

CLINICAL EFFECT OF AUTOLOGOUS PLATELET-RICH FIBRIN ON POST OPERATIVE WOUND HEALING: A RANDOMIZED COMPARATIVE CLINICAL STUDY

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ABSTRACT:

Surgical removal of impacted third molars is one of the most commonly performed minor oral surgical procedures in Oral and Maxillofacial surgical practice.

An uneventful and enhanced wound healing is desirable and critical in ascertaining quality of life after the third molar surgery. The use of platelet concentrates is one such technique that can aid in a better treatment outcome. Platelet concentrates are rich in various growth factors and are widely used in Oral and Maxillofacial Surgery for more superior wound healing. The shift in the therapeutic concepts from resection to regeneration has significantly influenced the practice of oral and maxillofacial surgery in the last quarter of the century.

This study demonstrates that Platelet rich fibrin (PRF) enhances soft tissue healing and accelerates bone formation.

Key words: Platelet rich fibrin, PRF, Platelet rich plasma, IL-1, IL-2, Bone Morphogenic proteins, Insulin derived growth factors.



INTRODUCTION:

Excogitation in the concept of tissue engineering has brought about a drastic improvement in healing response of tissues. Development of bioactive surgical additives is one of the greatest challenges of clinical research which has been used to regulate inflammation and increase the speed of wound healing process.^[1]

Bone grafting in oral and maxillofacial surgery can be traced back to Mayan civilization where they used mother pearl to reconstruct bony defects using them as implants in tooth bearing areas of the jaws.^[2]

Socket healing is a highly co-ordinated sequence of biochemical, physiologic, cellular and molecular responses involving numerous cell types, growth factors, hormones, cytokines and various proteins directed towards restoring tissue integrity and functional capacity after injury.^[3]

In 1974, Ross et al. were the first to describe the growth factors present in the platelets. After activation of the platelets trapped within the fibrin matrix, growth factors are released which stimulate the mitogenic response in the bone periosteum during normal wound healing for bone repair.^[4]

Platelet-rich fibrin was first developed by Choukroun et.al in 2001 in France. Platelet-

rich fibrin (PRF) belongs to a new generation of platelet concentrate, with simplified processing and without biochemical blood handling. Platelet Rich Fibrin is an immune and platelet concentrate collecting on a single fibrin membrane, containing all the constituents of a blood sample favourable to healing and immunity. PRF consists of a fibrin matrix polymerized in a tetra molecular structure with incorporation of platelets, leucocytes, cytokines and circulating stem cells.^[5]

The reasoning for the use of PRF is structured around 4 fundamental events of cicatrization, namely, angiogenesis, immune control, circulating stem cells trapping, and wound-covering epithelialization. All of the known clinical applications of PRF highlight an accelerated tissue cicatrization due to the development of effective neovascularization, accelerated wound healing with fast cicatricial tissue remodelling, and nearly total absence of infectious events.^[14]

Aims and objectives:

- 1.To evaluate clinical effects of autologous Platelet Rich Fibrin on post operative wound healing.
- 2.To evaluate the post-operative sequelae after surgical extraction of impacted mandibular third molar.
- 3.To assess post-operative infection after surgical removal of third molars.
- 4.To assess radiographically the post-surgical bone healing after insertion of Platelet Rich Fibrin into surgically removed third molar extraction socket.

MATERIALS AND METHODS:

Sample size:

A total of 40 cases with impacted mandibular third molar between 20 to 40 years of age were selected randomly for the study.

Inclusion Criteria:

- Impacted mandibular third molars
- Patients between 20 to 40 years of age.
- Physically and medically fit patients without any known source of infection from oral cavity.

Exclusion Criteria:

- Patients with severe systemic diseases (eg. diabetes, hypertension, bleeding disorders, kidney and liver diseases, etc).
- Pregnant and lactating mothers
- Patients with habit of smoking or tobacco chewing

Subjects were divided into two groups:

Group 1 (Control group): After surgical removal of impacted mandibular third molar, wound closure was done by suturing without placement of Platelet-Rich Fibrin.

Group 2 (Experimental group): After surgical removal of impacted mandibular third molar, Platelet Rich Fibrin was prepared and placed into the extraction socket followed by wound closure with suturing.

