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Original Article

Evaluation of platelet derived growth factor-BB levels in injectable platelet rich fibrin coated graft using gingival pedicle split thickness tunnel technique for isolated gingival recession: A randomized controlled trial

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ABSTRACT

Background: Use of injectable-platelet rich fibrin (i-PRF) in the field of periodontal regeneration is quite well known due to its efficacy. The study was aimed to evaluate the platelet derived growth factor-BB (PDGF-BB) levels in cases of isolated gingival recession using gingival pedicle split thickness tunnel technique (GPST) and de-epithelialized gingival graft (DGG) with or without coating it with i-PRF.

Methodology: 20 patients exhibiting Miller's class I/II isolated gingival recession were selected for this parallel arm randomized controlled trial. Recipient site was prepared using GPST technique, free gingival graft was harvested and de-epithelialized, further graft was coated with i-PRF and wound fluid samples from surgical site were collected at baseline, 3rd day and 7th day for group A. The same procedure without coating the graft in i-PRF was carried out for group B. Clinical parameters like probing depth, recession depth, recession width, width of keratinized gingiva, wound healing index (WHI), complete root coverage was recorded at baseline and after 4 months.

Results: Significant intergroup difference was seen in WHI and the levels of PDGF-BB from baseline to the last estimated time point ($p < 0.05$).

Conclusion: The study emphasizes on the use of novel GPST technique in conjunction with DGG coated with liquid PRF, which has shown sustained release of PDGF-BB resulting in better wound healing.

1. Introduction

Gingival recession (GR) terminology defines the defect in which root exposure occurs due to apical relocation of the marginal gingiva beyond the cemento-enamel junction (CEJ). Broadly based on the extent it is classified into generalized and localized/ isolated GR, many authors classified this defect under various categories. GRs are commonly seen in individuals suffering from trauma from occlusion, aberrant frenal attachment, and thin gingival phenotype with a prevalence in middle-age groups (>70% in 20–55) Merijohn (2016). The primary indication to treat GRs are patients' esthetic demand, to reduce dentinal hypersensitivity, and recently many patients are concerned about the thin phenotype which makes a way for perioplastic surgeries. Various treatment modalities have been put forth to date to correct GR, the surgical approach has given successful outcomes using various techniques and materials. These modalities are frequently employed in maxillary and mandibular anterior regions to correct these defects Imber and Kasaj (2021). Amongst all the proposed treatments connective tissue graft (CTG), has shown consistently better results in GR coverage in combination with any technique, nevertheless techniques such as coronally advanced flap (CAF), lateral pedicle flap (LPF), tunneling (TUN) has shown notable results.

Recently Agusto et al 2019 proposed a novel technique, a combination of LPF and TUN, known as the gingival pedicle split thickness tunnel technique (GPST) with the rationale that combining both the techniques will provide abundant blood supply and nourishment to the graft placed on the exposed root surface (Agusto et al., 2019) An alternative to gold standard biomaterial CTG, is de-epithelialized gingival graft (DGG) which has proven to be equally efficacious and provides dense, firm, stable tissue, but less literature is available about its application. Bakhishov et al. (2021) Another most acceptable biomaterial, platelet concentrates chiefly the second generation of it, platelet

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rich fibrin (PRF) has abundant platelets, growth factors, and cytokines which help in early tissue healing. Fernandes et al. (2021) Miron in 2017, based on the concept of lower speed centrifugation, invented i-PRF which is extensively utilized in dentistry due to its regenerative potential, wound healing, and bactericidal properties. (Miron RJ et al., 2017) Recently studies have shown despite being the liquid formulation, i-PRF has shown a greater release of growth factors almost up to ten days (Miron RJ et al., 2017; Varela et al. (2019)). In 1974, platelet regenerative potential gained attention, and it was Ross et al. who first elucidated the release of growth factors from platelets. Ross et al. (1974) Amongst all the released growth factors (GFs), PDGF is one of various established polypeptide cytokines which regulate the growth, differentiation, and activation of diverse cell types and serves as an initiator which directs tissue repair Raica and Cimpean (2010).

Based on the literature search by the authors, this is the first study that has used i-PRF to coat DGG for the treatment of isolated GR using GPST technique, to evaluate the wound healing by assessing the release of PDGF-BB at several time points. Thus, objective of the study was to biochemically and clinically evaluate the effect of i-PRF with DGG using GPST in the treatment of isolated GR.

