Clinical evaluation of the effect of platelet rich plasma on the coronally advanced flap root coverage procedure

Sharashchandra Madhavarao Biradar1, Ashok Satyanarayan1, Arun J Kulkarni2, Basavaraj Patti3, Sunil K Mysore4, Ambika Patil5

1Department of Periodontics and Oral Implantology, Manasarovar Dental College and Hospital, Bhopal, Madhya Pradesh, 2Department of Periodontics and Oral Implantology, MIDSR Dental College Hospital and Research Centre, Latur, Maharashtra, 3Department of Public Health Dentistry, DJ College of Dental Sciences and Research, Modinagar, Uttar Pradesh, 4Department of Oral Medicine and Radiology, Guru Nanak Dev Dental College and Research Institute, Sunam, Sangrur District, Punjab, 5Department of Oral Medicine and Radiology, Albadar-Dental College and Hospital Research Centre, Gulbarga, Karnataka, India

ABSTRACT

Background: Coronally advanced flap (CAF) has been shown to effectively treat gingival recession. Platelet rich plasma (PRP), containing autologous growth factors, has been shown to promote soft tissue healing. The aim of this clinical study was to determine whether the addition of an autologous PRP to a CAF when compared to a CAF alone would improve the clinical outcome for treatment of multiple gingival recessions.

Materials and Methods: Thirty patients with Miller’s class I and class II buccal recession defect were randomly assigned to control (CAF alone) or test (CAF with PRP) groups. Recession depth (RD), recession width (RW), probing depth (PD), clinical attachment level (CAL), width of keratinized tissue, wound healing index, percentage of root coverage was assessed. Patients were followed at 2, 4, 12 and 16 weeks post-surgery. Statistical analysis for intra and inter group comparisons was done using Wilcoxon sign rank and Wilcoxon rank sum tests respectively. \( P < 0.05 \) denoted statistical significance.

Results: The differences between the test and control groups were not significant with respect to all the clinical variables. The RD at 16 weeks was significantly reduced from 2.88 ± 0.69 to 0.76 ± 0.24 mm in control group (\( P < 0.05 \)) and from 2.95 ± 0.43 to 0.76 ± 0.35 mm in the test group (\( P < 0.05 \)). The mean percentage of root coverage was 73.1 ± 7.3 in the control group and 75.0 ± 8.3 in test group (\( P < 0.05 \)). The CAL gain was 3.17 ± 0.84 mm within the control group and 3.17 ± 0.79 mm within test group.

Conclusion: Within the limitations of this study, PRP with CAF can provide an early healing of soft tissues, but does not provide clinically measurable improvement in the final therapeutics outcome in CAF.

Key Words: Gingival recessions, platelet rich plasma, guided tissue regeneration, periodontal mucogingival procedures have been used, including creation of free gingival grafts, laterally positioned

INTRODUCTION

Gingival recession defects are typically treated by periodontal plastic surgery to correct or eliminate the deformities of the gingival mucosa.[1] Various

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flaps or coronally positioned flaps, as well as guided tissue regeneration (GTR) and connective tissue grafting.[2] The coronally advanced flap (CAF) is a simple pedicle flap that can be utilized for root coverage and does not require graft harvesting. However, some data have shown that root coverage associated with CAF used alone is unstable in the long time, where it shrinks from 89% in 1-month postoperatively to 58.8% after 6 months.[3] The potential limitation of CAF is the limited gain in the apico-coronal direction of the keratinized tissue, which is an important parameter in recurrence of gingival recession.[6] Therefore, data indicate that the CAF used alone is a less-optimal technique to achieve root coverage despite having advantage of generating low morbidity.[5]

Coronally advanced flap resulted in the re-formation of epithelial and connective tissue attachment with minimal bone repair. Thereafter, various adjunctive agents have been applied to promote healing and further enhance clinical outcome. These include root conditioners (e.g., citric acid,[6] tetracycline[7]), GTR (Bio-resorbable membrane,[8] collagen membrane[9]), enamel matrix proteins,[10] subepithelial connective tissue graft,[11] recombinant growth factors, and platelet rich plasma (PRP).[12] According to Marx et al. who first reported applications and clinical benefits of PRP in 1998, it is “a volume of autologous plasma that has a platelet concentration above the baseline”.[13] PRP has become a focus of current studies due to its potential to accelerate wound healing.[14] It is derived from concentrated platelets, allowing it to deliver a greater concentration of autologous growth factors including platelet derived growth factor, transforming growth factor-β, vascular endothelial growth factor, epithelial growth factors, insulin like growth factor.[14]