A detailed case history of the patient was obtained. An informed consent was obtained from the patient regarding the

surgery as well as the use of PRF after removal of lower third molars.

Evaluation Criteria:

All patients were evaluated post-operatively on day 1, day 3 and day 7 for:

1. **Pain** – Evaluated by Visual Analog Scale
2. **Swelling**–Evaluated by measurement from tragus of ear to corner of mouth and from lateral canthus of eye to angle of mandible
3. **Trismus** – Evaluated by measuring interincisal mouth opening using vernier calliper
4. **Post operative infection-** Grading scale (Johnson JT, Myers and Thearle, et al, 1984)
5. **Osseous healing**-Estimation of post operative socket healing using OPG and grey scale histogram.

Method:

A detailed case history was obtained.

All patients were explained in detail about surgical procedure and possible complications associated with the same.

A written consent was obtained from all patients before surgery was carried out.

Routine blood investigations were done.

Radiographic evaluation was done with the help of orthopantomogram (OPG).

Pre-operative:

Facial Measurement: Inter-incisal mouth opening using vernier calliper

Surgical technique:

Patient's face was prepared with povidone iodine 5% for skin and 1% for oral mucosa and draped.

Anaesthesia:

Pterygomandibular nerve block was given using 2 ml of 2% lignocaine with 1:80000 adrenaline.

Incision:

Standard Ward's incision was used. The incision began in the buccal sulcus in the region of mandibular second molar and passed upwards to the distobuccal line angle of second molar at gingival margin. The incision was then extended cervically behind the tooth to middle of the distal surface of the second molar. From this point single stroke incision was taken posteriorly and buccally over the external oblique ridge.

Reflection of mucoperiosteal flap:

The sharp end of periosteal elevator was inserted at the anterior end of incision and interdental papilla reflected. The blunt end used underneath the periosteum to reflect the soft tissue as a complete mucoperiosteal flap.

Osteotomy:

Osteotomy around the crown of the impacted third molar was done with handpiece and stainless steel burs. Bone was removed under constant irrigation

with normal saline to prevent thermal damage.

Sectioning of tooth:

Odontosectioning was done to facilitate tooth removal and minimise the bone removal.

Extraction of tooth:

After all due procedures, the tooth was luxated with the help of straight elevator and then extracted with forceps employing minimum force.

PREPARATION OF PLATELET RICH FIBRIN

1. 10 ml blood was drawn from the patient by venepuncture at the antecubital fossa.
2. This blood was transferred into autoclaved centrifugation tubes by

dividing it into 5ml each without an anticoagulant.

3. The tubes are then placed in a centrifugation machine at 3000 revolutions per minute for 10 minutes, after which it settles into the following layers—red lower fraction containing red blood cells, upper straw coloured cellular plasma, and the middle fraction containing the fibrin clot.
4. The upper straw coloured layer was then removed and the middle fraction collected, 2mm below the dividing line which is the Platelet Rich Fibrin.
5. Now the fibrin clot is held in the forceps placed into the extraction socket (Group 2) and flaps are approximated and sutured.