2. Methodology with materials

This is a prospective, parallel armed randomized controlled trial, including 20 subjects (12 Males and 8 females) with isolated Miller's class I/II GR. Subjects by following appropriate method of randomization divided into group A (n = 10), in which DGG coated with i-PRF was placed and group B (n = 10) subjects without coating DGG in i-PRF, both the groups were treated with GPST technique (Fig. 1). Procedure was explained verbally before surgery and written consent was taken from participants. The research protocol was accepted by the institutional review board (Ref No. BDC/EXAM/574/2020–21) prior to the commencement of the study, it is in agreement with the declaration of Helsinki 1975, revised in 2013. The trial is listed under Clinical Trial Registry of India (CTRI), (Ref No. CTRI/2021/11/037987). The trial was conducted between December 2021 to December 2022.

2.1. Study sample

The subjects from the age group 20—55 years (with mean age of 28.1 ± 4.9 years) from department of Periodontics, Bapuji dental college and hospital, Davangere were involved in the study. Systemically healthy individuals with Miller's class I/II GR were included. Pregnant, lactating mothers, smokers and tobacco users were omitted from the study.

2.2. Estimation of sample size and randomization

Using a formula power (1 − β) was set at 80%, and α was set at 5%, based on this original sample size obtained was 16, but considering 20% loss to follow up, final sample size included in study was 20, each group containing 10. Based on coin toss method of randomization subjects were allocated into group A and group B, Randomization and surgical intervention were carried out by different investigators. No blinding was considered in this study.

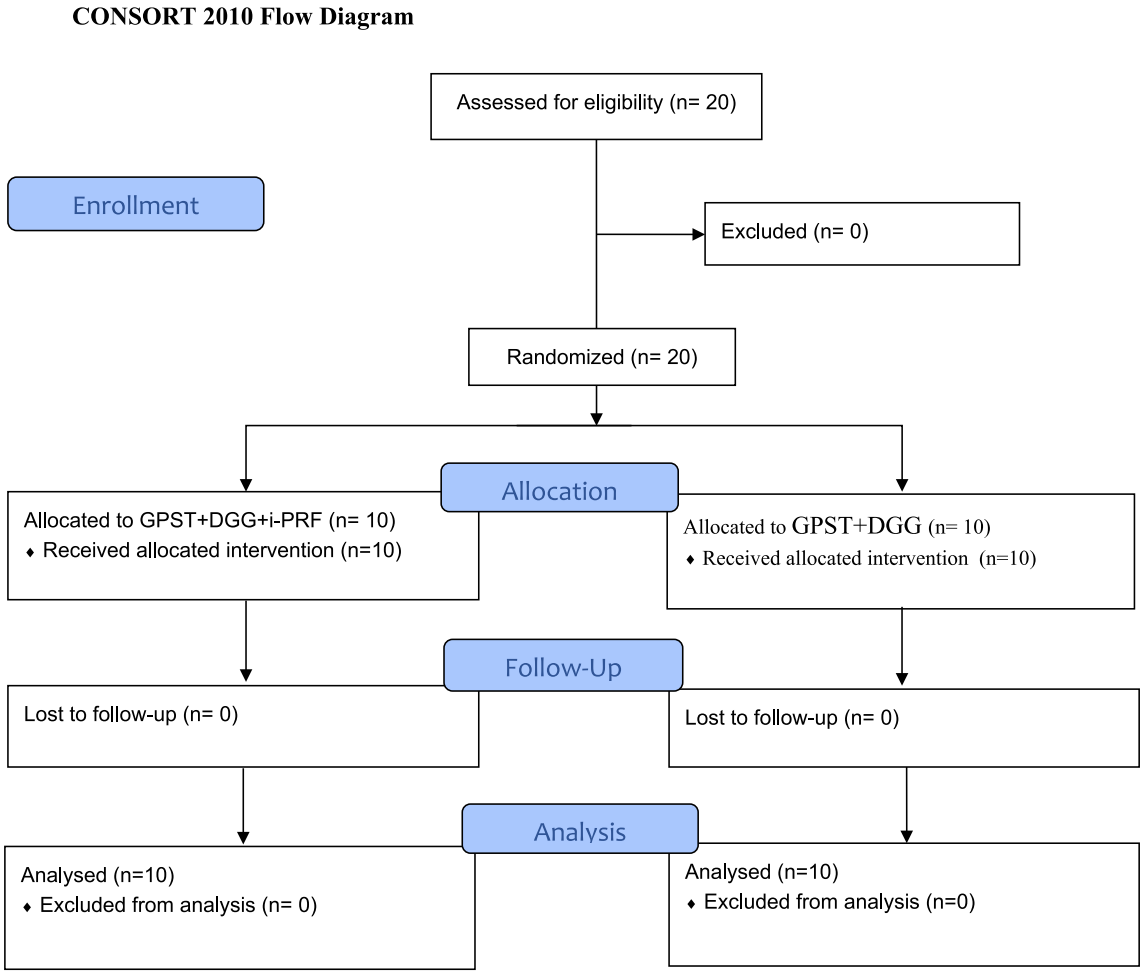

Fig. 1. Consort flow diagram.

