International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2019): 7.583

Osseointegration of Dental Implants Bioactivated with Stem Cells and Prp-an invivo Study

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Abstract: ***Statement of Problem: Many implant systems propose a delayed loading protocol for loading of implants. This waiting period of osseointegration of implants of 3 – 6 months is often cumbersome to both the dentist and the patient. Purpose: This study proposes to compare the effect of mesenchymal cells and PRP on reducing or eliminating the waiting period of osseointegration. Materials and Methodology: 5Healthy subjects were selected based on having more than one missing teeth in maxillary or mandibular arches, preferably both sides of arches. After CBCT and other hematological tests, implants were placed following standard protocols with PRP on one side and with the use of stem cells on the other side. Implants were placed and surgical protocol followed. The insertion force was maintained at 35N.Resonancefrequency analysis (RFA) was done with Ostell ISQ values were analyzed. Results: A Pair T Test was performed and significant difference was found in the mean RFA at 8th and 10th week on stem cell side suggestive of early osseointegration. Conclusion: It was concluded that the use of mesenchymal cells reduces the time interval for loading of implants from 12 weeks to 8 weeks.***

1. Introduction

The original Branemark[1] protocol was to perform a two stage implant surgery. The waiting period is often a drawback for both the patient as well as the implantologist. To reduce this waiting period, many procedures which enhance the osseointegration like bone grafts, platelet rich plasma; mesenchymal stem cells have been used.

Mesenchymal stem cells are known to produce bone, reduce inflammation and protect damaged and injured tissues. Dental stem cells have the properties of differentiation in the body and can be isolated and proliferated in the laboratory. The stem cells have the potential to divide and become further differentiated[2].

On the other hand platelet rich plasma has haemostatic activity, and contains an abundance of growth factors and cytokines that can affect inflammation, angiogenesis, stem cell migration, and cell proliferation which can in turn produce bone and affect the osseointegration[3].

Based on this hypothesis, the study has been designed to evaluate the effect of allogenicmesenchymal cells and platelet rich plasma on the osseointegration of dental implants when introduced during osteotomy. The stability of the dental implants is determined using osstell device which utilize resonance frequency analysis at the bone implant interface[4.]

2. Aim and objective of the study

The aim and objective of the present study was to comparatively evaluate the effect of mesenchymal stem cells and platelet rich plasma on reduction in waiting period of osseointegration of dental implants.

Dental pulp derived allogenicmesenchymal stem cells and PRP were chosen for the study. The primary and secondary stability of dental implant was evaluated using Resonance Frequency Analysis (RFA) with Osstell ISQ Device.

Summary:
The objective of the present study was to evaluate the effect of osseointegration on dental implants activated with stem cells and PRP. The study revealed significant results. The use of stem cells had an accelerating effect on the osseointegration of dental implants leading to the reduction in time interval required for loading dental implants. However in the PRP group considering the RFA Values osseointegration happened four weeks later than the stem cell group.

3. Conclusion

Within the limitations of this In Vivo study, following conclusions were drawn:

1) Stem cells have reduced the osseointegration time of implant when compared to PRP.
2) Stem cells have definite positive influence on osseointegration process.
3) PRP has proved to have no significant difference on osseointegration process.
4) Further studies are needed to ascertain the efficacy of stem cells in osseointegration.

To the best of our knowledge this is the first study conducted comparing stem cells and PRP for their role in osseointegration.

4. Materials and Methodology

4.1 Materials

1) Nobel biocare replace tapered select dental implants, healing abutment and prosthetic abutment.
2) Physio dispenser.
3) Nobel biocare replace surgical kit.
4) Mouth mirror, probe and tweezer.
5) Dental pulp derived mesenchymal stem cells.
6) Sterile centrifuge tubes.
7) Sterile pipettes.
8) Cold saline.
9) Suture material and suture needles.
10) Bard parker handle and blade no 11, 12, 15.

11) Model and surgical stent.
12) Informed consent.
13) CBCT.
14) Local anesthetic solution, containing 2 percent lignocaine hcl with 1:80000-epinephrine and 2ml disposable syringes.

The study was conducted in Dept. of Implantology Sri Sai College of Dental Surgery. Ethical and research committee clearance was obtained. Patients were selected for the study if they satisfied the inclusion, exclusion criteria and informed consent was obtained.

