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Original Article

Dentistry Section

Effect of Platelet-rich Fibrin on Soft-tissue and Hard-tissue Healing following Surgical Extraction of Mandibular Third Molar: A Prospective Interventional Study

SUDIP CHAKRABORTY[1], CHAYAN KUNDU[2], SAMIRAN GHOSH[3], RICHI BURMAN[4], AVISHEK DE SARKAR[5], GOURAB MANDAL[6], SOMAK SAHA[7]

ABSTRACT

Introduction: Surgical removal of mandibular third molars is one of the most frequent clinical tasks conducted by dental surgeons. This surgery is associated with the possibility of delayed and complicated soft-tissue and hard-tissue healing. Local incorporation of bioactive materials (such as growth factors and blood products) has been attempted to promote faster and better healing. Platelet-rich Fibrin (PRF) is the latest development among blood-derived products and is widely used to enhance hard and soft-tissue healing.

Aim: To estimate the effect of PRF on soft-tissue and hard-tissue healing following the surgical extraction of mandibular third molars.

Materials and Methods: This prospective interventional study was carried out in the Department of Oral and Maxillofacial Surgery at Guru Nanak Institute of Dental Science and Research, Kolkata, West Bengal, India. The study was conducted between May 2022 and January 2024. Patients who required extraction of impacted mandibular third molars were divided into two groups (group I and group II) by alternate selection methods. PRF was placed in the empty sockets of group I patients following the surgical extraction of the third molar, while the sockets of group II patients were allowed to heal without PRF. Soft-tissue healing evaluation was performed using the parameters of the healing index on the 3rd, 7th, 14th, and 28th post-extraction days by two blind observers. Hard-tissue healing was evaluated using the Cone Beam Computed Tomography (CBCT) findings on the 1st month and 3rd month postoperatively. Changes in empty socket volume, Bone Density Units (BDU) of the new bone, and the type of new bone were analysed. Statistical analysis was carried out using IBM Statistical Package for Social Sciences (SPSS) Statistics for Windows, version 26.0 (Armonk, NY: IBM Corp). A p-value of <0.05 was considered significant.

Results: Twenty-six patients were included in the final analysis. They were equally divided into the interventional group (group I, with PRF) and the control group (group II, without PRF). The demographic data and difficulty index of group I were similar to those of group II. The corrected Chi-square test of independence was performed to compare soft-tissue healing. A highly significant association was found on the 3rd postoperative day (p-value=0.03), 14th postoperative day (p-value=0.013), and 28th postoperative day (p-value=0.002), indicating that group I consistently demonstrated improved healing compared to group II. The volume of the empty socket of the extracted third molar was measured using CBCT on the 1st and 3rd postoperative months. In both groups, the volume reduced significantly, but the reduction was more pronounced in group I than in group II (p-value <0.0001). The quality of bone formation (measured by BDUs) was significantly better in group I than in group II (p-value=0.043). However, the type of bone formation was similar for both groups.

Conclusion: PRF appears to be beneficial and effective in promoting postoperative soft-tissue and hard-tissue healing following the surgical extraction of mandibular third molars.

Keywords: Bone density unit, Bone formation, Growth factor, Healing index

INTRODUCTION

Third molars are the last teeth to erupt in the oral cavity [1]. The mandibular third molars, whether they have erupted or are impacted, should be removed if they are causing symptoms, are diseased, or are anticipated to cause issues under dentures [2]. Dental surgeons frequently perform surgery to extract mandibular third molars. A research study found that almost 90% of patients awaiting surgery in oral and maxillofacial surgery hospitals were waiting for third molar surgery [3]. This surgery carries the risk of various postoperative complications such as pain, trismus, infection, alveolar osteitis, and the possibility of delayed and complicated soft-tissue and hard-tissue healing [4]. To reduce these complications, various strategies are employed. The pre-, per-, and postoperative use of analgesics, antibiotics, corticosteroids, mouthwashes, topical gels, cryotherapy, and ozone therapy are some of these [5].

Platelet-rich Fibrin (PRF) is the latest development in blood-derived products and is widely used to enhance healing [6,7]. PRF is obtained from plasma by spinning autologous peripheral blood in a centrifuge, which triggers the coagulation process and activates platelets. PRF was pioneered in 2000 by Choukroun J et al., [8]. It offers numerous clinical advantages by naturally creating a fibrin scaffold that aids in clot formation, acts as a framework for tissue regeneration, and preserves growth factors and stem cells [9]. Dentistry has extensively used PRF for several years, particularly in procedures such as implant dentistry and alveolar surgery. Reports suggest that the use of PRF in the socket after extraction accelerates tissue healing following third molar surgery [10]. However, evidence remains limited, and the results are still controversial [11-13].

The present study aimed to assess the effect of PRF on soft-tissue and hard-tissue healing following mandibular third molar surgery.