2.3. Presurgical intervention

Following the examination, all subjects underwent professional scaling and oral prophylaxis. After the satisfactory oral hygiene maintenance surgeries were carried out. The following clinical parameters were taken into consideration at baseline and 4 months after surgery.

- Probing depth (PD)
- Recession depth (RD)
- Recession width (RW) (determined at the level of CEJ)
- Width of keratinized gingiva (WKG)
- Complete root coverage (CRC) (at the end of 4 months)
- Wound healing index (WHI) at the recipient site was recorded at baseline, 7th and 14th day post-surgery. Huang et al. (2005)
- Wound fluid samples were collected from operated site at baseline, 3rd day and 7th day postoperatively used for assessment of levels of PDGF-BB.

All the above mentioned parameters were recorded by the single investigator, in order to avoid interexaminer variation. PD, RD, WKG were recorded (Table 1) on the midbuccal tooth region using colorvue UNC-12 probe*, and summed up to the nearest number.

2.4. Surgical intervention

All the surgeries were performed by the same operator. In both the groups the recipient site was anaesthetized using 2% lignocaine with 1:80000 adrenaline.

2.5. Preparation of recipient site (Agusto m et al., 2019)

Procedure begins with the horizontal incision (greater than the RW) which was connected distally to the vertical incision extending beyond the mucogingival junction, at the end of which cut back preparation was done. The care was taken to place the horizontal incision 1 mm away from the gingival margin of adjacent tooth, with this split thickness pedicle flap was elevated without disrupting the homogeneity. On the other side full thickness tunnel was prepared, followed by detaching the interdental papilla. (Fig. 2).

Table 1
Assessment of clinical parameters in both the groups.

N = 10	GROUP A (GPST + DGG + i-PRF) Mean ± SD	GROUP B (GPST + DGG) Mean ± SD
PD		
BASELINE	3.00 ± 0.00	3.00 ± 0.00
4 MONTHS	0.60 ± 0.51	1.00 ± 0.00
RD		
BASELINE	3.30 ± 0.67	3.00 ± 0.66
4 MONTHS	0.10 ± 00.31	0.40 ± 0.51
RW		
BASELINE	2.600 ± 0.69	3.00 ± 00.66
4 MONTHS	0.10 ± 0.66	0.40 ± 0.51
WKG		
BASELINE	3.00 ± 0.66	3.00 ± 00.81
4 MONTHS	4.30 ± 0.67	3.80 ± 1.03
CRC		
4 MONTHS	96%	86%
WHI		
7th day	1.5 ± 0.52	1.70 ± 00.48
14th day	1.00 ± 0.00	1.50 ± 0.52.
PDGF-BB		
BASELINE	94.86 ± 28.99	92.17 ± 10.92
3rd DAY	90.92 ± 26.26	78.95 ± 11.72
7th day	82.72 ± 29.64	62.73 ± 15.23

2.6. Preparation of i-PRF[6] & donor site (DGG & DGG + i-PRF)

Prior to harvesting, 10 ml blood was withdrawn and centrifuged at 700 rpm for 3 min (60g) without any anticoagulant, [†] upper transparent liquid was then collected.

After achieving adequate anaesthesia at the donor site, the template was prepared which was thrice the RW in length and equal to RD in width. The template was carried to the palatal site and graft was harvested. The harvested graft was de-epithelialized outside keeping in between two autoclaved tongue depressors. For group A participants DGG was placed into the prepared i-PRF for 15 min, after which i-PRF enclosed DGG was placed on to the recipient site. Ucak Turer et al. (2020) The graft was tucked inside the tunnel and was secured with mattress suture, on the other side graft was stabilized using two simple interrupted sutures. The pedicle flap was moved passively over the graft providing additional vascularity and sutured with the adjacent keratinized gingiva. (Figs. 3 and 4) Damp gauze was dabbed at the site to dissolve the blood clot which might interfere with the plasmatic circulation. The same procedure without coating the DGG in i-PRF was carried out for group B. (Fig. 5).