Inclusion criteria

1) Patients within the age group of 25 - 55 years.
2) Bilateral edentulous or completely edentulous either maxillary arch or mandibular arch

Exclusion criteria-

1) Patients were excluded from the study if they presented with any of the following condition; history of bleeding disorder, renal failure and HIV infection.
2) Patients with metabolic bone disorders like osteoporosis and with uncontrolled endocrine disorder.
3) General contraindication to implant surgery.
4) Patients with psychiatric problems.
5) Acute or chronic inflammation of the area intended for implant placement.
6) Patient with oral parafunctional habits like bruxism.
7) Patients with self-declared pregnancies or intention to become pregnant.
8) Patients who have undergone radiation therapy.

4.2 Methodology

- The study was a randomized control trial. A total of 5 patients were selected to be treated with dental implants for their bilaterally missing edentulous condition, the edentulous space could be in either maxilla or mandible.
- On the site where platelet rich plasma was used to activate the implant, Whole blood was obtained by venepuncture. This was centrifuged. After which, the lower 1/3rd of the tube had Platelet Rich Plasma and upper 2/3rd had Platelet-Poor Plasma (PPP). At the bottom of the tube, platelet pellets were formed. Platelet poor plasma was removed and the platelet pellets were suspended in a minimum quantity of plasma (2-4 mL) by gently shaking the tube.
- After preparation of platelet rich plasma, the procedure on the edentulous site was begun with administration of local anesthetic containing 2% lignocaine hydrochloride using 2ml disposable syringes followed by scrubbing the area of the face using betadine and surgical asepsis was followed.
- A mid crestal incision was made using number 15 B.P blade; followed by intrasulcular incision extending one tooth medially and other teeth distally. The implant to be placed was 4.3mm x10 mm, an initial round drill of 2 mm was used to mark the position of implant. This was followed by 2.0mm pilot drill at 950 rpm till it reached a depth of 10 mm, the length was confirmed with depth gauge, followed by use of 3.5mm shaping drill till it reached 10 mm. Osteotomy finally ended with 4.3 diameter finishing drill till it reached 10 mm. Drilling was performed with internal irrigation drills.
- The prepared PRP was activated and the implant was dipped in activated PRP for 5 min and solution was loaded and injected in the osteotomy site. The implant was placed mechanically with help of fixture mount with insertion force ranging from 35-40 N/CM. After which cover screw was placed and site was approximated using black braided silk.

Implant placement at stem cell site

- The procedure on the edentulous site began with administration of local anesthesia containing 2% lignocaine hydrochloride with 2ml disposable syringe, followed by scrubbing the area of the face using betadine and surgical asepsis was followed.
- A mid crestal incision was made using number 15 B.P blade which was followed by intrasulcular incision extending one tooth medially and distally. The implant to be placed was 4.3mm x 10 mm, an initial round drill of 2mm to mark the position of implant. This was followed by 2.0mm pilot drill at 950 rpm till it reached a depth of 10 mm, the length was confirmed with depth gauge, followed by use of 3.5mm shaping drill till it reached 10 mm. Osteotomy finally ended with 4.3 diameter finishing drill till it reached 10 mm. Drilling was performed with internal irrigation drills.
- After the final drill at the osteotomy site was made the stem cells were centrifuged in a sterile environment. The cryovial containing stem cells was removed from special device and defrosted. Defrosting was done with constant moderate agitation by rubbing the cryovial between, both the palms, until ice in cryovial was no longer visible.
- The content of vial was transferred into sterile centrifuge tube. And 15 ml of saline was added to centrifuge tube placed in cooling centrifuge. Another sterile centrifuge tube containing the saline of similar quantity was used as counter weight and placed in opposite direction. Centrifuge was started at 1000 rpm and the rpm was increased by increment of 500 rpm every 2 min till it reached 3500rpm. It was gradually brought back to zero by reducing increment of 500 rpm every 2 min. After the centrifuge was stopped, the vial contents were examined.
- The supernant solution was discarded and the transparent pellet at the bottom contained stem cells.
- The first wash removed the Dulbecco modified eagle medium, dimethylsulfoxide, fetal bovine serum.
- 15 ml of saline was used and procedure was repeated and the supernatant solution was discarded.
- 2ml of saline was then mixed with the pellet to form a solution.
- Implant was dipped in the solution for 5 min. Following which it was loaded in sterile syringe and injected into the osteotomy site.