Many studies have demonstrated that PRP enhances early maturation of bone, new bone formation and increases bone density,[15] early graft maturation,[16] early healing of bone around implants,[17] treating periodontal defects,[18] maxillary sinus augmentation,[16] ridge augmentation procedures,[16] and repair of peri-implant defects. Furthermore, PRP can be considered a safe method of growth factor utilization due to its autologous origin.[19] Depending on the initial platelet counts, it is common to achieve the platelet concentration three times higher than the baseline.

Several clinical and histological studies reveal that a specific platelet-concentrated therapeutic concept could be an encouraging medium for the improvement of soft tissue healing and regeneration in periodontology and implantology.[20] Considering this background, this study has been undertaken to evaluate the role of PRP on coronally advanced root coverage procedure.

**MATERIALS AND METHODS**

A randomized controlled trial approved by the Ethics Committee of the AME’S Dental College Hospital and Research Centre Raichur India, with registry number AME/Perio/201-2008 was performed on 30 patients both male and female in the age group of 18-45 years. Total sample was divided into two groups. Subjects with Millers Class I and Class II facial gingival recession of both maxillary and mandibular arches were randomly assigned to either test group (PRP + CAF) or control group (CAF only).

Inclusion criteria were patient who are systemically healthy; nonsmoker; willing to comply with the study related procedures; more than 18 years; ability to maintain good oral hygiene; maxillary or mandibular incisor, canines, premolars and molars with Class I or Class II gingival recession; gingival thickness more than 0.5 mm as measured 2 mm apical to the gingival margin; width of keratinized gingiva more than 2 mm and recession depth (RD) more than 2 mm. Subjects with previous surgical attempts to correct the gingival recession; history of periodontitis and orthodontic treatment; long-term (more than 2 weeks) use of antibiotics in past three months; known allergy to materials used; subject with compromised healing potential; subjects with active infectious diseases (Hepatitis, Tuberculosis, HIV etc); patients taking steroids or medications known to cause gingival enlargements; pregnant; mucosal disorders like high frenal attachments and ulcers; mal-aligned teeth and overhanging restorations were excluded.

**Pre-surgical procedure**

The compliance of the patient was sought. All the patients were given oral hygiene instructions. Standardized oral hygiene procedure to be incorporated by all the participants was advocated. For ease and quick acceptance, the roll method was instructed and demonstrated in the patients’ mouth with Colgate total toothpaste and medium toothbrush in front of the mirror. Brushing was advocated once a day throughout the study period. Manual scaling and root planning was done. Oral hygiene was satisfactory...
after 3 weeks of preparation. The patients were subjected for CAF root coverage procedure with or without PRP.

**Surgical procedure**

All the procedures were performed by the single operator under local infiltration of 2% lignocaine combined with 1:100,000 epinephrine. Lignox 2%, Indico remedies Ltd, Mumbai, India. Standard surgical procedures for CAF were performed.[15] The exposed root surface was thoroughly planed using periodontal curettes to obtain a smooth and hard surface. Patients in test group received PRP over the root surface while those in the control group received no further treatment. Flaps were then coronally advanced to cover the exposed root, and double sling suture were placed with 4-0 resorbable suture material. Gentle pressure is applied at the surgical site with moistened gauze to achieve hemostasis and a close adaptation of the flap to the underlying surface. Surgical dressing with Coe-pak (GC America INC, ALSIP, IL 60803, USA) was used. All patients were given standard postoperative instructions and were recalled at 2, 4, 12 and 16 weeks interval [Figures 1 and 2].