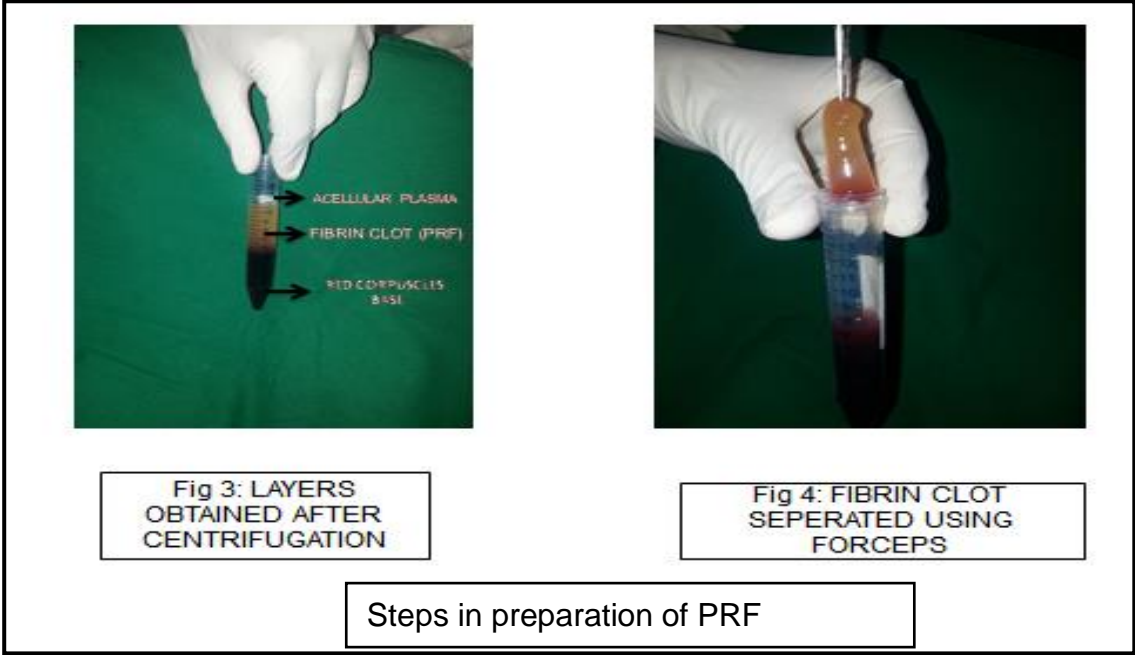
STEPS IN PREPARATION OF PLATELET RICH FIBRIN



FIG 1: DRAWING OF BLOOD AT THE ANTECUBITAL FOSSA



FIG 2: PLACING THE BLOOD IN THE CENTRIFUGE AND CENTRIFUGING AT 3000RPM FOR 10 MINUTES



RESULTS:

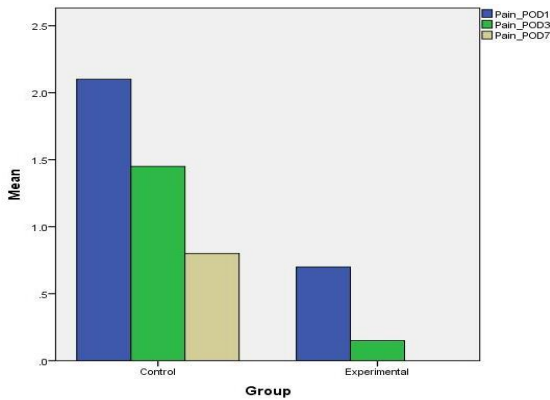
PAIN: (graph 1)

Pain was assessed on visual analog scale (VAS) rated 0, 1, 2, 3, 4, 5 (no pain, slight pain, mild pain, severe pain, very severe pain, extremely severe pain) on 1st post-operative day (POD1), 3rd post-operative

day (POD3) and 7th post-operative day (POD7).

Statistically significant results were seen post operative day 1 and post operative day 7.

Meanwise comparison of pain according to VAS scale on postoperative day 1, day 3 and day 7 between control and experimental group



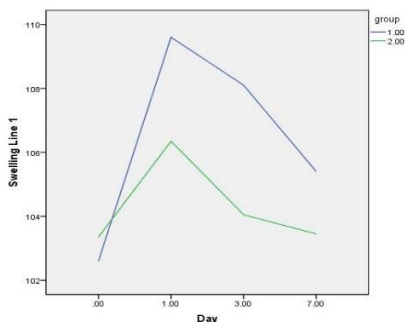
(Graph- 1)

SWELLING (Graph 2, Graph 3)

Swelling was measured with silk thread to record facial reference lines(Line-1 and Line2) on preoperative, 1stPost-operative day (POD1), 3rd Post-operative day (POD3) and 7th post-operative day (POD7).

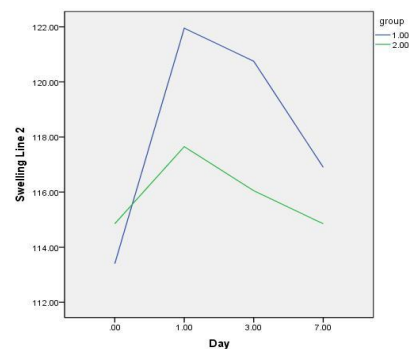
Swelling in experimental group(PRF group) was significantly less as compared to control group(non-PRF group).