2.7. Post surgical instructions

Postoperatively Zerodol P (Aceclofenac 100 mg & Paracetamol 325 mg) was prescribed. Patients were refrained from brushing for first week at the treated site, use of chlorhexidine mouthwash was recommended (0.12%), after that atraumatic brushing was advised to maintain optimum oral hygiene. Patients were recalled on 3rd, 7th, 14th day after surgery for recording of parameters, after that patients were followed up once in a month for clinical evaluation until 4 months.

2.8. Collection of wound fluid

Wound fluid was collected in both the groups from the surgical site. Before collection the area was damped with gauze to remove excess saliva. Filter paper [‡] was placed at the corners of graft placement and then collected samples were immediately transferred to Eppendorf tubes containing transport media. Samples were stored at −80℃ until sent for analysis, sample collection was done immediately after surgery, 3rd and 7th day post-surgery. Morelli et al. (2011).

3. Results

A total of 20 patients (20 GR defects) were analysed, as there were no drop outs in the study, data for all the patients has been included for statistical analysis. Primary outcome was to evaluate the effectiveness of i-PRF coated DGG for the management of isolated GR and secondary outcome was to measure the PDGF-BB levels released from i-PRF coated DGG at the surgical site. Data from baseline to different time points is summarized in Table 1. All the clinical values were tabulated and subjected for appropriate statistical evaluation.

Statistical analysis was carried out using recognized software[§] at 95% CI and 80% power to the study. Shapiro-Wilk test was done to check for normal distribution of the data. Descriptive statistics was performed in terms of mean, standard deviation. Intergroup and intragroup comparison was done by using unpaired *t* test and paired *t* test at baseline and 4 months respectively. Repeated measures ANOVA was applied to check significant difference in PDGF-BB levels between Baseline and 4 months in Group A and Group B. Results were considered significant for 'p' value < 0.05 and p' value > 0.05 to be non-significant.

1) PD: From baseline to 4 months no significant difference was seen on inter and intragroup comparison.
2) RD: Significant difference was observed in both the groups from baseline to 4 months ($p < 0.05$), but no significant difference was noted amongst the ($p > 0.05$).

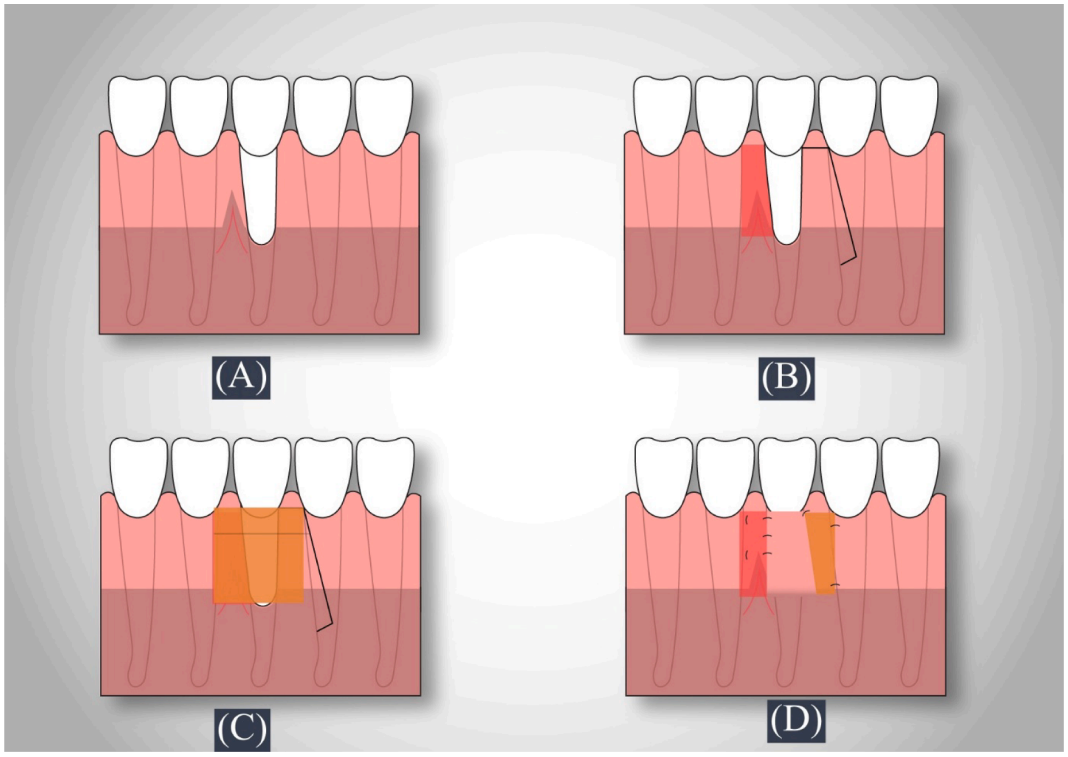

Fig. 2. Illustration of GPST technique.

