Original Article – Comparative Study

Soft Tissue Healing and Bony Regeneration of Impacted Mandibular Third Molar Extraction Sockets, Following Postoperative Incorporation of Platelet-rich Fibrin

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Abstract

Introduction: Surgical removal of impacted mandibular third molars is one of the most commonly performed dentoalveolar surgeries by dental surgeons around the globe. It is known to be associated with clinically significant postoperative morbidity including swelling, pain, trismus, fever, and infection. In addition, the residual bony defect takes 7 months to 1 year to gradually fill with bone and to reossify. **Aims and Objective:** (1) To carry out a prospective study to evaluate differences in soft tissue healing and bony regeneration of impacted mandibular third molar extraction sites, with and without the incorporation of autologous platelet-rich fibrin (PRF) within the surgical wounds. (2) To also compare the incidence of short- and long-term posttreatment complications in both cases. **Materials and Methods:** Sixty patients were randomly inducted into two groups, consisting of 30 patients each. The first group, which served as the study group, consisted of patients in whom fresh autologous PRF were placed within the extraction site immediately following the surgical removal of the impacted mandibular third molar, before suturing of the mucoperiosteal flap. The second group, which served as the control froup, included those patients in whom the mucoperiosteal flaps were closed without incorporation of PRF within site.Both groups were evaluated and compared for postoperative pain, swelling, trismus, soft tissue healing, as well as bone fill of the extraction socket. **Results:** It was found that the study group in which autologous PRF had been incorporated into the operative site exhibited quick and complication-free soft tissue healing as well as a much quicker reossification and bone fill of the extraction socket, as compared to the control group in which no PRF was used. **Conclusion:** Incorporation of PRF within extraction sockets of impacted third molars proved to be beneficial for patients, yielding a quicker postoperative recovery with fewer complications such as postoperative swelling and edema, pain, and trismus; better overall postoperative results in terms of faster soft tissue healing as well as an earlier bony regeneration.

Keywords: Bone fill, mandibular third molar extraction sockets, platelet-rich fibrin, platelet-rich plasma, reossification

Introduction

Surgical removal of impacted mandibular third molars is one of the most commonly performed dentoalveolar surgeries by oral and maxillofacial surgeons and dental surgeons around the globe. It is known to be associated with clinically significant postoperative morbidity including swelling, pain, trismus, fever, and infection. The surgical removal of the mandibular third molar involves reflection of the mucoperiosteal flap for access, removal of overlying bone, sectioning the tooth (odontectomy), delivery of the tooth, debridement of the socket, and closure of the soft tissue flap. This leads to a significant surgical insult resulting in postoperative inflammatory response ranging from pain and swelling to acute trismus, fever, etc. In some cases, other less frequent complications, e.g., infection, nerve damage, have also been reported. In addition, the residual bony defect takes several months to a year to gradually fill with bone and to reossify.[1]

The importance of growth factors in enhancing wound healing has become the focus of research in the present day. Three

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key elements, namely scaffolds (collagen, bone mineral, etc.), signaling molecules (growth factors), and cells (osteoblasts, fibroblasts) facilitate regeneration of particular tissues during the healing process, particularly in the reossification of bony defects and cavities.[1] Platelet-rich fibrin (PRF) belongs to a new generation of an immune and platelet concentrate collecting on a single fibrin membrane, containing all the constituents of a blood sample favorable to healing and immunity.[1] Though platelet and leukocyte cytokines play an important part in the biology of this biomaterial, the fibrin matrix supporting them certainly constitutes the determining element responsible for the real therapeutic potential of PRF. Four fundamental events of cicatrization, namely, angiogenesis, immune control, circulating stem cells trapping, and wound-covering epithelialization, have been shown to hasten and augment healing, by virtue of development of effective neovascularization, accelerated wound closing with fast cicatricial tissue remodeling, and nearly total absence of infectious events. Platelets isolated from peripheral blood are an autologous source of growth factors delivered in high concentrations to the site of bone defect or a region requiring augmentation.[1,2] Growth factors stored in the α-granules of platelets include platelet-derived growth factor (PDGF), insulin-like growth factor, vascular endothelial growth factor, and transforming growth factor-β (TGF-β). When platelets in a concentrated form are added to graft materials, a more predictable outcome is derived. PRF was first described by Choukroun *et al.*[1] in France as an agent stimulating the chemotactic migration of human mesenchymal stem cells to the injury site.[2,3]

Some of the applications of PRF in dental surgery have been bone grafting for dental implants, onlay and inlay grafts, guided bone/tissue regeneration techniques, sinus lift procedures, ridge augmentation procedures, and closure of cleft, lip, and palate defects. It has proved to be of immense value in the repair of bone defects created by small cysts, bone defects following removal of impacted canines, repair of fistulas between the sinus cavity and mouth, etc. Although a few studies have been conducted on the efficacy of platelet-rich plasma (PRP) on reossification of bone defects created by removal of teeth, a lacuna exists on the use of PRF on soft tissue and bony healing following impacted third molar extractions.