Meanwise comparison of swelling on postoperative day 1, day 3 and day 7 between control and experimental group Line 1



(Graph- 2)

Meanwise comparison of swelling on postoperative day 1, day 3 and day 7 between control and experimental group Line 2



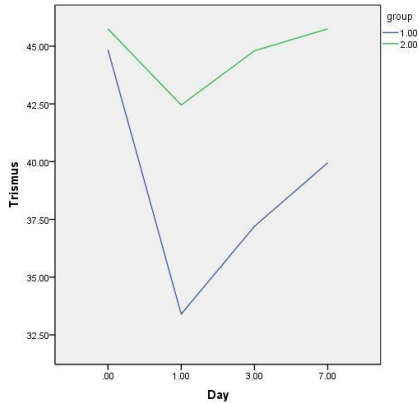
(Graph- 3)

TRISMUS (graph 4)

Trismus was assessed with vernier calliper measuring interincisal distance on preoperative, 1st Post-operative day (POD1), 3rd(POD3) and 7th(POD7).The preoperative measurements were taken as base line value.

The results show statistical significant results on post-operative day 1 and post-operative day 7.

Meanwise comparison of trismus on postoperative day 1, day 3 and day 7 between control and experimental group

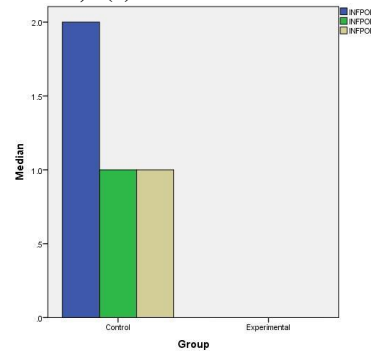


(GRAPH-4)

POST OPERATIVE INFECTION: (graph 5)

Post-operative infection was assessed on a grading scale of 0-5, given by Johnson JT, Myers en Thearle ,et al,1984 1st Post-operative day (POD1), 3rd (POD3) and 7th (POD7). Statistically significant results were seen on post-operative day 1 and post-operative day 7.

Median comparison of post-operative infection on postoperative day 1, day 3 and day 7 between control and experimental group



(GRAPH 5)

OSSEOUS HEALING (graph 6)

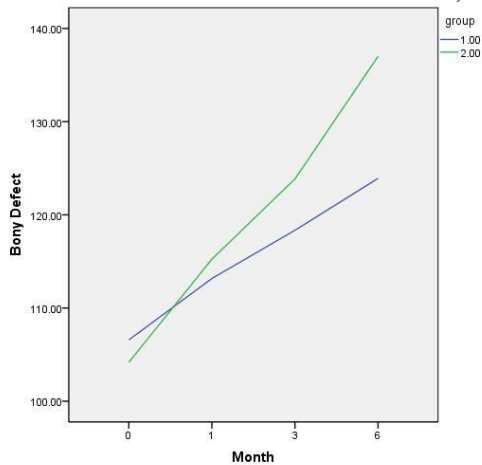
Osseous healing was assessed by taking orthopantomogram (OPG) immediate post-operatively, at 1 month, 3months and 6 months interval.

Index of relative bone healing was assessed by measuring the relative bone density of the pixels of bone defect and the surrounding bone.

It was measured with the help of computer software Adobe photoshop v.8 (on gray scale of 255 tonalities) using gray level histogram at immediate post-operatively,1 month, 3months and 6 months interval.

The results show statistical significant results of index of bone healing at intervals of 1 month, 3months and 6months.

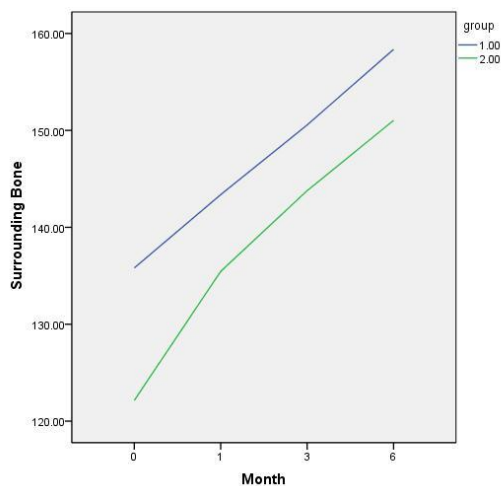
Meanwise comparison of bone density of the defect immediate – postoperatively, 1 month ,3 months and 6 months interval between control and experimental group.