RFA

The primary stability of implant was recorded with ostell ISQ device. The smart peg was screwed into the implant with the help of peg mount. The mount was disengaged after securing the smart peg in implant. The RFA instrument was activated after which transducer probe was placed maintaining a distance of 3mm from smart peg at an angle of

90 degree and 3mm away from soft tissue. The two measurements were taken buccolingually and mesiodistally. Each implant was measured twice from different angles i.e. at 90 degrees and parallel to the crest. The measured value was displayed given on implant stability quotient.

The values range from 1-100. Where in 100 being the highest degree of stability.

After the readings were displayed the smart peg was removed with help of smart peg mount. Healing abutment was placed over the dental implant; mucoperiosteal flap was sutured with Black braided silk suture 3-0 with eyed stainless cutting edge 2/3rd circle suture needle.

Recall visit-

Secondary Implant stability was recorded in each site of implant placement i.e. on platelet rich plasma site and stem cells site at 2nd 4th 6th 8th 10th 12th week of implant placement.

5. Discussion

Dental implants are now scientifically accepted and predictable treatment for complete and partially edentulous patient's. Successful osseointegration was a prerequisite for a successful dental implant. Osseointegration of dental implants is time dependent wherein there was an asymptomatic rigid fixation of an artificial material as stated by **Branemark P et al [1] and L Linder et al[5].**

The **Nobel biocare replace select tapered dental implants** of size 4.3 x 10 (RP) was used for the present study because of its characterized rough surface resulting from drilling, which provides it with characteristic unevenness, which was repeated showing a clear orientation across the anisotropic implant surface. This type of surface creates greater roughness to facilitate cell adhesion and thus accelerate implant osseointegration.

Regenerative medicine had given medicine an opportunity of reproducing organs in laboratory and further implanting the same into the region of interest. One of the biomedical approaches in the regenerative medicine is stem cells. The human tooth and its surrounding tissue contain different stem cell population that can be distinguished as following;

1) Dental pulp stem cells (DSPC)
2) Stem cells from exfoliated deciduous teeth (SHED)
3) Stem cells from the apical papilla (SCAP)
4) Dental follicle precursor cells
5) Periodontal ligament stem cells

Stem cells were used for in the study for the following reasons:

1) They have higher cell proliferation ability
2) Osteogenic differentiations
3) Lower apoptosis
4) Different expression level of cytokines
5) As stated by **Huai Juan Ren et al (2016)[39]** stem cells induce vascular endothelial growth factor, fibroblast growth factor, keratinocyte growth factor and hepatocyte growth factor
6) They are non-phagocytic

Friedenstein et al (1968) stated that the stem cells could differentiate into bone, cartilage, adipose tissue, tendon and muscle. The cells used in the study were hypo immunogenic which is in conformity with the study done by **Kaseem et al (2004)[8]** and thus allogenicmesenchymal stem cells transplantation was possible. Mesenchymal stem cells used in the study had no immune response which is in conformity with the study done **by Katarina Le Blanca et al (2003)[10].**

Another aspect of Regenerative medicine is platelet-rich plasma (PRP). Its use is based on the potential of the plasma to release multiple wound-healing growth factors and cytokines which are responsible for increasing cell mitosis, increasing collagen production, recruiting other cells to the site of injury, initiating vascular in-growth and inducing cell differentiatiation.

Platelet rich plasma (PRP) and Platelet rich in growth factor (PRGF) were introduced in 2001. PRP is the combination of seven native growth factors within a normal clot as the carrier. The clot is composed of fibrin, fibronectin and vitronectin, which are cell adhesion molecules required for cell migration such as is seen in osteoconduction, wound epithelialization and osseointegration.