This study aims to compare the rate of soft tissue and bony healing as well as the incidence of short- and long-term postextraction complications, following impacted mandibular third molar extractions, between two subject groups, one with and the other without the incorporation of PRF within the extraction site. Clinical comparison of the postextraction morbidity in terms of pain, swelling, edema, and trismus as well as radiographic comparison of the rate of "bone-fill"/reossification of the bony defect would be carried out to establish the efficacy of autologous PRF in soft tissue healing and regeneration of bone in mandibular third molar extraction sockets.

Aims

To evaluate, compare, and document the clinical differences in soft tissue healing and radiographic differences in "bone fill"/reossification of postsurgical bony defects, following transalveolar extractions of impacted mandibular third molars, with and without incorporation of platelet-rich fibrin within the extraction sockets and to compare the incidence of short-term and long-term posttreatment complications in both cases.

Objectives

i. To compare the postoperative pain using a standard scale
ii. To compare the postoperative swelling using standard measurements
iii. To compare the postoperative trismus at various time intervals
iv. To compare the periodontal health distal to mandibular second molar using periodontal probing at various time intervals
v. To compare the quality of bone healing at the mandibular third molar sockets using IOPA radiographs at various time intervals (at the end of 8 weeks).

Materials and Methods

The study was carried out at our Centre from October 1, 2016, to September 30, 2017. Volunteers were randomly recruited from the patients reporting to the outpatient department who fulfilled the inclusion criteria. A total of 60 patients were included in the study. These were randomly divided into two groups: Group 1 and Group 2, consisting of 30 patients each. Group 1, which served as the study group, consisted of patients in whom fresh autologous PRF was placed within the extraction socket immediately following the surgical removal of the impacted mandibular third molar, before suturing of the mucoperiosteal flap. Group 2, which served as the control Group, included those patients in whom the mucoperiosteal flaps were closed without incorporation of PRF within the socket.

Inclusion criteria

Skeletally matured adult patients above 18 years of age, with clinically and radiologically (mesioangular, distoangular or horizontally) impacted mandibular third molars teeth, presenting with one or more of the following features:

1. Clinical or radiographic evidence of pathology (pericoronal abscess, widening of follicular space)
2. Recurrent pericoronitis
3. Food impaction
4. Proximal caries of the adjoining molar
5. Cheek biting
6. Chronic or recurrent pain in the region
7. Restriction in mandibular movements.

