ISSN Print: 2394-7489
ISSN Online: 2394-7497
Impact Factor (RJIF): 7.85
IJADS 2026;12(1): 09-13

www.oraljournal.com
Received: 07-11-2025
Accepted: 10-12-2025

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PRP Augmented Iliac Corticocancellous Graft: Enhanced volume retention and stability in Bilateral Alveolar Cleft Reconstruction

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DOI: https://www.doi.org/10.22271/oral.2026.v12.i1a.2317

Abstract
Background and objectives: Secondary alveolar bone grafting for treatment of alveolar clefts is an established procedure. Platelet rich plasma, a relatively new concept in maxillofacial surgery, contains a high concentration of growth factors which help faster regeneration of bone across clefts. PRP mixed with autogenous cancellous bone grafts have been used for grafting of alveolar clefts. Several methods of radiography have been employed for quantitative assessment of outcomes of SABG. Universally accepted methods of estimation are yet to be established. The degree to which these estimates agree with each other is open to question.
Methods: PRP mixed with autogenous iliac bone was used as graft material for performing SABG in 12 patients with bilateral clefts. The volume of bone regenerated and percentage of bone support to cleft adjacent teeth was assessed on 3D computerized tomography were analyzed.
Results: Pre-operative and 9 month post-operative height of bone support and percentage coverage of root surface for central incisor and canine adjacent to cleft as assessed on 3D CT were 41.56% ± 15.38 and 86.78% ± 5.78 (central incisor)a nd 52.38% ± 21.31 and 88.33% ± 8.08 (canine) respectively.
Conclusions and interpretation: Autogenous cancellous bone grafting with PRP is a reliable procedure for SABG in bilateral cleft alveolus patients. 3 Dimensional CBCT's radiographs give a correct estimate of the three dimensional bone support of cleft adjacent teeth.

Keywords: Secondary alveolar bone grafting, Platelet rich plasma, Cleft alveolus, Bone regeneration, 3D CT, Volumetric analysis

Introduction
The presence of bilateral cleft lip and palate has the potential to significantly alter facial form and structure. The central third of the face is distorted by the bilateral cleft, and restoring the normal facial form is one of the primary goals for the reconstructive surgeon. Alveolar bone grafting is important because it provides bone for the erupting lateral incisor and canine. Secondary alveolar bone grafting (SABG) was first described by Boyne and Sands in 1972 and has since become the standard surgical protocol for alveolar bone reconstruction [1].
This bone grafting procedure is performed with the aim of constructing a homogeneous alveolar arch and stabilizing the maxillary arch, providing support for tooth eruption, eliminating oronasal fistulas and improving the aesthetic results [2]. SABG in bilateral cleft cleft lip palate is a complex surgical procedure involving raising of soft tissue flaps from both the oral and nasal side. The complex anatomic situation increases the risk of wound dehiscence. The cancellous bone is non-vascularized and vulnerable for infection. Many sources of bone both autogenous and alloplastic have been studied, compared, highly profiled and abandoned, but fresh autogenous cancellous bone is ideal because it supplies living, immunocompatible bony cells that integrate fully with the maxilla and are essential for osteogenesis [1-3]. A major complication in 30-75% of cases of surgical treatment of alveolar cleft is resorption of bone graft [4]. In order to overcome these problems a novel technique for the treatment of alveolar clefts using Platelet rich plasma for the regeneration of bone when mixed with autologous cortico-cancellous iliac bone graft in cleft alveolus, is suggested.

Platelet rich plasma (PRP) is a new application of tissue engineering and is a storage vehicle for growth factors like PDGF, TGF-β1, and TGF-β2 which influence bone regeneration. PRP is defined as high concentration of platelets in a small volume of autologous plasma [5, 6]. This being an autologous product, concerns about immunogenic reaction and disease transmission are eliminated [6, 7].

The present study is conducted with the aim to evaluate the efficacy of PRP in regeneration of bone when mixed with autologous cortico-cancellous iliac bone graft in cleft alveolus, and to assess the same using three-dimensional (3D) CT scans.