The initiation of bone formation starts with the release of Platelet Derived Growth Factor (PDGF), Transforming growth factor (TGF) and Insulin like growth factor (IGF). Vascular endothelial growth factor VEGF promotes endothelial growth proliferation **(Anupama Raheja et al 2015[13])**

In the present study PRP was selected because of its following features;

1) It is an extract of patients own blood
2) It is capable of promoting clot formation and release growth factors leading to osseointegration of the dental implant. This may reduce the osseointegration period which is in conformity with the study done by **AnupamaRaheja et al (2015)[13].**

The current study determined the effect of osseointegration when activated with platelet rich plasma and with stem cells by verifying the stability of the implants. The primary stability of implants was a crucial factor in determining the success of dental implant rehabilitation. One of the predictable and non invasive methods was application of resonance frequency analysis. Resonance frequency analysis (RFA) was introduced to provide objective measurement of primary stability and monitor implant stability over the healing period.

Traditionally, the ISQ values are known to vary between a range from 1 to 100. The ISQ value is directly proportional to the implant stability. Therefore, higher the ISQ value, higher is the stability. Stability of an implant plays a key role in achieving osseointegration. Therefore it was hypothesized that determination of a primary stability threshold might be relevant to predict the osseointegration and hence prognosis of the given implant.

In the present study 5 NOBEL BIOCARE REPLACE SELECT dental implants were placed. Implants were divided

into 2 groups. Group 1 implants were placed using stem cells. Group 2 implants were placed with PRP.

The manufacturer recommended surgical protocol was followed for Stem cells and platelet rich plasma. The stem cells were centrifuged in the cooling centrifuge and isolated before use following strict infection control protocol. Platelet rich plasma was prepared after withdrawing blood and it was centrifuged using acid dextrose tubes. After preparation of platelet rich plasma, it was loaded in a sterile disposable 2cc syringe. Immediately after the implant drilling protocol, the implants were dipped with stem cells and PRP on the contra lateral side for 5 minutes before being torqued to the bone. The primary stability for each patient was recorded on the day of surgery using RFA.

The primary stability at the time of implant placement on both stem cells group and PRP group followed a similar pattern. There was a decrease in RFA in PRP group suggestive of early osteoclastic activity, where in on the stem cells side osteoclastic activity starts at 4th week with a fall in RFA values. The means RFA on the buccolingual side of stem cells group after implant placement was **75** when compared to **74 ISQ** in the PRP group. This suggests good primary stability on both sides obtained from mechanical compression of bone**.**

In the present study good primary stability was a prerequisite for osseointegration and secondary stability of dental implants. There was a significant difference between the buccolingual RFA values on stem cells and PRP in the eight and tenth week with mean RFA of **78** on stem cells and **76** on the PRP group. The results of the present study showed significant difference at eight and tenth week when **p<0.05.** RFA value less than 40 is suggestive of implant failure as stated by **Esposito et al (2008)[16].**

With reference to patient N1 the highest RFA in the stem cell group was seen on the day of placement which indicates primary stability obtained due to mechanical compression of bone. In 2nd week (84-84) the RFA was not altered which was due to delay in migration of osteoclasts leading to delay in bone resorption. During 8th week (82-80) the RFA indicates formation of compact bone leading to osseointegration. The lowest RFA was observed at 4th week (75-76) which occurred once the bone resorption process started. The highest value of RFA in the PRP group was obtained immediately after placement which indicates good primary stability. At 12th week (80-82) the RFA indicates the formation of compact bone leading to osseointegration and lowest was observed at 2nd week (74-72) which could be due the initial bone resorption due to migrating osteoclast.

With reference to patient N2 the highest RFA in the stem cell site was observed immediately after placement indicating primary stability obtained by mechanical compression of bone .The highest was also recorded at 8th, 10th and 12th week (78-78) which could be due to transformation of hollow osteocytic lacunae to intact lacunae which indicates osseointegration .The lowest RFA was recorded at 2th week (70,72)due to the migrating osteoclasts causing bone resorption. The highest RFA in the PRP group was obtained immediately after placement. At 12th week (72-75) the RFA indicates formation of compact bone and thus osseointegration .The lowest RFA was recorded at 4th week (63-67) which indicates delay in osteoclastic activity due to the presence of growth factors.

