week to 2Week	2.865	.105	-1.877	22	.074 NS	-1.50000	.79931	-3.15766	.15766
Change 2Week to 4Week	2.316	.142	-2.755	22	.012 **	-2.33333	.84686	-4.08961	-.57705
Change 4Week to 6Week	.611	.443	5.613	22	.000***	2.16667	.38599	1.36617	2.96717
Change 4Week to 8Week	1.717	.204	5.215	22	.000***	2.50000	.47937	1.50584	3.49416
Change 6Week to 8Week	.278	.603	.733	22	.472 NS	.33333	.45505	-.61038	1.27705
Change 8Week to 12Week	.440	.514	3.162	22	.005*	1.66667	.52705	.57364	2.75969

NS Not significant p>0.05, * Significant p<0.05, ** Highly significant p<0.01, ***Very highly significant p<.001

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Table 5: Intra group comparison of means of ISQ values between two time intervals Group A by paired t-test

Paired Samples Test									
Group		Paired Differences					t	df	P value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Group A	Baseline - Week 1	2.333	.492	.142	2.020	2.646	16.416	11	.000***
	Baseline - Week2	4.667	.778	.225	4.172	5.161	20.765	11	.000***
	Baseline - Week 4	2.333	1.155	.333	1.600	3.067	7.000	11	.000***
	Baseline - Week 6	.000	1.206	.348	-.766	.766	.000	11	1.000 NS
	Baseline - Week 8	-3.333	1.155	.333	-4.067	-2.600	-10.000	11	.000***
	Baseline - Week 12	-4.833	1.403	.405	-5.725	-3.942	-11.930	11	.000***
	Week 1 - Week2	2.333	1.155	.333	1.600	3.067	7.000	11	.000***
	Week2 - Week 4	-2.333	1.670	.482	-3.394	-1.272	-4.841	11	.001**
	Week 4 - Week 6	-2.333	.778	.225	-2.828	-1.839	-10.383	11	.000***
	Week 6 - Week 8	-3.333	1.155	.333	-4.067	-2.600	-10.000	11	.000***
	Week 8 - Week 12	-1.500	1.168	.337	-2.242	-.758	-4.450	11	.001**

NS Not significant p>0.05, * Significant p<0.05, ** Highly significant p<0.01, ***Very highly significant p<.001

Table 6: Intra group comparison of means of ISQ values between two time intervals Group B by paired t-test

Paired Samples Test									
Group		Paired Differences					t	df	P value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Group B	Baseline - Week 1	-.083	2.392	.690	-1.603	1.436	-.121	11	.906 NS
	Baseline - Week2	3.750	3.279	.946	1.667	5.833	3.962	11	.002**
	Baseline - Week 4	3.750	2.958	.854	1.871	5.629	4.392	11	.001**
	Baseline - Week 6	-.750	2.379	.687	-2.261	.761	-1.092	11	.298 NS
	Baseline - Week 8	-4.417	2.392	.690	-5.936	-2.897	-6.397	11	.000***
	Baseline - Week 12	-7.583	2.314	.668	-9.054	-6.113	-11.351	11	.000***
	Week 1 - Week2	3.833	2.517	.726	2.234	5.432	5.277	11	.000***