Materials and Methods

The study was conducted in Department of Oral & Maxillofacial Surgery. HKES's S. Nijalingappa Institute of Dental Sciences and Research, Kalaburgi. The procedures followed were in accordance with the ethical standards of the Institutional ethical clearance committee on human experimentation and with the Helsinki Declaration (2003). Approval was taken from S Nijalingappa Institute of Dental Sciences and Research Institutional Ethics Committee.

Source of data

Patients presenting to the Department of Oral and Maxillofacial Surgery for bilateral alveolar cleft repair (with or without oronasal fistula) were included. Participants were limited to the mixed dentition age group (8–13 years). Patients with prior alveolar cleft surgery or immunocompromised conditions were excluded. After proper case selection patients were subjected to preoperative blood investigations, radiographs and informed written consent were taken from all the patients/ Guardians. Simultaneously preoperative Cone beam Computed Tomography was taken to evaluate the size and margins of the bone defect. (Fig 1)

Surgical procedure

All surgeries were performed under general orotracheal anesthesia. Of the two surgical teams, one operated on the cleft alveolus and the second prepared bone graft from anterior iliac crest simultaneously. Corticocancellous bone graft was harvested. After scrubbing and painting with Cetrimide 20%, Normal saline and Povidone iodine 5%, patient was draped. After local infiltration of the cleft region with local anaesthetic containing adrenaline a subperiosteal incision was made posterior or distal to the labial portion of the alveolar cleft, around the bony rim of the cleft and extended to cervical limits of the canine tooth and adjacent teeth and was carried posteriorly and advanced to mucobuccal fold on molar region. The subperiosteal mucosal flap outlined by this incision was undermined and raised from the bone. Similar incision was made on the anterior or mesial portion of the cleft and extended along the distal aspect of central incisor tooth. The flap was undermined subperiosteally to the region of the frenum. More tissue was retained on the palatal side. Only minimal raising of mucoperiosteal flaps in the cleft region was performed and pushed palatally. The nasal mucoperiosteum from the walls of the bony cleft was gently raised, mobilized and sutured with interrupted 6-0 or 5-0 absorbable polyglactin sutures thus achieving the closure of oronasal fistula and reconstruction of nasal floor. This established the nasal boundary of the area to be grafted. The superficial cortical bone layer in the cleft was refreshed with scalpel blade to improve the incorporation of bone graft. On the palatal side the mucoperiosteal flaps on either side of cleft side were sutured. The harvested iliac corticocancellous bone chips were mixed with PRP gel which was tightly packed in the cleft region avoiding dead space and also ensuring reconstruction of pyriform rim. (Fig 1) The mobilized buccal and labial mucosal flaps were extended over the grafted area and sutured in place with interrupted 4-0 absorbable polyglactin suture. The sutures were everted toward the oral cavity achieving water tight closure of the surgical wound.