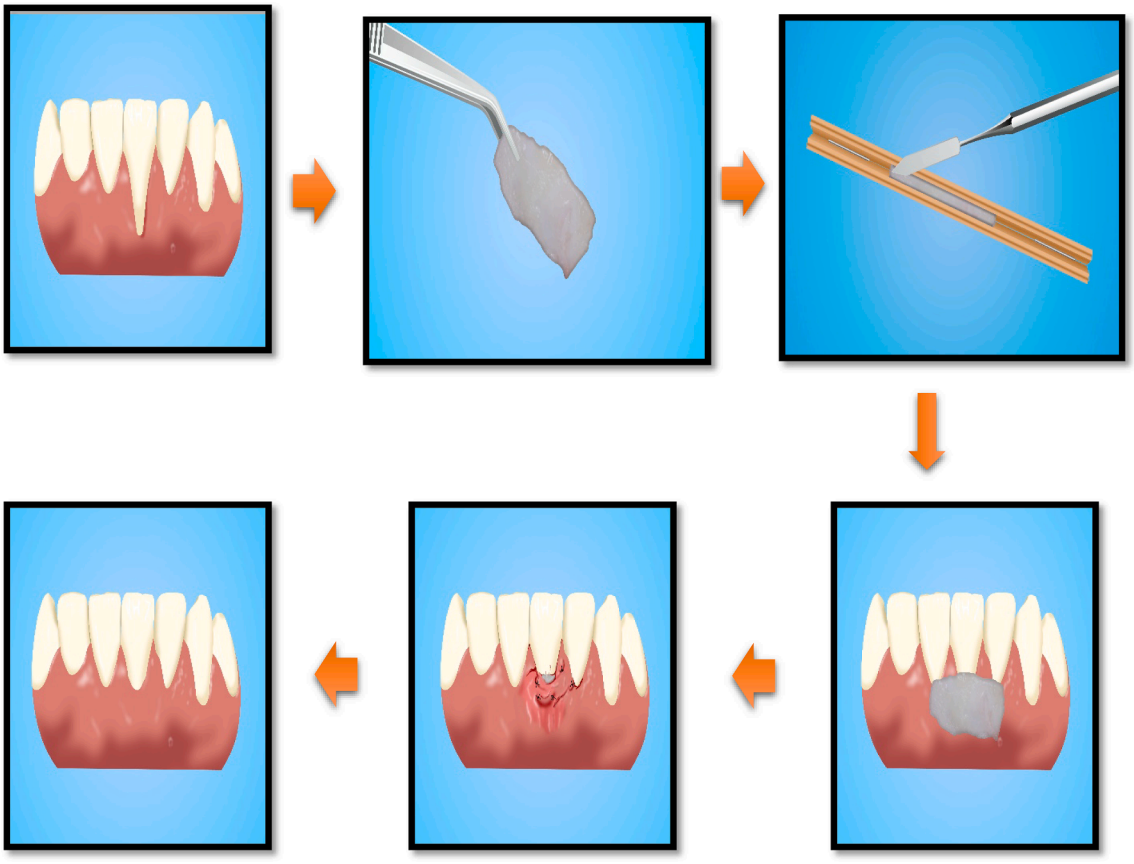

Fig. 3. Schematic presentation of steps followed in the study.