Exclusion criteria

1. Age below 18 years
2. Patients with very poor oral hygiene and/or generalized chronic destructive periodontitis

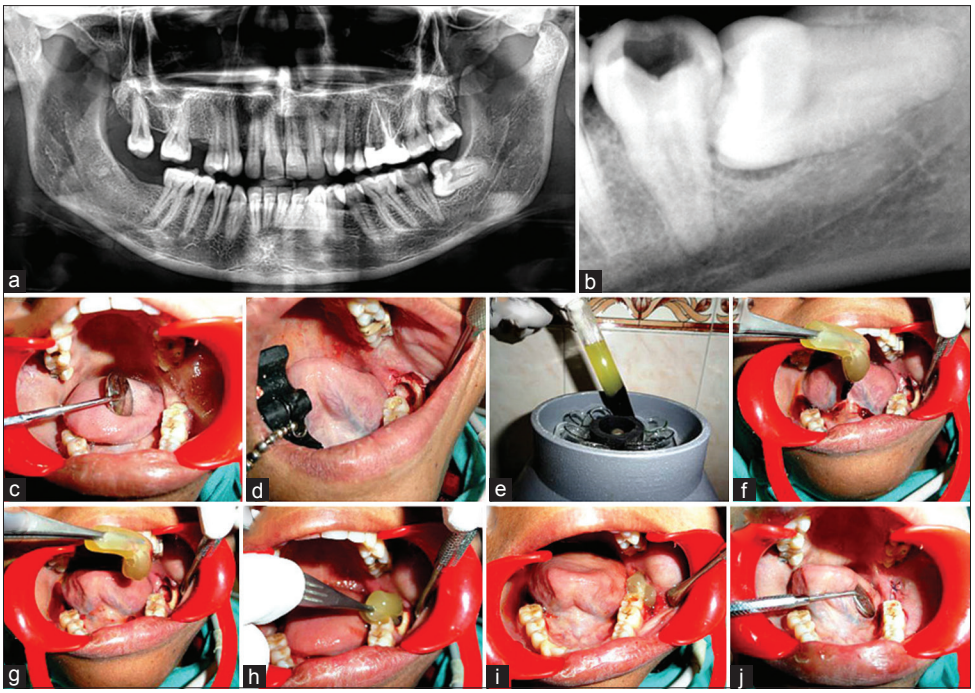


Figure 1: (a and b) Horizontally impacted 38 (c and d) transalveolar extraction under local anesthesia. (e) Autologous platelet-rich fibrin prepared using a tabletop centrifuge. (f-j) Red blood cell layer at the bottom of the centrifugation strata removed and the platelet-rich fibrin segment placed into the extraction socket, followed by flap closure

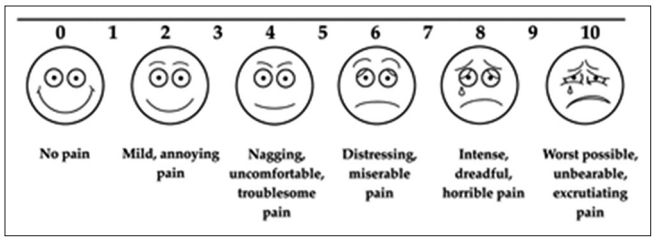


Figure 2: Ten Point Visual Analogue Scale for evaluation of post-surgical pain

3. Medically compromised patients systemically contraindicated for surgery.

Preoperative assessment

All the study subjects underwent a thorough clinical and radiological examination which included:

1. Maximum mouth opening with Vernier's caliper (inter-incisal distance)
2. Periodontal health by measuring pocket depth distal to mandibular second molar using William's periodontal probe
3. Extraoral and intraoral photographs
4. Orthopantomogram
5. Intraoral periapical radiograph (IOPA) of the impacted mandibular third molar
6. Routine hematological investigation.

Intraoperative procedure

Transalveolar surgical extraction of the impacted third molar [Figure 1a-c] was carried out in all the 60 patients [Figure 1c and d], followed by establishing adequate hemostasis at the surgical site. In patients from Group 1, autologous fresh PRF prepared using a tabletop centrifuge [Figure 1e and f] was incorporated into the extraction socket just before closure of the mucoperiosteal flap [Figure 1f-j]. In patients from Group 2, no adjunctive material/procedure was used before suturing the mucoperiosteal flap.

The PRF membranes were prepared as described by Choukroun *et al*.[1] The required quantity of blood (20 ml) was drawn into two 10 ml test tubes without an anticoagulant and centrifuged immediately. Blood was centrifuged using a tabletop centrifuge for 12 min at 2700 rpm or for 10 min at 30,000 rpm**.** The following three biological phases or layers form in the resultant product [Figure 1e].

1. The lowermost layer consisted of coagulated red blood corpuscles layer at the bottom of the centrifugation tube,
2. The intermediate/middle layer comprised the rigid and elastic PRF clot/gel
3. The topmost layer was the supernatant serum consisting of acellular platelet-poor plasma.

The PRF clots were retrieved from the tubes and incorporated into the extraction sites, while the red blood cell gel detached and discarded.

Ibuprofen 400 mg + paracetamol 325 mg and amoxicillin 500 mg TID for 5 days were prescribed for all patients.