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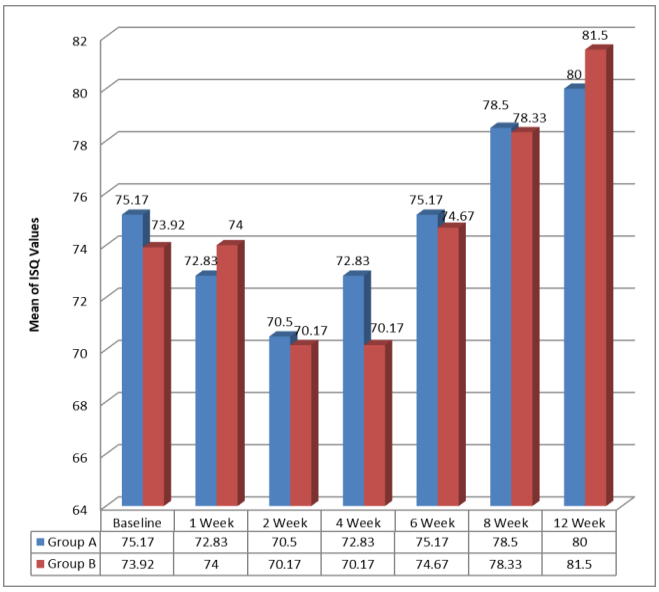
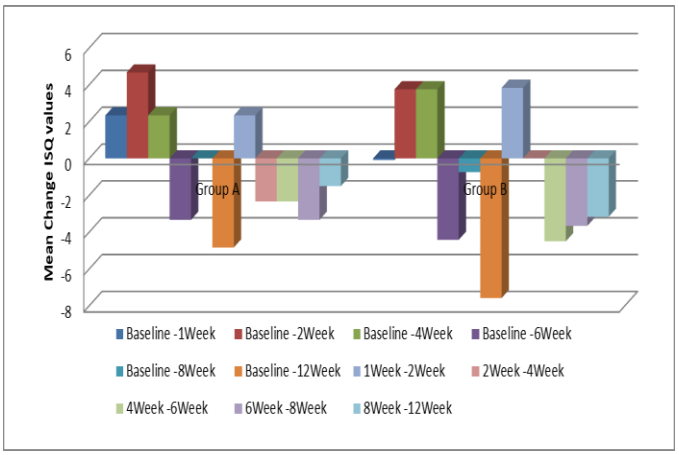
	Week2 - Week 4	.000	2.412	.696	-1.533	1.533	.000	11	1.000 NS
	Week 4 - Week 6	-4.500	1.087	.314	-5.191	-3.809	-14.339	11	.000***
	Week 6 - Week 8	-3.667	1.073	.310	-4.348	-2.985	-11.837	11	.000***
	Week 8 - Week 12	-3.167	1.403	.405	-4.058	-2.275	-7.816	11	.000***

NS Not significant p>0.05, * Significant p<0.05, ** Highly significant p<0.01, ***Very highly significant p<.001

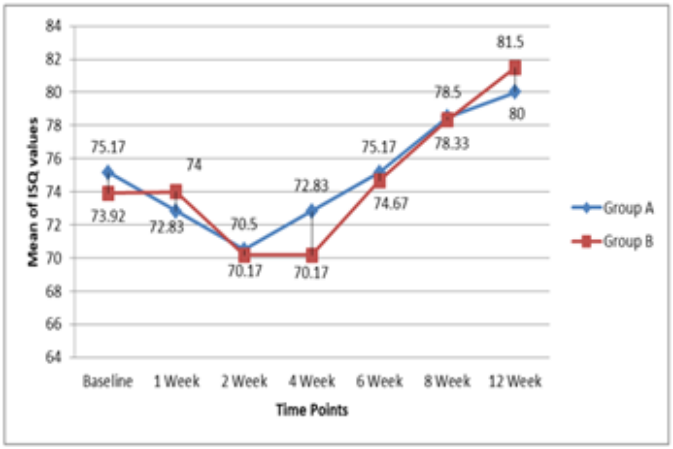
Graph 1: The comparison of Mean ISQ values in Group A & Group B at Different time intervals.



Graph 2: The comparison of change in Mean ISQ values in Group A & Group B at different time intervals.



Graph 3: The comparison of ISQ values in Group A & Group B at different time points.



Discussion

The concept of Osseointegration given by Branemark and co-workers in Europe in 1950 said that chambers made of the metal titanium could become permanently incorporated with bone. That is, the living bone could become so fused with the titanium oxide layer of the implant that the two could not be separated without fracture.[2]

Today, dental implants are used for supporting fixed prosthetic rehabilitation and removable over dentures. For a successful osseointegration of an end osseous dental implant placed in mandible at least 3 months without loading is important.

Different implant systems exist at present that vary in shape, dimension, surface materials, surface topography, surface chemistry, wettability, thread design, bulk, implant abutment connection and surface modifications.

The stability of the implant at the time of placement and during the development of the osseointegration process

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are the two major issues governing the implant survival. Implant stability is a mechanical phenomenon related to local factors such as bone quality, quantity, type of placement technique and type of implant used.

In our study a better version of L-PRF i.e. the Advanced platelet-rich fibrin (A-PRF) was used hypothesising that A-PRF releases a significantly higher amount of growth factors compared with L-PRF and would further improve early healing and faster osseous integration of implants, as according to the studies in A-PRF the number of leucocytes includes more neutrophils, which can help in differentiation of monocyte/macrophage.

To conclude, significant results were obtained at end of week 12 in Group B indicating that use of A-PRF might have a long term benefit. Since our sample size was small and duration of study was less further clinical and histological studies are required to get more precise results about the effect of PRF on osseointegration.