MATERIALS AND METHODS

This prospective interventional study was carried out in the Department of Oral and Maxillofacial Surgery at Guru Nanak

Institute of Dental Science and Research, Kolkata, West Bengal, India. The study was conducted after approval from the Institutional Ethics Committee (IEC) of Guru Nanak Institute of Dental Science and Research between May 2022 and January 2024 (ref. no. GNIDSR/IEC/21-24/09). The ethical principles of the World Medical Assosciation (WMA) Declaration of Helsinki were followed. Following the inclusion and exclusion criteria, patients were selected from those requiring extraction of impacted mandibular third molars.

Sample size calculation: The sample size for this study was calculated using G*Power software. For a one-tailed independent samples t-test, with a statistical power of 0.8, an alpha of 0.05, and Cohen's effect size of 0.9, the calculated sample size was 16 for each group.

Inclusion criteria: Patients included in the study were those aged 18 to 30 years, willing to follow all study procedures, with an impacted tooth free of any pathology or active infection, and a Pederson's difficulty index of the concerned third molar between 3 and 6 [14].

Exclusion criteria: Patients undergoing chemotherapy or radiotherapy, taking any medications that could interfere with healing (e.g., bisphosphonates, steroids), or having systemic diseases were excluded from the study.

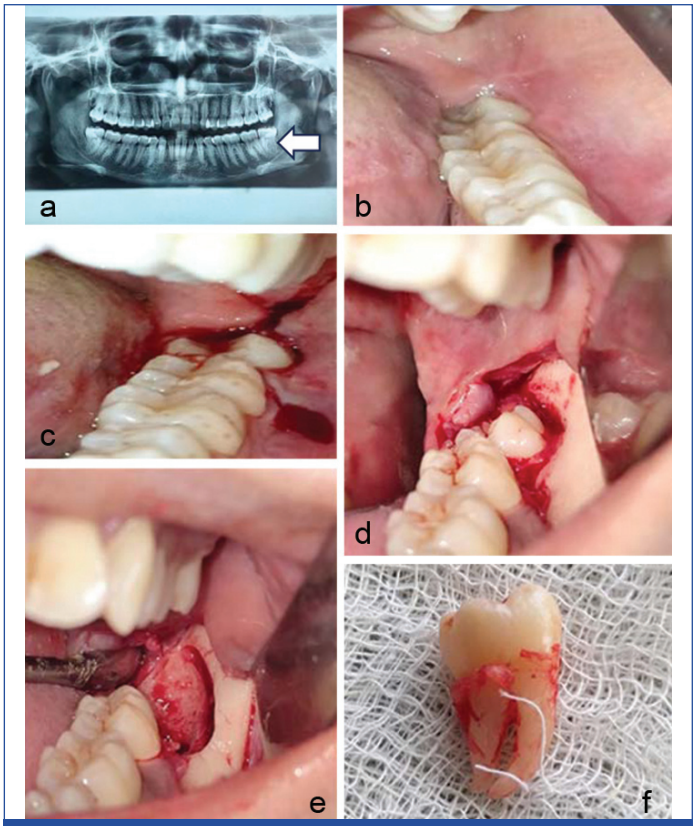
Participants read and signed an informed consent form. Initially, 38 patients gave their consent, but 12 patients (6 from each group) did not attend for regular follow-up. Therefore, a total of 26 patients were included in the study, with each group comprising 13 patients.

Participants were allocated into two groups by alternate selection method:

- **Group I** (interventional group, n=13): PRF was placed in the extraction socket after the surgical removal of impacted mandibular third molars.
- **Group II** (control group, n=13): PRF was not placed in the extraction socket after the surgical removal of impacted mandibular third molars.

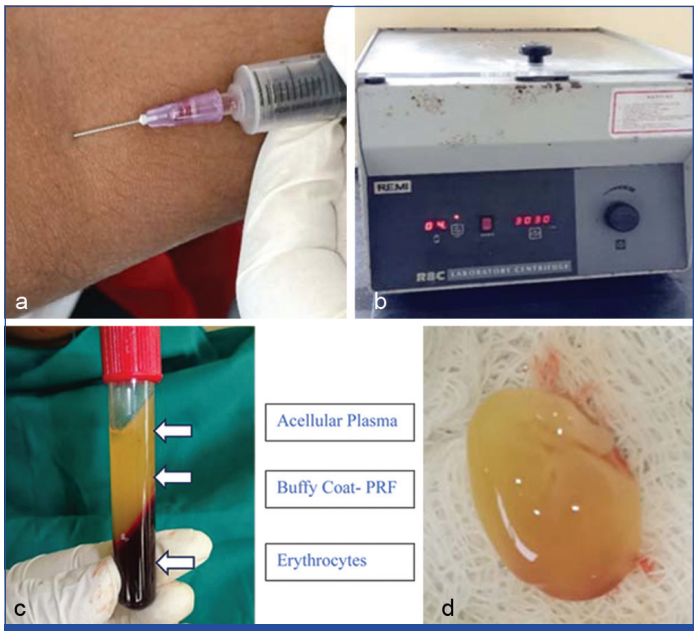
Study Procedure

All extractions were performed by the same surgeon, following the standard surgical protocol. For the extraction of group I patients, a Ward's incision was made to raise the soft-tissue flap, followed by bone removal using a rotary cutting instrument and tooth extraction using elevators [Table/Fig-1a-f].