With reference to patient N3 the highest RFA was recorded immediately after placement which indicates good primary stability obtained due to mechanical compression of bone. The highest RFA was also recorded at 8th, 10th and 12th week (75-75) which is due to rapid transformation of hollow osteocytic lacunae to intact lacunae which indicates osseointegration .The lowest was observed on 2nd week (70-70) due to osteoclast migrating at the site of placement and causing bone resorption. However on the PRP group the highest RFA was at 12th week (73-74) which is due to delayed transformation of hollow osteocytic lacunae to intact lacunae which indicates delayed osseointegration. The lowest RFA was recorded at 4th week (70-70) this is due to delay in osteoclastic activity due to presence of growth factors.

With reference to patient N4 highest RFA in the stem cell site, was obtained at the time of placement which could be due to mechanical compression of bone. At 8th, 10th, and at 12th week (79-78) the RFA was due to rapid transformation of hollow osteocyticlacunae to intact lacunae which indicates osseointegration. The lowest RFA was recorded at 2nd week (72-74) which could be explained by osteoclasticresorption. Whereas the highest RFA on PRP group was recorded immediately after placement which could be explained by mechanical compression of bone leading to good primary stability. At 10th and 12th week (77-78) the RFA value can be explained by delayed transformation of hollow osteocytic lacunae to intact lacunae which indicates delayed osseointegration. The lowest RFA was recorded at 4th week (72-71) this is due to delay in osteoclastic activity due to presence of growth factors.

With reference to patient N5 the highest RFA in the stem cell group was obtained immediately after placement which indicates good primary stability obtained due to mechanical compression of bone. At 8th, 10th, 12th week (80-80) the RFA could be explained by increased transformation of hollow osteocytic lacunae to intact lacunae which indicates osseointegration. The lowest was recorded in 2nd week (69-70) which is due to migration of osteoclasts to the site of placement and cause bone resorption. The highest RFA in the PRP group was found only at the time of placement (80-80) which indicates good primary stability obtained due to mechanical compression of bone. Whereas the lowest RFA was at 4th week (70-72) this is due to delay in osteoclastic activity due to presence of growth factors.

In the stem cells region, the primary and secondary stability coincides with eight week suggestive of a decrease in the time interval of osseointegration from standard of 12thweek to 8th week.

The results in the present study can be explained by their direct ability to generate osteoprogenitors and osteoblasts and simultaneous introduction of bone morphogenic protein 2 (BMP2) and other osteoinductive growth factors. The addition of supporting growth factors has become

particularly relevant when bone repair occurs.

In the present study when implants were activated with platelet rich plasma and followed for 12 weeks it was noticed that there was a favourable tissue response and decreased osteoclastic activity for few weeks. This however did not contribute to reduction in time required for osseointegration of dental implants. It is not in conformity with the study done by **AnupamaRaheja et al (2015)[13].**

In this study Platelet rich plasma (PRP) when used for bone regeneration around dental implants, demonstrated no statistically significant differences when PRP was used ($P > 0.05$), and Plasma alone did not enhance bone regeneration which is in conformity with the study done by **Casati et al(2006)[13] and Stwart et al (2002)[7].**

In the PRP group the osseointegration occurred at twelve weeks suggestive of standard time taken to achieve osseointegration. These results may be explained by the apparent different cell responses in the earliest osseointegration stages. Firstly the implant roughness led to significantly increased wetting and protein absorption, which in turn favoured cell migration and adhesion

The **mean buccolingual stability** on the stem cell group at the time of placement was 75 when compared to RFA of 74 in the PRP group. At the second week the RFA was 73 at the stem cell group and where as it was 70 in the PRP group. When recorded at 4th week the RFA on stem cell group was 69 whereas in PRP the RFA was 70.In the 6th week the RFA on stem cell group was 72 however in the PRP group the RFA was 71. There was a significant difference at the 8th and 10th week when $p<0.05$ between buccolingual RFA values in stem cells and PRP group. The mean RFA on the stem cell group recorded highest RFA of 78 and in the PRP group the RFA was 70. During the 10th week the RFA on the stem cell group was 76 whereas it was 72 in the PRP group. Lastly in the 12th week the RFA on the stem cell group was 76 and whereas in the PRP group the RFA recorded were 74.