**Platelet rich plasma preparation**

Prior to the surgery, 10 ml of venous blood was drawn from patient randomized to the test group. The blood was then dispensed into 10 ml sterilized tubes with 1.5 ml 10% tri-sodium citrate, which functioned as anticoagulant. The tubes were placed into the REMI digital centrifuge for PRP preparation (REMI Laboratory centrifuges, Digital Speed Meter and Timer without Head (Model R8C), 8 × 15 ml, swing out head with graduated glass tubes). The technique used in this study followed the guideline proposed by Gonshor (2002).[12] Briefly, after counter balancing the tubes, the first spin was made at 1300 rpm for 10 min. The result was a separation of the whole blood into blood cells and plasma layer. The plasma layer and upper most part of the cell layer were withdrawn using needle and syringe, and placed into 10 ml sterilized tube that had no anticoagulant. Then, the sediment was centrifuged at 2000 rpm for 10 min. The second spin separated PRP at the bottom and clear straw colored serum at the top. The serum was removed. The PRP layer was then expressed into a sterile container and prepared for use. The clot initiator was a mixture of 1.5 ml calcium chloride and autogenous thrombin. The protocol of PRP activation was adding calcium chloride/thrombin in 10:1 ratio. After a few seconds, the activated PRP gel was applied to the test sites as a coating membrane. Platelet count was performed to compare the number of platelets in whole blood and PRP. Platelet levels were determined by a hematology analyzer RMD Mediaids Limited, New Delhi, India [Figure 3].

**Post-surgical care**

The patients were advised not to brush the treated areas for 3 weeks and to avoid excessive muscle tractioning, chewing on, or trauma to these areas. Patients were prescribed a 0.12% chlorhexidine digluconate mouthwash twice daily for the tested period. Analgesics (ibuprofen) were prescribed if indicated. At day 10-12, sutures were removed, and the areas were professionally cleaned as a supragingival prophylaxis with a rubber cup at

![Figure 1](image1.png)

**Figure 1:** (a) Control group — pre operative view. (b) Control group-4 week postoperative view. (c) Control group-16 week postoperative view. (d) Control group — flap coronally advanced and secured in position with suture.

![Figure 2](image2.png)

**Figure 2:** (a) Test group — Preoperative view. (b) Test group — after application of platelet rich plasma. (c) Test group-4 week postoperative view. (d) Test group-16 week postoperative view.
low speed. At 3 weeks, brushing with a very soft toothbrush was reinstituted.

**Measurements**
All measurements and photographs were done by a single blinded examiner who had been calibrated prior to the study (kappa = 0.92). Patients were called for measurements and photographic documentation pre-surgically and after 2, 4, 12 and 16 weeks postoperatively. At baseline and at 16 weeks following surgical treatment, all variables were recorded. Clinical parameters assessed were probing depth (PD), clinical attachment level (CAL), RD, Recession Width (RW) and Width of Keratinized Tissue (WKT), wound healing index (WHI), percentage of root coverage of individual sites within control and test groups. Williams graduated probe, divider, and vernier caliper were used for all measurements. To test the reliability of the assessments, the measurements of three patients were randomly re-examined.

**Statistical analysis**
The recorded data were compiled and entered in a spreadsheet computer program (Microsoft Excel 2007) and then exported to data editor of SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics included computation of percentages, means, and standard deviations. The variables were assessed for normality using Kolmogorov-Smirnov test and were found to be nonnormally distributed. Hence, nonparametric test were considered; for intra group comparison-Wilcoxon sign rank and for inter group comparison Wilcoxon rank sum. \( P < 0.05 \) denoted statistical significance.

**RESULTS**
A total of 30 patients participated in the study. The age range of patients was 18-45 years in both control and test groups. In control group, six mandibular and nine maxillary teeth were treated. In test group, four mandibular and eleven maxillary teeth were treated. The mean PD, CAL, RD, RW and WKT for both the test and control groups at the baseline are summarized in Table 1. The difference between the test and control groups was not significant with respect to all the clinical variables. The mean RD reduction after 16 weeks of surgery was 2.12 ± 0.5 mm within the control group and 2.19 ± 0.25 mm within test group. The mean percentage of root coverage was 73.1 ± 7.3 within the control group and 75.1 ± 8.3 within test group. The mean reduction in the PD was 0.87 ± 0.40 within the control group and 0.90 ± 0.51 within test group. The mean CAL gain was 3.17 ± 0.84 mm within the control group and 3.17 ± 0.79 mm within test group. The mean reduction in the RW was 2.19 ± 0.47 mm within the control group and 2.59 ± 0.76 mm within test group. WKT was increased about 0.52 ± 0.28 mm within control group and 0.48 ± 0.18 mm within test group and percentage of root coverage from baseline to 16th week was 75% and 73.1% for test and control group respectively. All the variables showed no statistically significant difference between control and test group [Table 2].