[Table/Fig-1]: Treatment protocol for group I patients: a) Orthopantomogram (OPG) X-ray showing impacted 38; b) Clinical view of impacted 38; c) Ward's incision for exposure; d) Flap elevation and removal of bone; e) Empty socket after tooth extraction; f) Extracted tooth.

Under aseptic techniques, 10 mL of blood was drawn intravenously from the median cubital vein of group I patients' forearms. This was transferred to centrifugal vials for the preparation of PRF. The blood sample was taken in a tube without anticoagulant and was immediately centrifuged at 2700 rpm for 12 minutes [15]. A yellow-coloured fibrin clot, containing platelets, formed in the middle of the tube, between the red blood cell layer at the bottom and the acellular plasma at the top. This was removed from the tube using sterilised tweezers, and the attached red blood cells were scraped off and discarded [Table/Fig-2a-d].



[Table/Fig-2]: PRF preparation: a) Aspiration of blood; b) Aspirated blood taken in a test-tube and placed in the centrifuge machine; c) Yellow-coloured PRF prepared after centrifugation of blood; d) PRF.

This yellow-coloured PRF was placed in the empty sockets of group I patients after the extraction of third molars, and closure was completed using interrupted sutures made from 3-0 Mersilk [Table/Fig-3a-c]. For the extraction of group II patients, a Ward's incision was made to raise the soft-tissue flap. Bone removal was performed using a rotary cutting instrument, and the tooth was removed using elevators. Closure was completed with interrupted sutures made from 3-0 Mersilk, without placing PRF inside the socket [Table/Fig-4a-g].

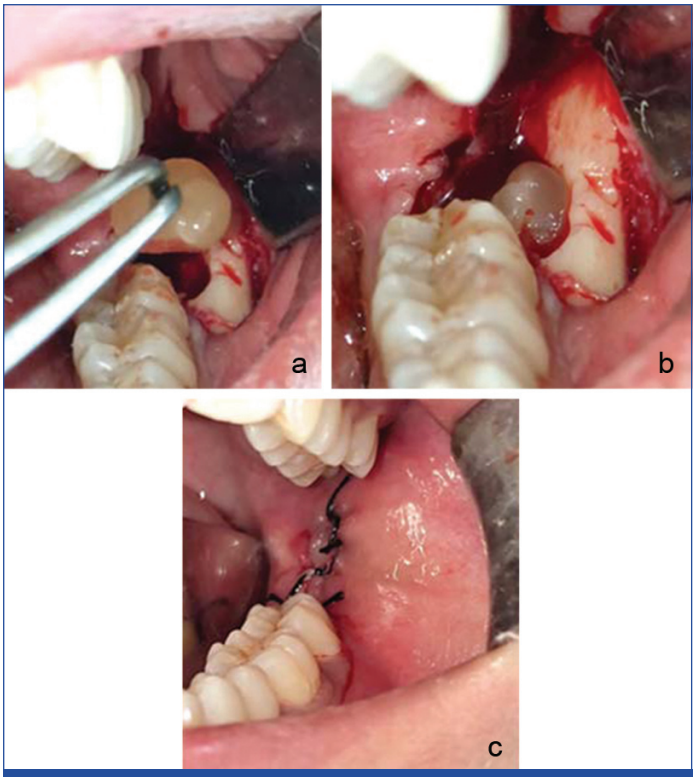
A standard conventional drug regimen and postoperative instructions were prescribed for both groups. The drug regimen consisted of:

Drug regimen was as followed:

- Capsule Amoxicillin (500 mg) eight-hourly for seven days;
- Tablet Metronidazole (400 mg) eight-hourly for seven days;
- Tablet Paracetamol (1000 mg) eight-hourly for seven days;
- Tablet Pantoprazole (40 mg) once every 24 hours before breakfast for seven days.

The difficulty level of each extraction was recorded by the surgeon according to the modified Parant scale [16].

Soft-tissue healing evaluation: This was assessed clinically by two blinded observers on the 3rd, 7th, 14th, and 28th days postextraction, using the healing index proposed by Landry R et al., [17]. This index is based on tissue colour, the presence of bleeding on palpation, epithelialisation of wound margins, presence of granulation tissue, and suppuration. It grades the wound on a



[Table/Fig-3]: Placement of PRF in group I patients: a) Placement of PRF; b) PRF placed in the socket; c) Closure by interrupted suture.

scale of 1 to 5, where 1 indicates very poor healing and 5 indicates excellent healing. Frequency distribution of group I and group II patients was recorded based on this evaluation across each follow-up period [Table/Fig-5a-d].