During the evaluation of RFA on the buccolingual side, the highest RFA was recorded at 8th week on stem cells. The lowest RFA in stem cells group was recorded in the 2nd week and on PRP side at the 4th week. These results could be attributed to the nature of stem cells that favours vertical bone augmentation which is in conformity with the study done by **ArashKhojashteh et al (2011)[26];** the other reason is its ability to migrate to tissue injury sites and differentiae into osteoblasts **.** These RFA values are suggestive of early osseointegration and secondary stability of dental implants at the stem cells side compared to PRP side. The RFA values followed the similar pattern mesiodistally on both stem cells and PRP sides.

The primary stability RFA values were recorded at the time of implant placement and were found to be similar to the secondary stability RFA values which were recorded at the completion of osseointegration. The ISQ values on the stem cell group at 2nd, 8th and 10th week with mean RFA were about 73 in 2nd week, 78 in 8th week and 76 in 10th week. The lowest RFA was obtained in stem cell group during 4th week suggesting a delayed initial osteoclastic activity when compared to lowest RFA in second week for PRP group. The delay in the first 2 weeks of implant placement could be explained due to the presence of growth factors.

The osteoclastic activity could be observed during the 4th week where the osteoclasts migrating to the site of placement undergoes the process of osteoclasticresorption and remodelling.

During the 6th and 8th week RFA gradually increased by cell differentiatiation into osteoblasts which formed woven bone. And since then at 12th week the RFA on PRP group was stabilized and there was marked increase in formation of intact osteocytes .This newly formed bone presented the morphological characteristics of compact bone which indicated osseointegration.

In the present study, the ISQ values of primary stability achieved at the time of dental implant placement were closely similar to secondary stability ISQ values attained after osseointegration of dental implants. There was a dip in the ISQ values during the 4th week in the stem cell group and 2nd week for the PRP group suggestive of the osteoclastic activity happening at the bone – implant interface. Osteoclastic activity in the 4th week in stem cell group is suggestive of the presence of osteoclasts at the implant bed from the injected undifferentiated mesenchymal stem cells. The Buccolingual and Mesiodistal ISQ values indicating the secondary stability were stabilized at eight weeks on the stem cell group and the PRP group took 12weeks for the same.

The RFA method with the Osstell ISQ device has been claimed to be useful for monitoring implant osseointegration during the healing and helping the clinician to decide on an individual basis when to load an implant. The implicit assumption was that, implants undergoing osseointegration are supposed to increase their stability with time or atleast maintain it **.**A second assumption was that implants having achieved a higher primary stability might be loaded earlier than implants with lower ISQ values.

Neither a defined cut off ISQ value had been validated until now through documented studies to determine the threshold value that discriminates between a mobile and stable implant, nor a cut off ISQ value had been published so far to orient the clinician towards shorter or longer healing periods .The present study was designed to determine these values for Nobel biocare to replace select implants.

The data collected over the 12 weeks healing period led to determination of primary and secondary stability RFA values corresponding to the changes occurring at the bone–implant interface on both stem cells and PRP group. As per the results obtained in this study it can be concluded that stem cells can be used over PRP for achieving early osseointegration.

6. Results

N1

The table N1 denotes that the highest RFA in the stem cell group was seen on the day of placement and 2nd week (84-

84) and lowest was observed at 4th week (75-76), whereas highest value in the PRP group was obtained immediately and at 12th week (80-82) and lowest was observed at 2nd week (74-72).

	Patient Name- Hameeda			
	Stem Cells		PRP	
	B-L	M-D	B-L	M-D
Immediate	83	83	82	82
2th	84	84	74	72
4th	75	76	74	76
6th	78	76	76	76
8th	82	80	76	76
10th	80	80	78	77
12th	81	80	80	82

N2

The table N denotes that the highest RFA in the stem cell site was observed immediately after placement and at 8th,10th and 12th week (78-78)and lowest was recorded at 2th week (70,72), whereas the highest RFA in the PRP group was obtained immediately after placement, and at 12th week(72-75)and lowest was recorded at 4th week(63-67).