Conclusion

In order to reduce the Osseointegration time of dental implants a larger Bone to Implant contact(BIC) is required this can be achieved by chemical methods such as incorporation of inorganic phases on or into the titanium oxide layer and physical enhancement of the materials by increasing the level of roughness. Adding biologically active molecules such as growth factors, bone morphogenic proteins etc can be alternative method to accelerate the osseointegration in initial healing phase. This study evaluated and compared the change in secondary implant stability of end osseous dental implants when used with and without the of use of A-PRF in 24 edentulous sites. The prospective design of this clinical study includes evaluation of ISQ values at different time points to understand the pattern of healing. The results of the study indicate that in both the Groups implants were successfully osseointegrated at end of 12 weeks but with the use of A-PRF with implants a slight increase in stability was observed at 1week but which was not significant demonstrating that adding A-PRF did not add any beneficial advantage in early healing periods. However, it must be noted that to reach a more definitive conclusion further long-term clinical trials with larger sample size is required.

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Legend Figures

Figure 1: Armamentarium for Surgical Procedures

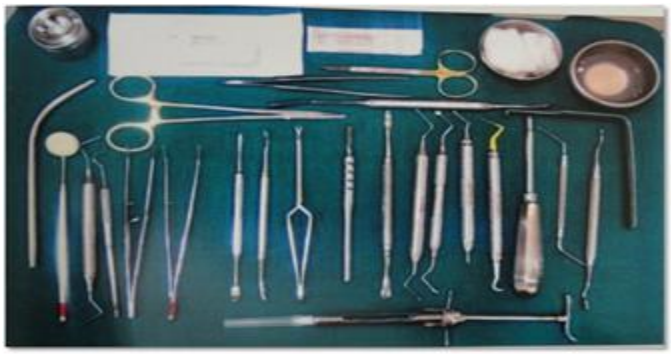

Figure 2: Adequate Ridge for Implant Placement

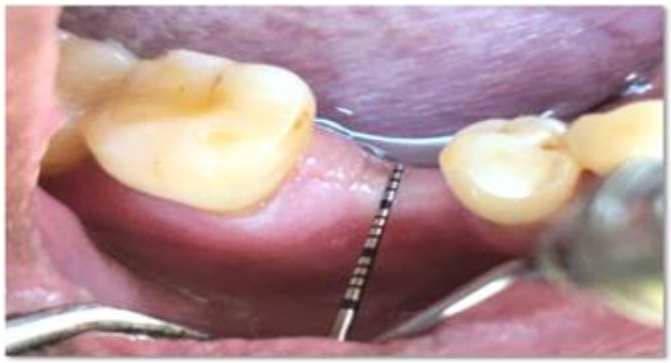

Figure 3: Full Thickness Mucoperiosteal Flap Reflected

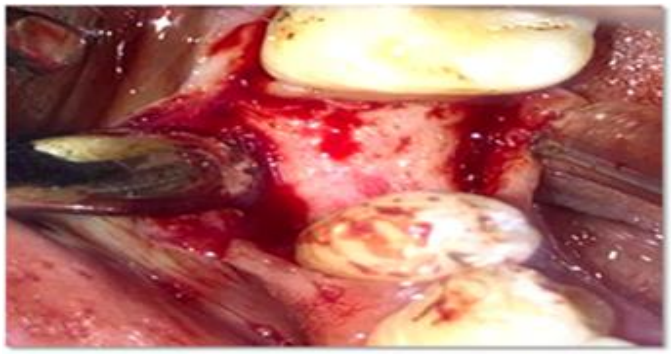

Figure 4: Centrifugation of Blood to Make A-Prf

Figure 5: PRF Obtaine

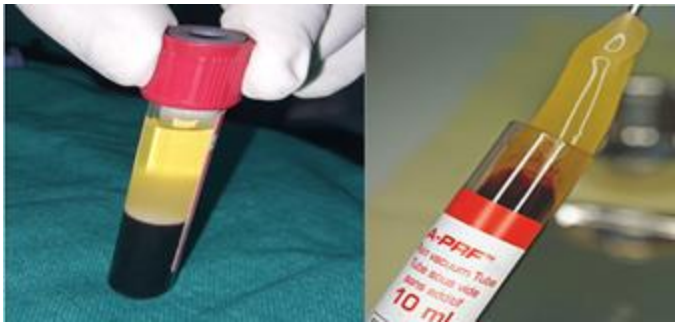

Figure 6: A-Prf Membrane with Serum Obtained From Compression of A-Prf.