Hard-tissue healing assessment: CBCT images were used to evaluate bone healing based on socket volume, bone density, and type of bone formation. Tomographic data were collected one month and three months after surgery. Volumetric analysis was performed using semi-automatic segmentation of the area of interest with the help of Sidexis-4 software (Dentsply, Sirona) in a Dicom viewer (Philips) [18]. The volume of the socket of the extracted third molar at the 1st postoperative month was analysed and reduced at the 3rd postoperative month due to new bone formation. The mean values of this volume reduction for group I (V1) and group II (V2) were compared statistically [Table/Fig-6a-d].

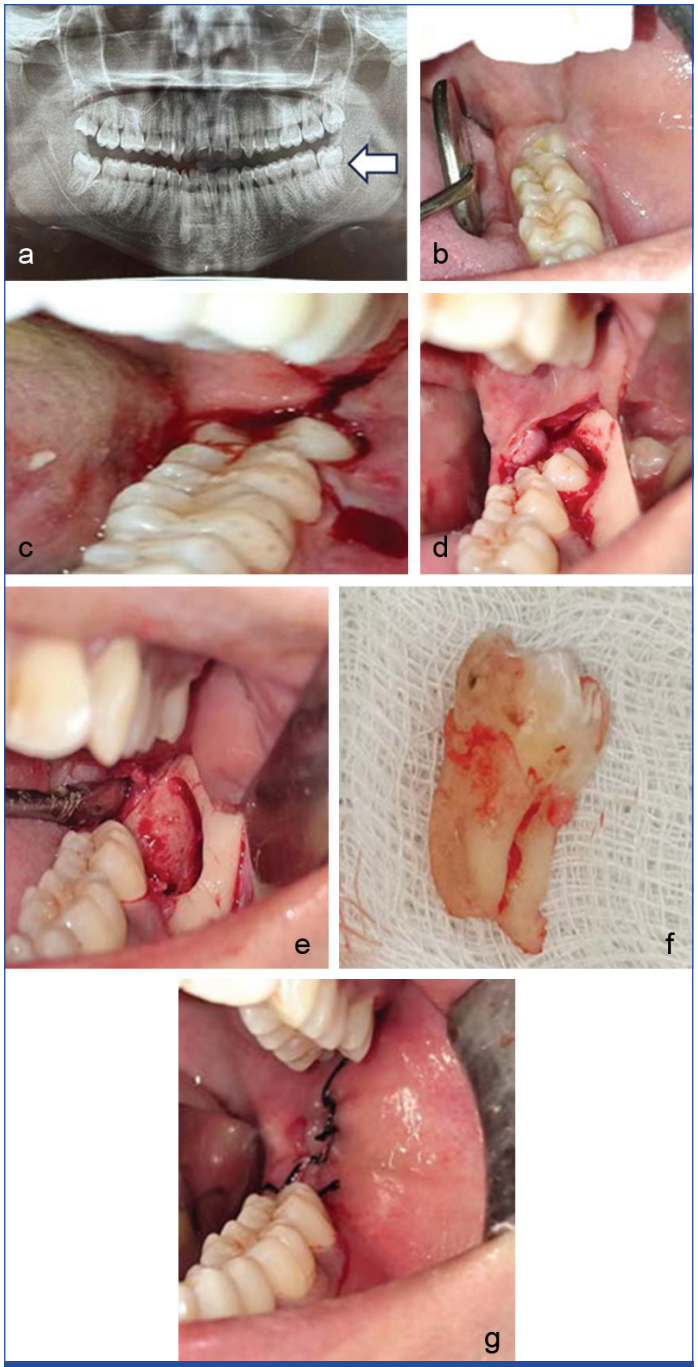
The same software and viewer were used to record the density of the newly formed bone. The software correlates the values of CBCT voxels with bone mineral content and displays the result in Bone Density Units (BDU). The BDU of the socket of the extracted third molar at the 1st postoperative month increased by the 3rd postoperative month due to new bone formation. The mean value of this difference in BDU for group I (BDU1) and group II (BDU2) was compared statistically.

The type of newly formed bone was classified according to Lekholm U and Zarb GA (1985) and Misch CE (1989) [19,20]. Lekholm U and Zarb GA classified bone into four types based on the thickness of the cortical bone and density of the trabecular bone, where type 1 is the best quality bone and type 4 is the worst quality bone. Misch E classified bone into five types based on the radiological density of the bone, where type 1 (D1) is the best quality bone and type 5 (D5) is the worst quality immature bone.

All measurements were performed by two radiologists who were blind to the group divisions.