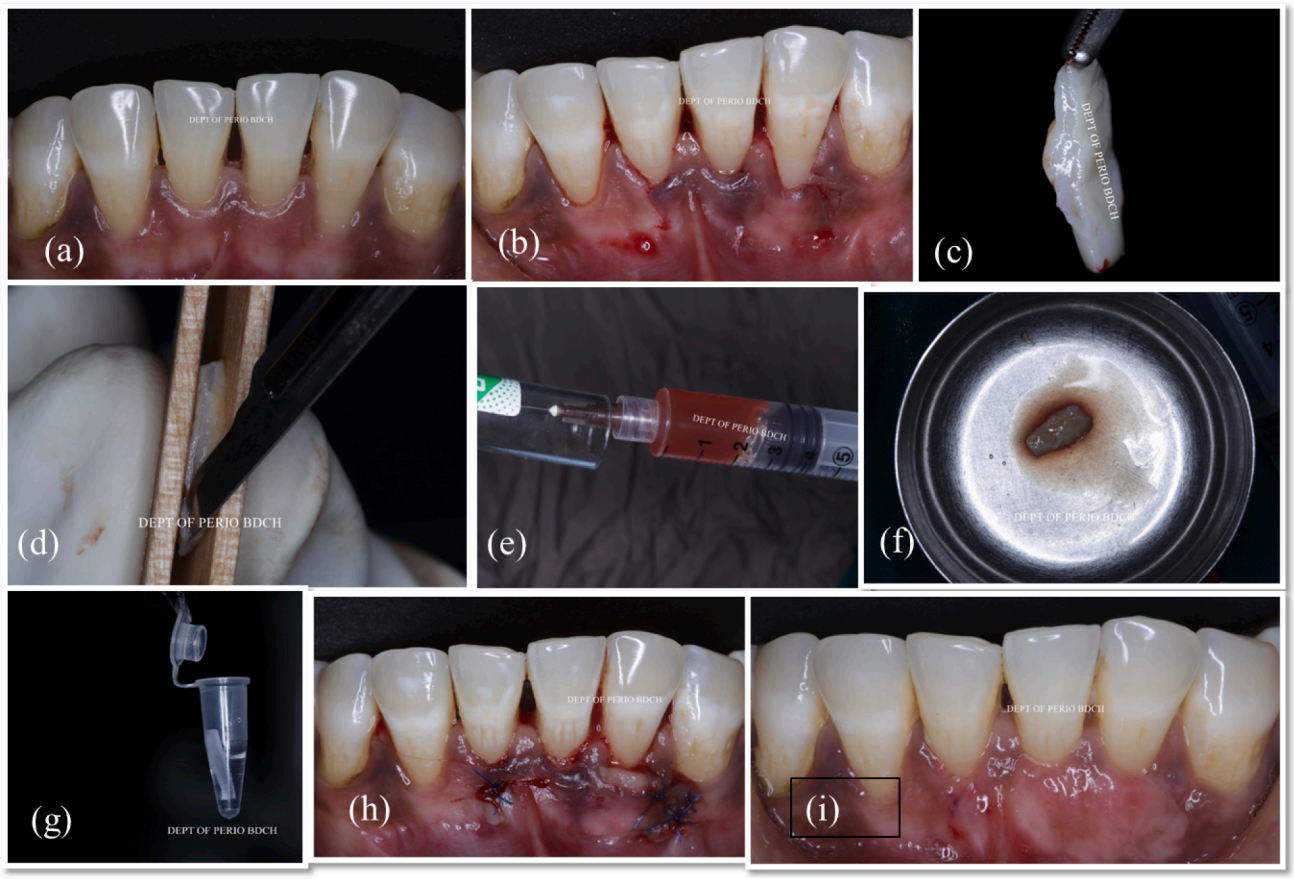

Fig. 4. GROUP A: (a) Preoperative view at baseline (b) Recipient site preparation using GPST technique (c) FGG harvested from the palate (d) De-epithelialized FGG using autoclaved tongue depressors (e) Prepared i-PRF collected in syringe (f) DGG coated with i-PRF (g) Collection of wound fluid from recipient site (h) Suturing recipient site (i) 14th day follow up.

3) RW: Significant reduction was found in both the groups post-operatively after 4 months, without any intergroup significant difference($p > 0.05$).
4) WKG: Significantly higher gain was observed 4 months post-surgery, with no significant difference between the groups ($p < 0.05$).
5) CRC: CRC was obtained in 9 out of 10 (96%) in group A and 8 out 10 (86%) in group B and respectively. A slight (not statistically significant) gain was seen in GPST + DGG + i-PRF patients in this parameter.
6) WHI: Highly significant difference was noted on 14th day between the groups.
7) PDGF-BB LEVELS: Significant difference was found amongst the groups since baseline to 7th day ($p < 0.05$).