	Patient Name- Sujuna			
	Stem Cells		PRP	
	B-L	M-D	B-L	M-D
Immediate	80	78	76	74
2th	70	72	72	74
4th	68	70	63	67
6th	71	78	67	67
8th	78	78	67	70
10th	78	78	69	75
12th	78	78	72	75

N3-

The table N3 denotes that the highest RFA was recorded immediately after placement and at 8th, 10th and 12th week (75-75) and lowest was observed on 2ndweek (70-70). However On the PRP group the highest RFA was at 12th week (73-74) and lowest RFA was recorded at 4thweek (70-70)

	Patient Name- Radha			
	Stem Cells		PRP	
	B-L	M-D	B-L	M-D
Immediate	74	74	72	70
2th	70	70	74	74
4th	70	71	70	70
6th	72	72	70	72
8th	75	75	72	74
10th	75	75	72	74
12th	75	75	73	74

N4-

The table N4 denotes that the highest RFA in the stem cell group was obtained immediately after placement, 8th, 10th 12th week (80-80) and lowest was recorded in 2nd week (69-70). Whereas on highest RFA in the PRP group was found only at the time of placement (80-80) and lowest was at 4th week (70-72).

	Patient Name- Sudarshan			
	Stem Cells		PRP	
	B-L	M-D	B-L	M-D
Immediate	80	80	80	80
2th	69	70	78	78
4th	72	72	70	72
6th	76	76	71	73
8th	80	78	73	74
10th	80	80	74	75
12th	80	80	75	75

N5

The table N5 denotes the highest RFA in the stem cell group was recorded immediate after placement and at 8th,10th and 12th week (75-70) and lowest at 4th week(71-68).however the highest RFA on the PRP side was recorded immediately after placement(76-72) and the lowest at 4th week(65-70).

	Patient Name- Rajeshwari			
	Stem Cells		PRP	
	B-L	M-D	B-L	M-D
Immediate	75	78	76	72
2th	75	72	70	70
4th	71	68	65	70
6th	72	70	66	72
8th	75	70	66	73
10th	75	70	68	73
12th	75	70	70	73

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N3

The table N3 denotes that the highest RFA in the stem cell site was observed immediately after placement and at 8th,10th and 12th week (78-78)and lowest was recorded at 2th week (70,72), whereas the highest RFA in the PRP group was obtained immediately after placement, and at 12th week(72-75)and lowest was recorded at 4th week(63-67).

	Patient Name- SUJUNA			
	Stem Cells		PRP	
	B-L	M-D	B-L	M-D
Immediate	80	78	76	74
2th	70	72	72	74
4th	68	70	63	67
6th	71	78	67	67
8th	78	78	67	70
10th	78	78	69	75
12th	78	78	72	75

N5-

The table N5 denotes that the highest RFA was recorded immediately after placement and at 8th, 10th and 12th week (75-75) and lowest was observed on 2ndweek (70-70). However On the PRP group the highest RFA was at 12th week (73-74) and lowest RFA was recorded at 4thweek (70-70)

	Patient Name- RADHA			
	Stem Cells		PRP	
	B-L	M-D	B-L	M-D
Immediate	74	74	72	70
2th	70	70	74	74
4th	70	71	70	70
6th	72	72	70	72
8th	75	75	72	74
10th	75	75	72	74
12th	75	75	73	74

N6-

The table N6, highest RFA in the stem cell site, was obtained at the time of placement, 2nd week, 8th, 10th, and at 12th week (79-78), and lowest RFA was recorded at 2ndweek (72-74). Whereas the highest RFA on PRP group was recorded immediately after placement, 10th and 12th week (77-78), lowest RFA was recorded at 4th week (72-71).

	Patient Name- NEERJA			
	Stem Cells		PRP	
	B-L	M-D	B-L	M-D
Immediate	79	79	77	77
2th	72	74	76	75
4th	74	74	72	71
6th	76	77	74	76
8th	79	78	76	76
10th	79	78	77	78
12th	79	78	77	78

N7-

The table N7 denotes that the highest RFA in the stem cell group was obtained immediately after placement, 8th, 10th 12th week (80-80) and lowest was recorded in 2nd week (69-70). Whereas on highest RFA in the PRP group was found only at the time of placement (80-80) and lowest was at 4th week (70-72).

	Patient Name- Sudarshan			
	Stem Cells		PRP	
	B-L	M-D	B-L	M-D
Immediate	80	80	80	80
2th	69	70	78	78
4th	72	72	70	72
6th	76	76	71	73
8th	80	78	73	74
10th	80	80	74	75
12th	80	80	75	75