STATISTICAL ANALYSIS

The collected data were tabulated in a spreadsheet using Microsoft Excel 2019, and statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp). A

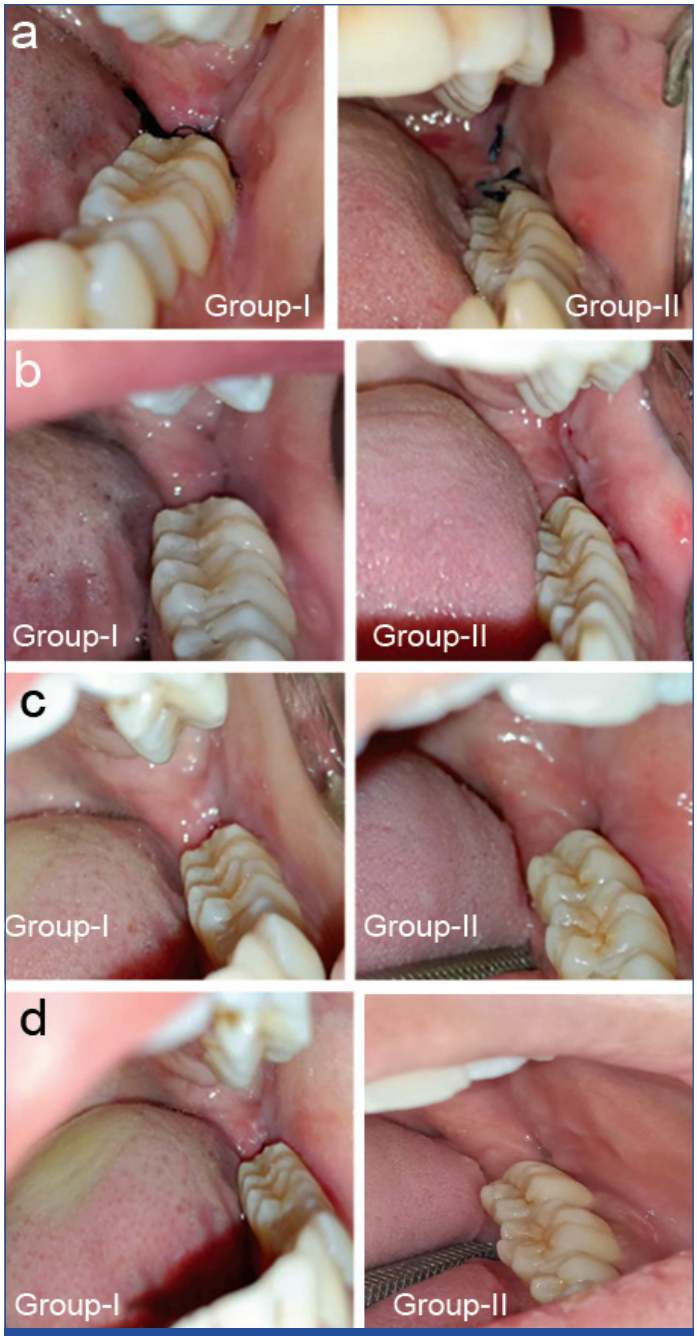


[Table/Fig-4]: Treatment protocol for group II patients: a) OPG X-ray showing impacted 38; b) Clinical view of impacted 38; c) Ward's incision for exposure; d) Flap elevation and removal of bone; e) Empty socket after tooth extraction; f) Extracted tooth; g) Closure by interrupted suture.

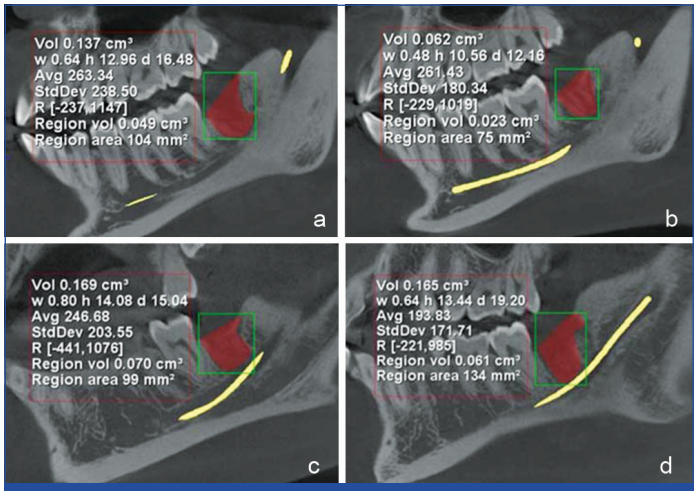
Chi-square test was conducted to evaluate the categorical variables. Inter-group comparisons for the improvement in the outcome parameters were performed using the Independent samples t-test. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 26 patients were equally divided into interventional (group I) and control (group II) groups. The demographic data (age, gender) of the groups were similar. The mean age of group I patients was 25.38±3.05 years, while that of group II patients was 25.38±2.43 years. In group I, 69.23% were male and 30.76% were female patients. In group II, 53.84% were male and 46.15% were female patients. The Pederson's difficulty index and the modified Parant scale (preoperative and postoperative assessment of the difficulty level of the extraction) of the test group did not significantly differ from the interventional group. The mean value of Pederson's difficulty index for group I was 4.69±0.91, while that of group II was 4.30±0.72. The mean value of the modified Parant scale was 3.07±0.72 for group I and 3.23±0.69 for group II [Table/Fig-7].