Results

Postoperative clinical and radiographic assessment

Postoperatively, all patients of both the groups were evaluated for incidence and magnitude of complications, such as follows:

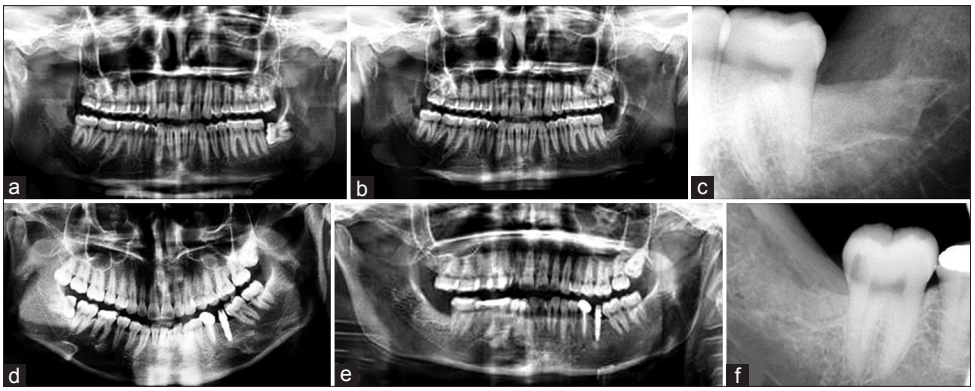


Figure 3: (Group 1) Orthopantomograms of two cases, preextraction (a and d) and 2 months postextraction with platelet-rich fibrin placement (b and e). (c and f) Intraoral periapical radiographs 2 months postextraction with platelet-rich fibrin placement showing dense and healthy bone fill in the extraction sockets

Figure 4: (a and b) (Group 1) Intraoral periapical radiographs of mandibular right third molar region (a) before extraction. (b) Two months postextraction with platelet-rich fibrin placement in extraction socket showing dense and healthy bone fill. (c and d) (Group 2) Intraoral periapical radiographs of mandibular right third molar region (c) before extraction. (d) Two months postextraction without platelet-rich fibrin placement showing incomplete bone fill/reossification of the extraction socket

Figure 5: (Group 2) Intraoral periapical radiographs of mandibular third molar region following routine extractions without platelet-rich fibrin placement in extraction sockets, (a and c) two months postextraction revealing radiolucent appearance of the root sockets. (b and d) Six months postextraction showing beginning of bone fill and trabeculations

1. Pain - 3rd postoperative day
2. Swelling - 3rd postoperative day
3. Trismus - 3rd postoperative day
4. Periodontal health-on 8th week
5. Bone healing - on 8th week.

Clinical assessment

Pain

Degree of postsurgical pain was evaluated using 10-point visual analog scale [Figure 2], with a score of "0" equaling "no pain" and "10" equaling "very severe pain."

Pain was evaluated on the 3rd postoperative day and recorded [Table 1] for both groups of patients.

Swelling

Degree of postoperative cheek swelling and edema was estimated by measuring the distances from the tragus to the soft tissue pogonion and from the tragus to the angle of the mouth. Swelling was evaluated on the 3rd postoperative day in both the groups and recorded as nil, mild, moderate, and severe [Table 1 and Graph 1].

Periodontal health of the second molar

The preoperative measurements of probing depth (PD) on the distobuccal aspect of the second molars were checked with William's periodontal probe with millimeter marking, by measuring pocket depth from free gingival margin to bottom of the pocket. It was then compared with postoperative measurements at 8th week following the extraction and the difference (increase or decrease) was recorded [Table 1 and Graph 2].

The preoperative baseline PD (PD1) and 8 weeks postoperative (PD2) were measured in both groups. They ranged from a mean ± SD of 2.41 ± 0.59 mm to 3.10 ± 0.88 mm, respectively, in Group 1 and from a mean ± SD of 2.41 ± 0.59 mm to 3.90 ± 0.88 mm, respectively, in Group 2.

Radiographic assessment (bone healing)

Periapical intraoral radiographs were obtained of the impacted mandibular third molar tooth sites before surgery, immediately