GRAPH-6)

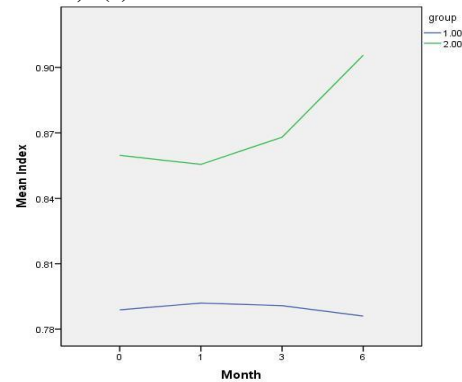
Bone density of the surrounding bone:
(graph 7)

Meanwise comparision of bone density of the surrounding bone immediate – postoperatively, 1 month ,3 months and 6 months interval between control and experimental group.



(GRAPH-7)

Meanwise comparision (graph 8) of index of relative bone density immediate-postoperatively, 1 month ,3 months and 6 months interval between control and experimental group.



(GRAPH-8)

DISCUSSION:

The development of bone graft material to replace bone still is a formidable challenge in modern dentistry. The search for an ideal bone substitutes has been widely pursued for more than 20 years. Various other bone grafts either autogenous, alloplastic or xenogenic have been used for replacing the lost bone. Various tissue engineering techniques have been recently introduced.

One such latest recent innovation in dentistry is the preparation and use of platelet concentrates like Platelet Rich Plasma (PRP) and Platelet Rich Fibrin (PRF), which is a concentrate of growth factors found in platelets. Platelets contain important growth factors, when secreted, are responsible for increasing cell mitosis, increased collagen production, recruitment of other cells to site of injury, initiating vascular in –growth and inducing cell differentiation. Using the concept that lot of platelets may be better, increasing the concentration of platelets at the wound site may enhance wound healing. Surgical sites enhanced with PRF have shown to heal two to three times faster than that of normal

surgical sites. Thus PRF and PRP can be used as a great adjunct to various surgical procedures.^[47]

Various studies have verified that platelet concentrates when combined with autogenous bone results in considerably faster radiographic maturation and histomorphologically denser bone regeneration.^[48,49]

The use of platelet concentrates has shown to be a valid technique for promoting bone regeneration following extraction of impacted third molar. PRF also offers improved quality and faster healing of both hard and soft tissues. It enhances cell adhesion and also induces osteoconduction because of its significant fibrin component. It is prepared in 10 minutes using a small amount of patient's own blood and a cell separator to sequester and concentrate the platelets.

Choukroun et al. in 2006 published a series of articles suggesting the properties and technique to prepare PRF. This technique is simple and free of any coagulants and anticoagulants. It is nothing other than patient's own centrifuged blood. Techniques include collection of blood sample in a glass coated plastic vacutainer and centrifuge it at 2700rpm for 10 minutes.

This study was aimed to evaluate the role of PRF in healing of the wound compared to the natural healing that takes place without using PRF in third molar sockets.

The study included a total number of 40 cases with impacted mandibular third molar

between 20 to 40 years of age selected randomly for the study. This study divided the subjects into 2 groups: GROUP 1 (Control group) and GROUP 2 (Experimental group). In this study PRF was prepared from 10ml of blood by dividing it into two 5ml tubes and centrifuging it at 3000rpm for 10 minutes. We found that PRF formed a gelatinous mass and could be inserted in the socket easily. It could also be squeezed to make membranes if required.^[5]

In this study, there was a statistical difference between the pain values in Group 1 and Group 2 with pain values being less in Group 2. Similar results have been demonstrated by Choukroun et al. in their study of clinical effects of PRF on tissue healing where they observed rapid healing of extraction sockets without pain, dryness or purulent complications by using PRF in extraction sockets.^[14]

The study showed soft tissue being comparatively better in Group 2 (PRF group). The swelling as measured by Line 1 and Line 2 was significantly less in Group 2 as compared to Group 1. The result found can be attributed to the fact that PRF results in amplification of growth factors. Similar results have been reported by Marco Mozzati et al.^[31] They reported significantly higher concentrations of inflammatory parameters such as IL-1B and IL-6 at the PRF sites. Also IL-10 and TGF- β 2 possessing anti-inflammatory property and inducing fibroblast proliferation were reported in significant concentrations.