[Table/Fig-5]: Images of soft-tissue healing in relation to extracted lower 3rd molar in different postoperative days: a) soft-tissue healing on post-op day 3; b) soft-tissue healing on post-op day 7; c) soft-tissue healing on post-op day 14; d) soft-tissue healing on post-op day 28.



[Table/Fig-6]: Comparison of socket volume of group I and group II patients at 1st and 3rd postoperative month: a) Socket volume of group I patient at 1st postoperative month; b) Socket volume of group I patient at 3rd postoperative month; c) Socket volume of group II patient at 1st postoperative month; d) Socket volume of group II patient at 3rd postoperative month.

Group I	Age (mean 25.38±3.05) years	Gender (M 69.23%, F- 30.76%)	Pederson's Difficulty Index [14] (mean 4.69±0.91)	Modified Parant scale [16] (mean-3.07±0.72)
Group II	Age (mean 25.38±2.43) years	Gender (M- 53.84%, F- 46.15%)	Pederson's Difficulty Index [14] (4.30±0.72)	Modified Parant scale [16] (3.23±0.69)
Group I vs Group II	p=0.35 ns Independent sample t-test	p=0.42 ns Chi-square test	p=0.29 ns Independent sample t-test	p=0.85 ns Independent sample t-test

[Table/Fig-7]: Demographic data and difficulty index [14,16].
ns: Not significant (p>0.05), *: Statistically significant (p<0.05), **: Highly statistically significant (p<0.01)

Soft-tissue healing assessment: The corrected Chi-square (χ^2) test of independence was performed to compare the frequency distribution of the study subjects across each follow-up period between the study groups, based on soft-tissue healing status. A highly significant association was found on the 3rd postoperative day (p=0.03*), 14th postoperative day (p=0.013*), and 28th postoperative day (p=0.002*), indicating that group I consistently showed improved healing compared to group II. Specifically, group I exhibited significantly better healing outcomes at all follow-up periods, with 46.2% showing "Good" healing on the 3rd postoperative day, 76.9% on the 7th postoperative day, 61.5% showing "Very Good" healing on the 14th postoperative day, and 38.5% achieving "Excellent" healing on the 28th postoperative day. In contrast, group II had higher proportions of "Poor" or "Very Poor" healing, particularly on the 3rd and 7th postoperative days, with 69.2% and 61.5%, respectively [Table/Fig-8].

Follow-up periods	Status	Group I n=13 n (%)	Group II n=13 n (%)	Total N=26 N (%)	p-value
3rd day	Good	6 (46.2%)	1 (7.7%)	7 (26.9%)	0.03*
	Poor	7 (53.8%)	9 (69.2%)	16 (61.5%)	
	Very poor	0	3 (23.1%)	3 (11.5%)	
7th day	Good	10 (76.9%)	4 (30.8%)	14 (53.8%)	0.05 ns
	Poor	3 (23.1%)	8 (61.5%)	11 (42.3%)	
	Very Poor	0	1 (7.7%)	1 (3.8%)	
14th day	Very good	8 (61.5%)	1 (7.7%)	9 (34.6%)	0.013*
	Good	5 (38.5%)	11 (84.6%)	16 (61.5%)	
	Poor	0	1 (7.7%)	1 (3.8%)	
28th day	Excellent	5 (38.5%)	1 (7.7%)	6 (23.1%)	0.002**
	Very good	8 (61.5%)	6 (46.2%)	14 (53.8%)	
	Good	0	6 (46.2%)	6 (23.1%)	

[Table/Fig-8]: Intergroup comparison of the soft-tissue healing by healing index of Landry R.
ns: Not significant (p>0.05), *: Statistically significant (p<0.05), **: Highly statistically significant (p<0.01)

Hard-tissue healing assessment: Socket volume: In group I, the volume at the 3rd postoperative month was less than that of the 1st month, and the difference (V1) was statistically significant (p-value=0.004*). In group II, the volume at the 3rd postoperative month was also less than that of the 1st month, but the difference (V2) was not statistically significant (p-value=0.17) [Table/Fig-9a]. On intergroup comparison (V1 vs V2), it was found that the decrease in the volume of the socket (cm^3) was significantly greater for group I than for group II (p-value <0.0001*) [Table/Fig-9b].