Table 1: Comparison of postoperative complications between the two groups

Average pain score (3rd postoperative day)		Postoperative swelling (3rd postoperative day)		Postoperative trismus (3rd postoperative day) (yes/no)		Increase in Periodontal Probing Depth (PD) in relation to 2nd molar (at 8 weeks)	
Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
1	4	Nil	Moderate	No	Yes	0.75	1.5
2	3	Nil	Mild	No	Yes	0.74	1.2
1	3	Mild	Moderate	No	Yes	0.76	1.7
1	3	Nil	Mild	No	Yes	0.78	1.6
1	2	Nil	Moderate	No	Yes	0.72	1.6
2	3	Nil	Mild	No	No	0.73	1.4
2	2	Nil	Moderate	No	No	0.74	1.3
2	5	Nil	Moderate	No	Yes	0.71	1.6
3	5	Mild	Mild	No	Yes	0.78	1.8
3	4	Mild	Mild	Yes	Yes	0.72	1.6
1	3	Nil	Mild	No	Yes	0.75	1.5
1	4	Nil	Moderate	No	No	0.78	1.5
1	4	Nil	Nil	No	No	0.73	1.4
0	3	Nil	Moderate	Yes	Yes	0.75	1.7
0	3	Mild	Nil	No	Yes	0.75	1.6
1	3	Nil	Severe	No	Yes	0.73	1.7
2	2	Mild	Mild	No	Yes	0.73	1.7
1	1	Mild	Mild	No	Yes	0.74	1.4
0	5	Nil	Moderate	Yes	Yes	0.69	1.3
2	6	Nil	Nil	Yes	No	0.68	1.5
2	4	Mild	Moderate	No	No	0.75	1.2
1	3	Mild	Moderate	No	Yes	0.74	1.4
1	3	Nil	Nil	No	Yes	0.72	1.3
1	5	Nil	Moderate	No	No	0.76	1.5
0	3	Mild	Moderate	Yes	No	1.1	1.4
2	2	Mild	Severe	Yes	No	1.2	1.4
3	1	Mild	Moderate	No	Yes	1.3	1.6
1	4	Nil	Severe	No	Yes	1	1.5
4	3	Nil	Mild	No	Yes	0.78	1.2
3	3	Nil	Moderate	No	Yes	0.75	1.3

PD=Probing depth

after surgical extraction, and at 2 monthly intervals, till 6 months. The radiographs were obtained with a standardized paralleling technique, using a radiovisiography unit. The bone healing of the third molar socket was assessed using IOPA radiographs using a standard periapical X-ray.

The criteria of bone healing and scoring system were based on modification of method used by Kelly *et al*. Two parameters namely overall density score and trabecular pattern score were assessed.

Overall density score

3 - Marked increase in radiographic density reaching normal limits.
2 - Moderate increase in radiographic density.
1 - Mild increase in radiographic density.
0 - Nil increase in radiographic density.

Trabecular pattern score

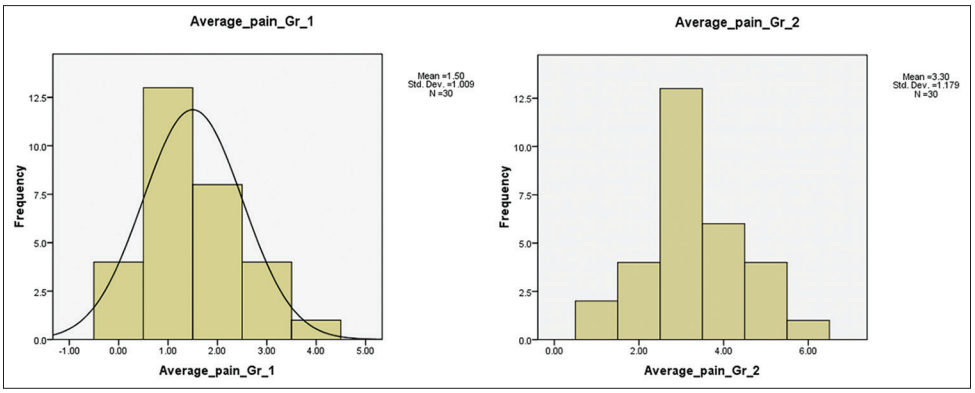
3 - All trabeculae substantially coarse.
2 - Mostly coarse and some fine trabeculae.
1 - Delicate, finely meshed trabeculae.
0 - Granular, nearly homogenous patterns; individual trabeculae essentially absent.

Bone healing in the two groups was evaluated and compared at the end of the 8th week [Table 2 and Graphs 3-5].