Intra group comparisons revealed significant difference within test and control group with respect to all the variables from baseline to 16th week [Table 3]. Mean WHI scores in patients within control group after 2 weeks was 1.6 ± 0.5 and after 16 weeks of surgery was 1, whereas in test group after 2 weeks of surgery was 1.3 ± 0.5 and 16 weeks after surgery was 1. Difference between control and test group after 2 weeks was not statistically significant.

| Parameter | Mean in mm | Control group | Test group | \( P \) |
|-----------|------------|---------------|------------|------|
| PD        | 2.27±0.37  | 2.27±0.62     | 0.95       |
| CAL       | 5.08±0.90  | 4.99±0.78     | 0.55       |
| RD        | 2.88±0.69  | 2.95±0.43     | 0.98       |
| RW        | 2.56±0.47  | 2.91±0.74     | 0.22       |
| WKT       | 1.93±0.60  | 2.09±0.73     | 0.57       |

* \( P < 0.05 \) is considered statistically significant. Test applied: Mann-Whitney U-test; PD: Probing depth; CAL: Clinical attachment level; RD: Recession depth; RW: Recession width; WKT: Width of keratinized tissue.
**DISCUSSION**

At the end of 16 weeks, statistically significant improvement in all the clinical parameters was observed in both control and test groups as compared to their respective baseline parameters. When compared to control group, the observed reduction in RD and RW in test group was not statistically significant. These results are in accordance with several studies,\(^{12,20,21}\) but they are contradictory to the results reported by Modica *et al.*\(^{10}\) Both test and control groups resulted in root coverage; however, the slight difference between them may be due to patient selection, defect selection, local anatomical factors such as shallow vestibule, prominent roots and quality of tissue.

The percentage of root coverage observed within test group (75%) when compared with the control group (73.1%) was not statistically significant. These results are in accordance with Spahr *et al.*\(^{22}\) and the outcome of our recession coverage procedure was successful from a clinical point of view.

The difference between the reduction in the PD and gain in CAL within control and test groups were not statistically significant. PD reduction depends on the tissue condition before and after the surgical procedure. Similar results are reported by several studies.\(^{10,12,23}\) The improvement in CAL suggests that recession coverage by CAF procedure does not result in pocket formation but in tissue attachment on the previously exposed root surface.

In comparison to control group, increase in WKT in test group was not statistically significant. Similar results were reported by several authors.\(^{12,24,25}\) This increase in WKT is considered to occur as a result of the proliferation of granulation tissue from the gingival connective tissue and the periodontal ligament. This outcome has been justified due to the genetically determined position of the mucogingival line, which, with time, reaches its original position and consequently causes an increase of WKT. The results from these clinical studies may in part justify the outcomes obtained in this investigation.

The PRP preparation technique used in the current study was a modification of Gonshor method\(^{12}\) where autogenous thrombin was added instead of bovine thrombin. Mean platelet recovered from our method was 46.52%. It was comparable with Ghonshor method that could recover 67.5%\(^{12}\). Less percentage of platelet recovery in our study indicates that this procedure is technique sensitive and requires learning skills. In our study, the average increase of platelet concentration in PRP was 17.4 times (4.7 minimum and 30.6 maximum). Recent advances in PRP technology are able to provide more consistent platelet concentration than those observed in this