4. Discussion

A variety of techniques along with different combinations of biomaterials have been described extensively in the literature for the management of isolated GR. Still, there is less information available on the usage of i-PRF along with DGG in such defects, which has shown a higher release of PDGF-BB which further promotes regeneration and faster wound healing. Hence, the present study aimed to compare and evaluate the levels of PDGF-BB with/without i-PRF application as an envelope for DGG together with GPST on wound healing and CRC obtained for isolated Miller Class I/II GR of mandibular anterior teeth. The results verified that both techniques (GPST + DGG + i-PRF and GPST + DGG) were equally effective in GR management, while biochemically, the release of PDGF-BB was found to be statistically significant in group A as well as better wound healing scores were seen in the same.

Taking the surgical site into consideration, mandibular incisors, due to a shallow vestibule and aberrant frenal attachment, coronal positioning of the flap becomes a challenge. In such cases, LPF provides an advantage without the displacement of MGJ. In the present study LPF in combination with TUN known as GPST was performed which combines the advantages of both by providing abundant blood supply and nourishment from all sides. Sculean and Allen (2018) (Wang and Stathopoulou (2019)). In a clinical trial done by Zuccheli et al, the use of DGG has shown similar results as of CTG. The obtained graft is denser, firmer, and equally stable with the advantage that even the most superficial connective tissue can be utilized. Zucchelli et al. (2010).

Eren and Atilla in their split-mouth study compared the efficacy of PRF along with CAF for isolated gingival recession, and they stated PRF can be used as an alternative biomaterial. Other forms of PRF, solid formulations have been used extensively over CTG or in comparison with CTG and have shown promising results. Eren and Atilla (2014) Miron et al in their study speculated that i-PRF favors the formation of a stable fibrin clots, which acts as a site of adherence for all cells. (Miron RJ et al., 2017) Fibrin acts as a center for growth factors for their sustained release which helps in prolonging bioactivity throughout the healing period. Wend et al., have assessed the impact of RCF (relative centrifugal force) on the release of GF and cell types present within i-PRF, and concluded that fluctuations in RCF, have shown RCF is inversely proportional to the release of GF. Keeping this notion in mind the same concept was applied in the study for the preparation of i-PRF. Wend et al. (2017).

The results of the current study stated that sites treated in group A have shown a higher CRC (96%) in contrast to group B (86%). The decline in PDGF-BB levels in the i-PRF group from baseline to the 7th