Follow-up periods	Group I (n=13)	Group II (n=13)
1st month	0.15±0.05	0.13±0.04
3rd month	0.08±0.03	0.11±0.04
p-value	0.004**	0.17

[Table/Fig-9a]: Intragroup comparison of the socket volume (cm^3).
Values present as Mean±SD; Paired t-test, ns: Not significant (p>0.05), *: Statistically significant (p<0.05), **: Highly statistically significant (p<0.01)

Group I (n=13)	Group II (n=13)	p-value
0.07±0.03	0.01±0.03	<0.0001**

[Table/Fig-9b]: Intergroup comparison of the decrease of socket volume (cm^3) from 1st to 3rd postoperative month.
Values present as Mean±SD; Independent samples t-test, ns: Not significant (p>0.05), *: statistically significant (p<0.05), **: highly statistically significant (p<0.01)

Bone density: In group I, the BDU at the 3rd month postoperatively was greater than that at the 1st month, and the difference (BDU1) was statistically significant (p=0.0041*). In group II, the BDU at the 3rd month postoperatively was also greater than that at the 1st month, but the difference (BDU2) was not statistically significant (p=0.38) [Table/Fig-10a]. On intergroup comparison (BDU1 vs BDU2), it was found that the increase in BDU was significantly greater for group I than for group II (p=0.043) [Table/Fig-10b].

Follow-up periods	Group I (n=13)	Group II (n=13)
1st month	229.31±97.31	181.38±62.42
3rd month	335.38±71.72	237.54±94.97
p-value	0.0041**	0.38 ns

[Table/Fig-10a]: Intragroup comparison of the Bone Density Units (BDU).
Values present as Mean±SD; Paired samples t-test, ns: Not significant (p>0.05), *: Statistically significant (p<0.05), **: Highly statistically significant (p<0.01)

Group I (n=13)	Group II (n=13)	p-value
106.08±64.04	56.15±55.61	0.043*

[Table/Fig-10b]: Intergroup comparison of the Bone Density Units (BDU) from 1st to 3rd postoperative month.
Values present as Mean±SD; Independent samples t-test ns: Not significant (p>0.05), *: Statistically significant (p<0.05), **: Highly statistically significant (p<0.01)

Type of bone formation: The type of bone formation was comparable for both groups in the 1st and 3rd postoperative months (p>0.05) [Table/Fig-11].

Follow-up periods	Classification followed	Types	Group I n=13 n (%)	Group II n=13 n (%)	p-value
1st month	Lekholm U and Zarb GA	I	1 (7.7%)	3 (23.1%)	0.27 ns
		II	12 (92.3%)	10 (76.9%)	
	Misch CE	I	1 (7.7%)	3 (23.1%)	0.54 ns
		II	11 (84.6%)	9 (69.2%)	
		III	1 (7.7%)	1 (7.7%)	
3rd month	Lekholm U and Zarb GA	I	3 (23.1%)	3 (23.1%)	1 ns
		II	10 (76.9%)	10 (76.9%)	
	Misch CE	I	0	1 (7.7%)	0.36 ns
		II	12 (92.3%)	12 (92.3%)	
		III	1 (7.7%)	0	

[Table/Fig-11]: Comparison of the type of bone formation [19,20].
Chi-square test, ns: Not significant (p>0.05), *: Statistically significant (p<0.05), **: Highly statistically significant (p<0.01)

DISCUSSION

The current study interventional study had two primary outcomes: soft-tissue and hard-tissue healing following the surgical extraction of lower third molars, with and without the placement of PRF in the empty sockets. Inclusion and exclusion criteria were designed to maximise the reliability of the study results by selecting two well-defined, homogeneous groups that allow for accurate and unbiased interpretation of the results. PRF was created by centrifuging autologous blood at 2700 rpm for 12 minutes. Chandra V et al., in a similar study, used the same method [15]. The demographic data of the groups were similar, which allowed us to rule out age and gender-related variables that might influence the study results. All surgeries were performed by the same surgeon, and the difficulty index of the third molars was comparable, resulting in negligible surgical injury-related heterogeneity. Several similar studies had larger sample sizes but involved more than one surgeon [21,22].

For soft-tissue healing assessment, the authors utilised Landry R et al.,'s soft-tissue healing index [17]. The present study is a complex subjective scoring system. To increase the accuracy of the assessment, two observers well-acquainted with the index independently conducted the evaluations. To ensure an unbiased assessment, both observers were blind to the group allocation. Landry's healing index is one of the most commonly used indices in oral surgery and has been employed in several similar studies [12,21,22]. In the present study, the results showed a marked improvement in soft-tissue healing after surgery when PRF was utilised.