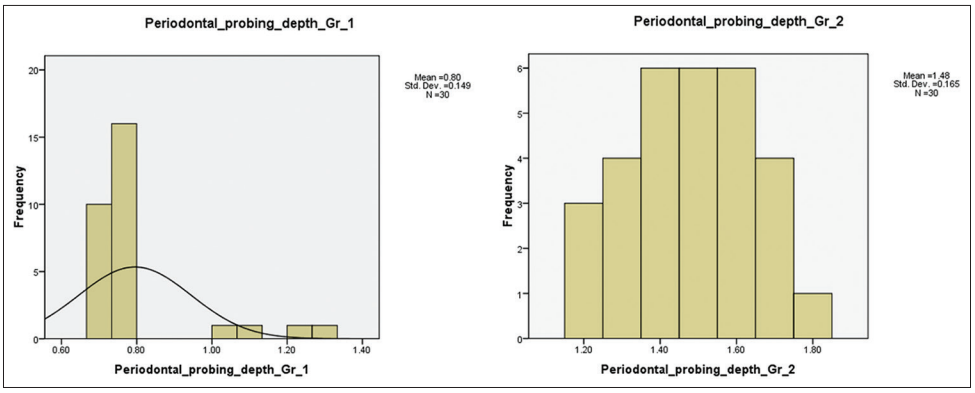
Statistical analysis

All the data was compiled and ANOVA test was used to analyze the same using SPSS software version 16 (Statistical Package for the Social Sciences 2016 for Windows-IBM corporation, NY, USA). At 95% confidence interval, the following test statistics for Group 1 and Group 2, respectively, were observed [Tables 3 and 4].

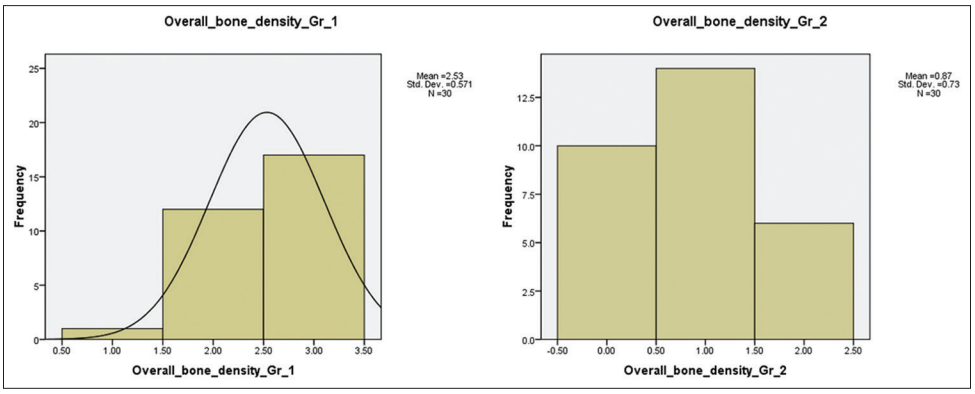
It was observed that incorporation of PRF within extraction sockets of impacted third molars proved to be immensely beneficial in patients in Group 1, resulting in quicker postoperative recovery clinically and reduced morbidity, with



Graph 1: Comparison of postoperative pain between the two Groups at 3 days postsurgery



Graph 2: Comparison of periodontal health of adjacent second molar between the two Groups at 8 weeks postsurgery



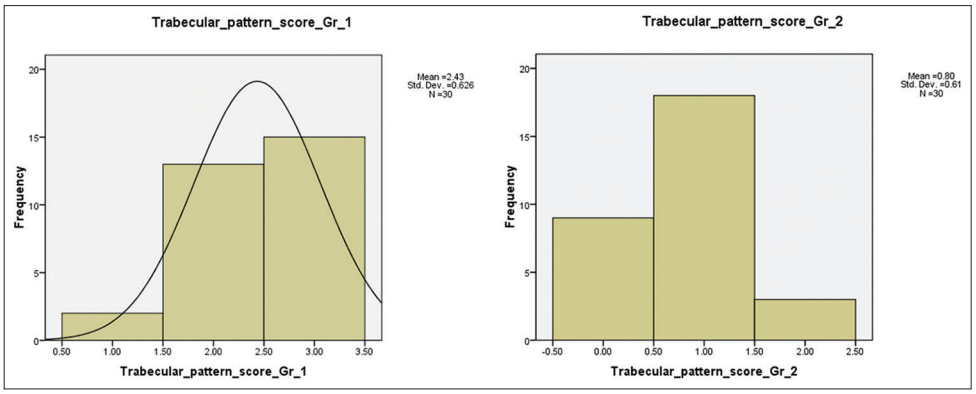
Graph 3: Comparison of overall bone density score of extraction sockets between the two groups at 8 weeks postsurgery

fewer postoperative complications such as pain, swelling, trismus, and periodontal pocket in relation to the adjacent second molar. There was also observed improved and faster bone regeneration, with good quality of bone fill in terms of bone density as well as trabeculations, of the extraction socket radiographically, within 8 weeks [Figures 3 and 4a and b]. On the other hand, patients in Group 2, who underwent surgical extraction without incorporation of PRF, exhibited increased frequency of postoperative complications such as pain, swelling, trismus, and deeper periodontal pockets in relation to the adjacent tooth. Further, the radiological evidence of bone fill of the extraction sockets at the end of 8 weeks was inferior as compared to Group 1 patients, in terms of bone density as well as trabeculations [Figures 4c, d and 5].