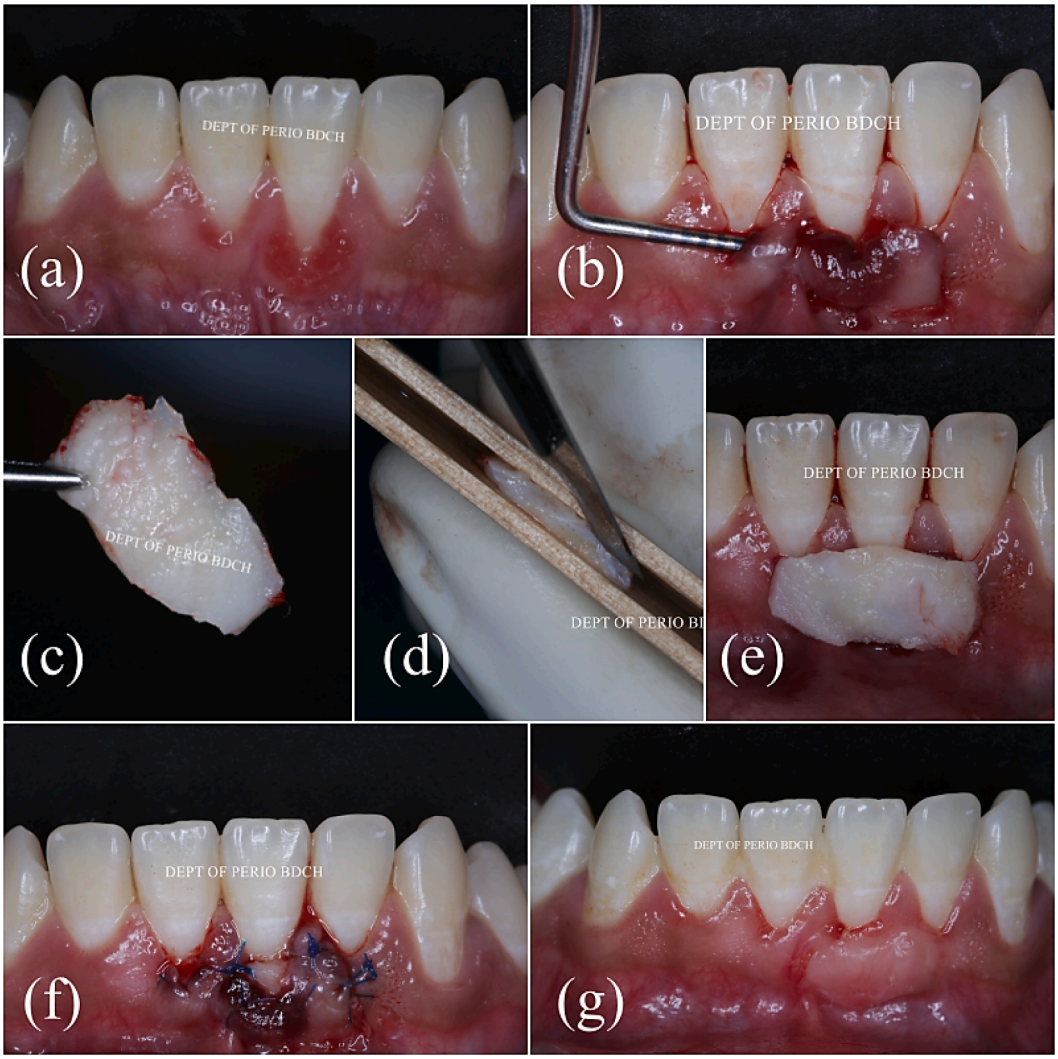

Fig. 5. GROUP B: (a) Preoperative view at baseline (b) Recipient site preparation using GPST technique (c) FGG harvested from the palate (d) De-epithelialized FGG using autoclaved tongue depressors (e) Placement of DGG at the recipient site (f) Suturing recipient site (g) 14th day follow up.

day (94.86 ± 28.99 ng/mL to 82.72 ± 29.64 ng/mL) was lesser, than that of non i-PRF group (84.86 ng/mL to 62.730 ng/mL). As platelets are the first cells to initiate tissue repair, they are an unlimited source of PDGF in the body. Peripherally circulating monocytes get chemotactically adhered to the wound site and get converted into wound macrophages and activate them. These activated cells contain m-RNA for all the isoforms of PDGF, as these macrophages and fibroblasts can be activated by PDGF a positive feedback loop operates within the wound which initiates platelet derived signals and triggers the cycle of events (extracellular matrix and GFs) forming completely healed wound which explains the improved healing in group A. Andrae et al. (2008) Although there are less literature and no clinical trials done to claim the clinical and biochemical benefits of i-PRF as a coating for DGG. Thus, it can be contemplated that the use of i-PRF acts as an envelope for DGG that which shows sustained release of GFs which ultimately fastens wound healing.

Other GR related parameters such as PD, RD, RW, WKG has shown similar results in both groups. After the thorough literature search as per our knowledge this can be acknowledged as the first clinical trial to compare and evaluate the efficacy of i-PRF coated DGG along with GPST for the management of isolated gingival recession. The combination of novel technique along with well accepted biomaterials can be used in different scenarios. In future various studies using different i-PRF protocols can be carried out to support its clinical benefits.

5. Limitations

The present study did not correlate the clinical findings with histopathological examinations. Due to the shorter duration of the study (4 months), the creeping attachment phenomenon (coronal migration of gingival margin after perioplastic surgeries over the denuded root surface area) cannot be observed. Subjects with limited mesio-distal dimensions which do not allow to obtain a pedicle with adequate horizontal width and very thin biotype may not be suitable and can be considered as a limitation of the technique.

6. Future perspectives

Future studies should focus on conducting more clinical trials with blinding techniques, investigating long-term outcomes, elucidating underlying mechanisms of action, assessing cost-effectiveness, and expanding the study population.

7. Conclusions

Within the limits of the study, isolated gingival recession can be effectively treated with GPST + DGG + i-PRF combination. As the applicability of the technique is clinician friendly it is recommended for clinical use.

A one-sentence summary

Miller's class I/II isolated gingival recession was treated using gingival pedicle split thickness tunnel technique with injectable platelet rich fibrin coated de-epithelialized gingival graft, and platelet derived growth factor-BB release was assessed and concluded that the release is more in PRF group compared to another which fastens wound healing.

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CRediT authorship contribution statement

Leena Patil: Visualization, Validation, Project administration, Formal analysis, Conceptualization. **Jayasheela M.:** Visualization, Methodology, Supervision, Conceptualization. **Triveni M.G.:** Writing – review & editing, Validation. **Gayathri G.V.:** Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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