The PRF contains a 6 to 8-fold supra-physiological level of growth factors in its fibrin matrix, which are released slowly into the local environment. The beneficial role of locally incorporated growth factors in tissue healing has been hypothesised based on evidence from in-vitro, in-vivo, and animal studies, as well as controlled trials [23]. Varghese MP et al., demonstrated greater healing outcomes in patients who underwent surgical extraction with PRF incorporation [21]. Other studies also showed similar results [22,23]. However, in a recent systematic review and meta-analysis, Ye L et al., did not find any convincing results regarding the effect of PRF on soft-tissue healing [12]. Conversely, a recent umbrella study by Yang H et al., found it beneficial for soft-tissue healing [24]. PRF releases Epidermal Growth Factor (EGF) for soft-tissue repair, Transforming Growth Factor beta (TGF-β) for cell proliferation and migration, and Vascular Endothelial Growth Factor (VEGF) for new blood vessel formation [25].

The bony healing was assessed using CBCT findings. CBCT, which is a less complex device with low operational costs and reduced radiation exposure, was employed to acquire three-dimensional images. Gray values obtained from CBCT are used in an analogue manner to the Hounsfield Unit (HU) values of a CT scan for the determination of mineral density, showing a linear relationship with the attenuation coefficients of the materials [18]. The tomographic data were collected one month and three months after surgery. Analysis of socket volume, BDU, and type of bone formation was performed using semi-automatic segmentation with the aid of Sidex-4 software. In a similar study, Santhoshi Revathy N et al., used this same software to analyse OPG findings [26]. OPG does not provide a three-dimensional view, making volumetric analysis impossible. Ritto FG et al., used software (ITK-SNAP) to analyse CBCT images for this purpose [13]. The same machine and identical image acquisition settings were used to enhance the validity of the results. To minimise human error, two radiologists, blinded to the group allocation, independently evaluated the images. Better bone healing was observed in group I patients where PRF was placed in the empty sockets. The current result aligns with the findings of two recent studies by Rathan ACL et al., and Sharma R et al., [27,28]. In a meta-analysis, Al-Hamed FS et al., found no beneficial effect of PRF on bone healing of extraction sockets from third molars, whereas Ye L et al., in a recent meta-analysis and review articles, supported the beneficial role of PRF in bone healing of third molar sockets [11,12].

The induction of new alveolar bone formation is possibly facilitated by the effect of PRF on RUNX2 expression, alkaline phosphatase activity, osteoblast differentiation, and matrix mineralisation [29]. Growth factors stimulate wound healing and tissue repair by promoting angiogenesis, cell proliferation, cell differentiation, chemotaxis, and collagenous and non collagenous protein synthesis, as well as bone matrix deposition [30].

Limitation(s)

The present study had a small sample size and a short follow-up period. Some patients did not attend regular follow-up appointments and were consequently not included in the final analysis, thereby

reducing the study's power. A follow-up period of six months or more should be employed for a better understanding of bone healing.

CONCLUSION(S)

Platelet-rich Fibrin is an autologous product that is very easy to procure, incurs little additional cost, and is safe to use. Its applications in facial aesthetics, wound healing, and dental implantology are already established. The present study study results indicate that the postoperative incorporation of PRF in the extraction socket improves both soft-tissue and hard-tissue healing after the surgical extraction of mandibular third molars. A multicentre and split-mouth study design with a longer follow-up period and a larger sample size for future research in this area is recommended. Additionally, scintigraphy and histological analysis of new bone formation could be incorporated into the study design for a better understanding of bone healing.

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PARTICULARS OF CONTRIBUTORS:

1. Professor, Department of Oral and Maxillofacial Surgery, Guru Nanak Institute of Dental Sciences and Research, Hooghly, Kolkata, West Bengal, India.
2. Postgraduate Student, Department of Oral and Maxillofacial Surgery, Guru Nanak Institute of Dental Sciences and Research, Hooghly, Kolkata, West Bengal, India.
3. Professor, Department of Oral and Maxillofacial Surgery, Guru Nanak Institute of Dental Sciences and Research, Hooghly, Kolkata, West Bengal, India.
4. Professor, Department of Oral and Maxillofacial Surgery, Guru Nanak Institute of Dental Sciences and Research, Hooghly, Kolkata, West Bengal, India.
5. Professor, Department of Oral and Maxillofacial Surgery, Guru Nanak Institute of Dental Sciences and Research, Hooghly, Kolkata, West Bengal, India.
6. Lecturer, Department of Oral and Maxillofacial Surgery, Guru Nanak Institute of Dental Sciences and Research, Hooghly, Kolkata, West Bengal, India.
7. Lecturer, Department of Oral and Maxillofacial Surgery, Guru Nanak Institute of Dental Sciences and Research, Hooghly, Kolkata, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Sudip Chakraborty,
Dankuni Government Housing, A2/14, Hooghly, Kolkata-712-331, West Bengal, India.
E-mail: sudip.chakraborty@gnidsr.ac.in

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