Mouth opening was evaluated with a vernier calliper by measuring interincisal mouth

opening. There was statistically significant difference on post-operative day 1, 3 and 7 with the p value of .000, .000 and .000 respectively. Results showed early return of normal mouth opening postoperatively in Group 2 (PRF group) compared to Group 1 (non-PRF group). The result was consistent with the findings from C. Ratiu, Diana Osvat, V. Miclaus, C. Ober who reported decreased incidence of pain, trismus, inflammation with the use of PRGF after surgical extraction of impacted mandibular third molars.^[45]

Post-operative infection was graded on scale of 0-5 given by Johnson and Myers et al there was significant statistical difference on post-operative day 1, day 3 and day 7 between Group 2 and Group 1 with infection being significantly reduced in Group 2. Marco Del Corso et al reported decreased infection of the operative site using PRF stating that the fibrin matrix promotes an effective neoangiogenesis, necessary for both wound healing and tissue remodeling and also immune drainage as the leucocytes can have a quick access to the surgical site protecting from it from infections.^[33]

In the study the indices of relative bone healing were calculated as the quotients of bone density of pixels in the area of bony defect and the area of the surrounding bone using orthopantomograph (OPG), measured with the help of computer software Adobe photoshop v.8 (on gray scale of 255 tonalities) using gray level histogram.^[50] Statistically significant results of index of bone healing at intervals of 1 month, 3 months and 6 months in Group 2 as compared to Group 1. C. Ratiu, Diana Osvat,

V. Miclaus, C. Ober reported accelerated healing of the osseous tissues by use of PRGF in extraction sockets in their study.^[45]

Sujeet Khiste and Ritam Naik Tari in their study concluded that bone morphogenic proteins enmeshed in the fibrin matrix have the ability to be released consistently highlighting the angiogenic, hemostatic and osteoconductive properties of PRF. BMPs enmeshed in fibrin clot are progressively released and are able to induce bone formation.^[1]

Marco Del Corso et al in their study on literature regarding PRF stated that both PRF and PRP increase bone regeneration when inserted in an extraction socket.^[33]

CONCLUSION:

The present study was an attempt to evaluate the clinical effect autologous of Platelet-Rich Fibrin (PRF) on post-operative wound healing compared to the natural healing that takes place without using PRF in third molar sockets.

The study comprised of 40 patients in the age group of 20-40 years, with impacted mandibular third molar were selected for the study. These patients were randomly divided into 2 groups:

- 1) Group 1 (Control Group): In this group, the extraction socket was left to heal naturally after primary closure.
- 2) Group 2 (Experimental Group): In this group, the extraction socket was filled with autologous Platelet-

Rich Fibrin gel and primary closure was done.

- The results of our study show statistical significance for pain reduction in the experimental group (PRF group) as compared to the control group (non-PRF group) on 1st and 7th post-operative day.
- Patients in the experimental group reported less swelling as compared to the control group.
- Patients in the experimental group demonstrated early post-operative function and recovery which were attributed to minimal post-operative trismus as compared to the control group.
- The results of the study showed statistical difference for post-operative infection in experimental group as compared to the control group on 1st and 7th post-operative day.
- Statistically significant difference in the relative index of bone density in the extraction socket at 1st, 3rd and 6th month post-operatively in both the groups, with higher index of bone density been observed in the experimental group.

The results of the above mentioned parameters signify better clinical efficacy with the use of autologous Platelet-Rich

Fibrin (PRF) after surgical extraction of impacted mandibular third molars.

We found the following advantages of PRF:

1. No biochemical handling of blood.
2. Simple and cost-effective process.
3. Ease of handling and adaptability.
4. Its adhesiveness and tensile strength for clot stabilization.
5. It contains a dense fibrin network that is highly osteoinductive.
6. Favourable healing due to slow polymerization.
7. More efficient cell migration and proliferation.
8. It has a supportive effect on immune system, without any immune response since it is autologous in nature.
9. It has hemostatic properties.
10. It improves wound healing.

Thus it can be concluded that Platelet Rich fibrin (PRF) enhances soft tissue healing and accelerates bone formation

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