### Table 2: Inter group comparisons of various variables

| Variables            | Groups | Mean ± SD | P   |
|----------------------|--------|-----------|-----|
| PD                   | Control| 0.87±0.40 | 0.45|
|                      | Test   | 0.90±0.51 |     |
| CAL                  | Control| 3.17±0.84 | 0.25|
|                      | Test   | 3.17±0.79 |     |
| RD                   | Control| 2.12±0.59 | 0.46|
|                      | Test   | 2.19±0.25 |     |
| Percentage of root   | Control| 73.1±7.3  | 0.46|
| coverage             | Test   | 75.0±8.3  |     |
| RW                   | Control| 2.19±0.47 | 0.06|
|                      | Test   | 2.59±0.76 |     |
| WKT                  | Control| 0.57±0.28 | 0.06|
|                      | Test   | 0.48±0.16 |     |

*P < 0.05 is considered statistically significant. Test applied: Mann-Whitney U-test (between test and control groups); PD: Probing depth; CAL: Clinical attachment level; RD: Recession depth; RW: Recession width; WKT: Width of keratinized tissue; SD: Standard deviation.*

### Table 3: Intra group comparisons from baseline to 16 weeks after surgery

| Variables | Groups | Baseline | 2 weeks | 4 weeks | 12 weeks | 16 weeks |
|-----------|--------|----------|---------|---------|----------|----------|
| PD        | Control| 2.27±0.37| —       | —       | 1.43±0.67| 1.4±0.21 |
|           | Test   | 2.27±0.62| —       | —       | 1.48±2.3 | 1.37±1.9 |
| RD        | Control| 2.88±0.69| 1.1±0.45| 1.0±0.12| 0.9±0.14 | 0.8±0.67 |
|           | Test   | 2.95±0.43| 0.9±0.13| 0.9±0.08| 0.8±0.07 | 0.8±0.76 |
| RW        | Control| 2.56±0.47| 1.1±0.77| 1.0±0.99| 0.9±0.12 | 0.8±0.47 |
|           | Test   | 2.91±0.74| 0.4±0.08| 0.4±0.74| 0.3±0.31 | 0.3±0.74 |
| WKT       | Control| 1.93±0.60| 2.1±0.45| 2.2±0.60| 2.4±0.89 | 2.5±0.49 |
|           | Test   | 2.09±0.73| 2.3±0.28| 2.4±0.73| 2.5±0.94 | 2.6±0.99 |
| CAL       | Control| 5.08±0.90| —       | —       | 1.96±0.99| 1.91±0.89|
|           | Test   | 4.99±0.78| —       | —       | 1.93±0.73| 1.81±0.78|

*Baseline versus 16 weeks significant, P ≤ 0.05. Test applied: Wilcoxon sign rank; PD: Probing depth; RD: Recession depth; RW: Recession width; WKT: Width of keratinized tissue; CAL: Clinical attachment level.*
study. However, despite the small sample size, no dose dependent effect of PRP on soft tissue wound healing could be detected.

Clinical evaluations of healing events are evaluated with recordings of healing index. WHI reached plateau 1-month after surgery in both groups suggesting uneventful healing regardless of PRP application. Recent systematic review and meta analysis reported that platelet concentrate does not benefit significantly in the treatment of root coverage.[26,27] Time of application after platelet activation plays an important role in tissue healing and regeneration. Hence, this factor may affect the final root coverage. Thus, preparation, application at the defect site and handling of PRP can be considered technique sensitive and need learning skills.

The present work has certain limitations that include small sample size and limited study duration (16 weeks). Gingival thickness before and after root coverage procedures is also important for success of root coverage procedure.[28] In the present study, thickness of the flap was not measured. Therefore, further studies with larger sample size and longer duration are needed to show the histological changes following PRP therapy and growth factors levels in PRP. CAF procedure has proved successful for root coverage in the present study. However, an additional benefit of PRP is not yet known. Comparative study with split mouth design on similar recession defects with or without PRP could provide further knowledge.

CONCLUSION

Soft tissue maintenance is the primary line of defense in protecting the tissue from bacterial infection. On the basis of the results of the present research, the additional use of PRP together with CAF technique for recession coverage showed no difference in the overall clinical outcome. A possible positive tendency of PRP utilization should be better evaluated in the studies involving larger sample size and longer duration. Since no histological evaluation was performed in the present study, the effect of PRP on overall regeneration capacity remains to be determined.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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