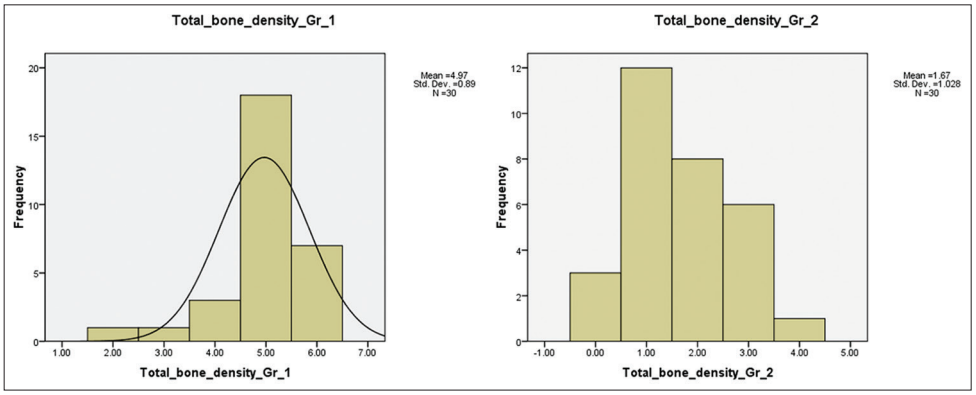
Discussion

Socket healing is a highly coordinated sequence of biochemical, physiologic, cellular, and molecular responses involving numerous cell types, growth factors, hormones, cytokines, and other proteins, which is directed toward restoring tissue integrity and functional capacity after injury.

Reconstruction of bony defects represents a challenging problem for the surgical community. Many defects in facial



Graph 4: Comparison of trabecular pattern score of extraction sockets between the two groups at 8 weeks postsurgery



Graph 5: Comparison of total bone density of extraction sockets between the two groups at 8 weeks postsurgery

skeleton may significantly impair proper prosthetic and functional rehabilitation of the stomatognathic system.[3]

Various glass materials, autografts, alloplastic materials have been tested to enhance socket healing and to minimize the postoperative sequelae after the third molar surgery. Platelet-rich growth factors are also very successful in stimulating bone regeneration and promote healing after the surgical removal of the third molar tooth. PRP is one of the platelet releasing growth factor that has been successfully used to accelerate soft-tissue and hard-tissue healing.

The growth factors present in PRP are well-known including TGF-1 and TGF-2, vascular endothelial growth factor, three isomers of Platelet Derived Growth Factor (PDGF), namely PDGF- AA, PDGF- BB and PDGF-AB, and endothelial growth factor. These growth factors are considered to have the ability to accelerate chemotaxis, mitogenesis, angiogenesis, and synthesis of collagen matrix and favor tissue repair when applied on bone wounds.

PRF is a newer material which is referred to as a second-generation platelet concentrate. It contains all constituents of a blood sample favorable for healing and immunity on a single fibrin membrane.[3]

The existing literature mainly deals with the use of PRP in extraction sockets as well as in other areas with nonplacement in control sites. However, there is limited information available concerning the healing of extraction sockets using PRF.

PRF can be considered as a healing biomaterial, consisting of a fibrin matrix polymerized in a tetramolecular structure, incorporated with platelets, leukocyte, and cytokines and stem cells, featuring all the necessary parameters permitting optimal healing. Despite the fact that cytokines trapped in PRF are gradually released and able to accelerate the cellular phenomenon, the structure of the fibrin network is the key element of all improved PRF healing processes.[1] All of the known clinical applications of PRF highlight an accelerated tissue cicatrization due to the development of effective neovascularization, accelerated wound closing with fast cicatricial tissue remodeling, and nearly total absence of infectious events. Preparation of PRF was first described by Choukroun *et al.* in 2006.[1] Studies have revealed that PRF could be an immune regulation node with inflammation retrocontrol abilities. This concept could explain the reduction of postoperative infections when PRF is used as surgical additive.[3] Studies have also shown that PRF, unlike the other platelet concentrates, would be able to progressively release cytokines during fibrin matrix remodeling; such a mechanism might explain the clinically observed healing properties of PRF.[4]