Figure 7: Implant Being Dipped into the Serum

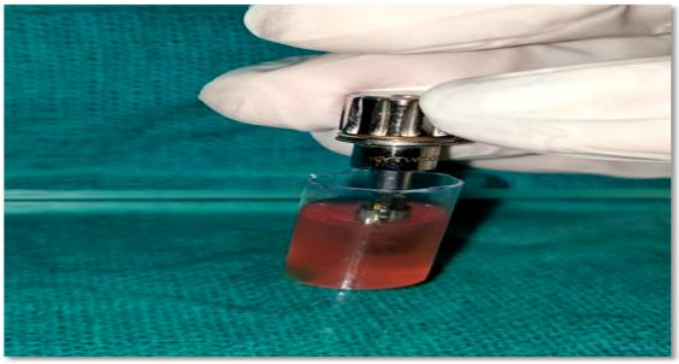

Figure 8: A-PRF Membrane Being Inserted Into Osteotomy Site.

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Figure 9: ISQ Value Being Recorded In Non-Contact Mode and Displayed On The Device.

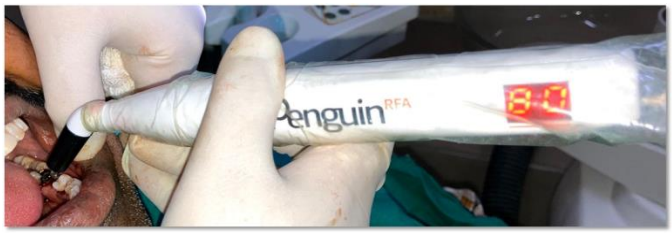

Figure 10: Sutures Given And Healing Abdutment Placed.

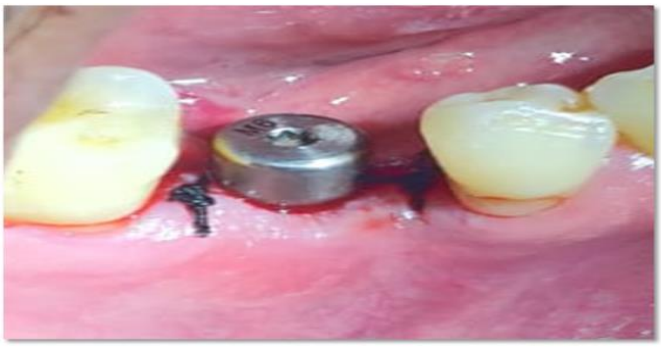

Figure 11: ISQ Value at Baseline

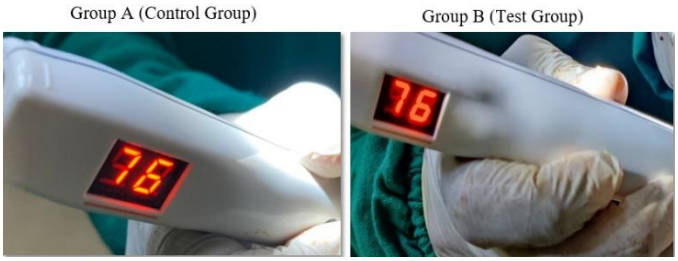

Figure 12: ISQ Value at 1 Week

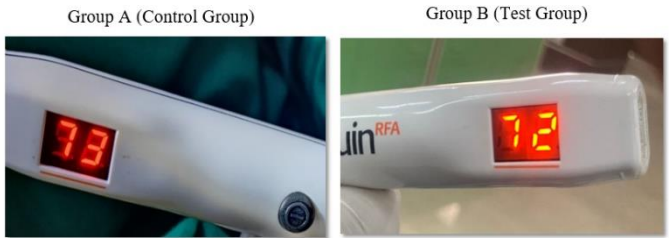

Figure 13: ISQ Value At 2 Week

Figure 14: ISQ Value At 4 Week

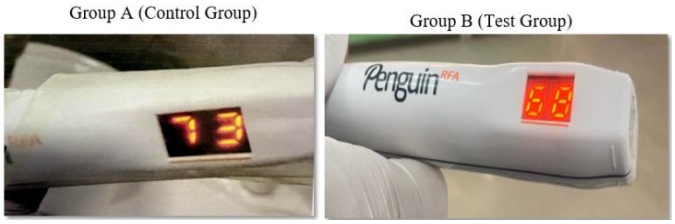

Figure 15: ISQ Value At 12 Week

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