A few studies have been carried out in dental implantology, examining the possible use of PRF as a grafting material in maxillary sinus augmentation procedures. PRF mixed with freeze-dried bone allograft has been shown to reduce healing time before implant placement.[5,6]

Table 2: Comparison of the bone fill and reossification between the two groups at the end of 8 weeks

Group 1			Group 2		
Overall density score	Trabecular pattern score	Total bone density score	Overall density score	Trabecular pattern score	Total bone density score
3	3	6	0	1	1
3	2	5	1	0	1
3	2	5	2	2	4
3	3	6	1	1	2
2	3	5	1	1	2
2	3	5	1	1	2
2	2	4	0	1	1
3	2	5	0	0	0
1	1	2	0	1	1
2	3	5	0	0	0
2	3	5	1	2	3
3	2	5	1	1	2
3	2	5	1	1	2
2	1	3	2	1	3
3	2	5	1	2	3
2	3	5	0	1	1
2	2	4	0	1	1
3	2	5	1	0	1
3	2	5	1	0	1
3	3	6	2	0	2
3	3	6	2	1	3
2	3	5	1	0	1
3	3	6	2	1	3
2	3	5	2	1	3
3	2	5	1	1	2
2	2	4	0	0	0
3		5	0	1	1
3	3	6	0	1	1
3	3	6	1	1	2
2	3	5	1	0	1

Table 3: Test statistics for Group 1

	Average pain in Group 1	Periodontal PD in Group 1	Overall bone density in Group 1	Trabecular pattern score in Group 1	Total bone density in Group 1
χ^2	14.333[a]	15.067[b]	13.400[c]	9.800[c]	34.000[a]
df	4	12	2	2	4
Asymptotic significant	0.006	0.238	0.001	0.007	0.000
Monte Carlo significant					
Significant	0.009[d]	0.255[d]	0.001[d]	0.010[d]	0.000[d]
95% CI					
Lower bound	0.007	0.246	0.000	0.008	0.000
Upper bound	0.011	0.263	0.001	0.012	0.000

PD=Probing depth; CI=Confidence interval

From a clinical standpoint, although this biomaterial appears to accelerate physiologic healing, the numerous perspectives of PRF have still to be clinically tested.[7] This initial research evaluating the effects of PRF on the soft and hard tissue healing of postextraction sockets of impacted third molars, both impartially and rigorously, will make it possible to plan several future PRF applications, particularly in the field of oral and maxillofacial surgery. An interesting study was carried out to evaluate the effect of PRF on the early bone healing process with bone scintigraphy based on technetium-99 m methylene diphosphonate uptake in the third molar extraction sockets.[8] It was demonstrated that PRF exhibits the potential characteristics of an autologous fibrin matrix,[9,10] with abundant fibrin and inflammatory cells. However, the study suggested that further investigation was required to determine whether the presence of crystal-like

Table 4: Test statistics for Group 2

	Average pain in Group 2	Periodontal PD in Group 2	Overall bone density in Group 2	Trabecular pattern score in Group 2	Total bone density in Group 2
χ^2	18.400[a]	5.000[b]	3.200[c]	11.400[c]	12.333[d]
df	5	6	2	2	4
Asymptotic significant	0.002	0.544	0.202	0.003	0.015
Monte Carlo significant					
Significant	0.004[e]	0.569[e]	0.211[e]	0.003[e]	0.014[e]
95% CI					
Lower bound	0.003	0.559	0.203	0.002	0.011
Upper bound	0.005	0.579	0.219	0.004	0.016

PD=Probing depth; CI=Confidence interval

particles on the outer surface of PRF actually alters bone and soft tissue healing.

The present study demonstrates the benefits of intraoperative incorporation of Autologous PRF in impacted third molar extraction sockets, resulting in clinically as well as radiographically appreciable postoperative results, in terms of soft as well as hard tissue recovery, healing, and regeneration.

Conclusion

In view of the fact that the use of PRF placed in the extraction socket resulted in reduced morbidity postoperatively, and improved bone healing radiologically, it is recommended that a larger sample may be studied in conjunction with a detailed and more objective radiological parameters and protocols to verify the findings of this study and possibly incorporate the use of PRF as part of standard extraction procedure especially for difficult extractions or for extraction of those teeth to be replaced using dental implants.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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