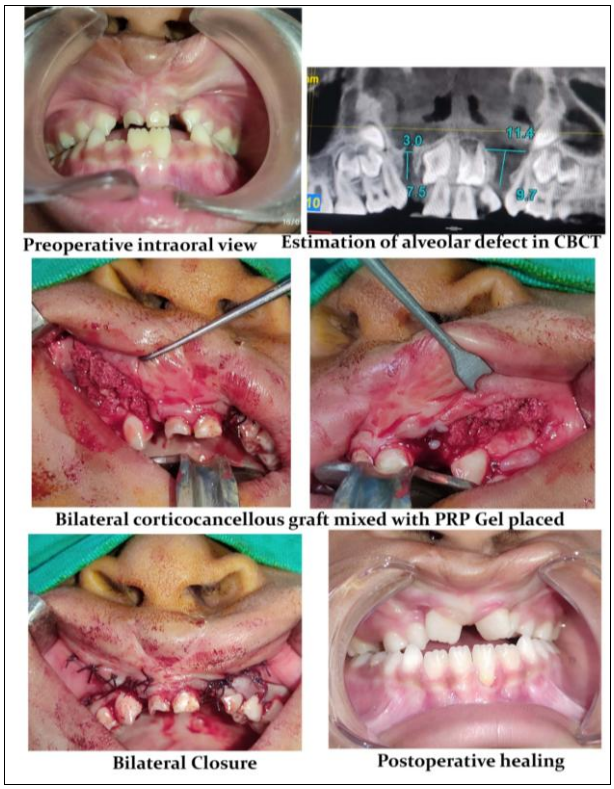

Fig 1: The mobilized buccal and labial mucosal flaps were extended over the grafted area and sutured in place with interrupted 4-0 absorbable polyglactin suture

Preparation of PRP gel [13]

Under aseptic techniques, 20 ml of venous blood was drawn from antecubital or femoral vein. This blood was mixed with 1.8 ml of CPDA (anticoagulant) in two separate glass test tubes. The test tubes were thoroughly shaken to ensure mixture of anticoagulant with the drawn blood. The whole blood was centrifuged at 3,600 rpm for 10 minutes. This lead to formation of 3 layers: An upper clear fluid, a middle Buffy coat, and a lower red fraction. The upper fraction and Buffy coat and about 2 mm of red cells were collected in a separate test tube and were centrifuged again at 2,400 rpm for 15 min. The supernatant yellow serum component was discarded and the remaining substance was the PRP solution. 2.5 ml of PRP solution was mixed with 0.08 ml of 10% calcium chloride. This resulted in clot formation and a clear fluid which was autologous thrombin. Thrombin was collected and 0.5 ml of thrombin was mixed with 3 ml of PRP solution and was kept in hot water bath for 2-3 min at 37^0 Celsius to yield PRP gel.

Analysis of results using CT scan

Each patient had a computerized axial tomogram taken of the face. Patient positioning was standardized with the maxillary alveolar crest parallel to the plane of the scan. Cuts of 1.5 mm were taken from the infraorbital rim of the gingival third of the crowns of the teeth. The total area scanned was approximately 3.0 to 4.0 cm high. Measurements and

reformatting were completed by a single operator to eliminate inter-operator variability. (Fig 2)

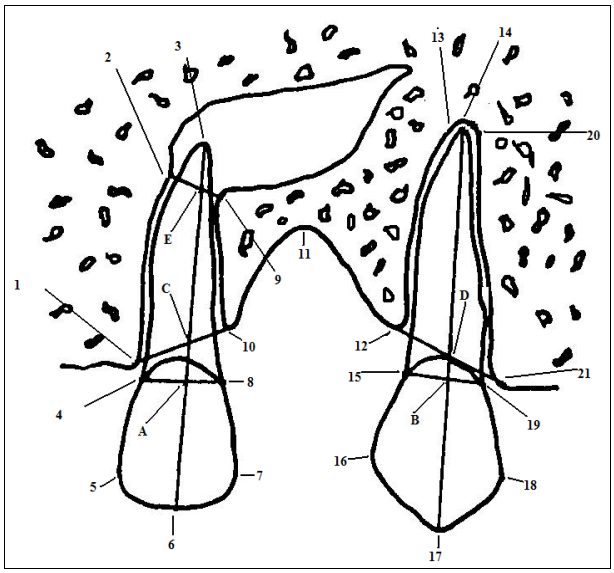

Fig 2: Digitized points and points for measurement on radiographs

Results

The collected data was analysed statistically using paired't' test, ANOVA test and Tukey's multiple posthoc test.

The comparison of pre-op and post–op period with respect to percentage of root coverage at central incisor and canine adjacent to alveolar cleft using CT by paired t-test shows preoperative mean of 41.5573 ± 15.3789 which increased to 86.7790 ± 5.7847 after 9 months follow up period for central incisor which was statistically highly significant (p=0.0000). (Fig 3) Similarly for canine the mean was 52.3827 ± 21.3075 preoperatively which increased to 88.3278 ± 8.80811. It was also statistically significant (p=0.0011).

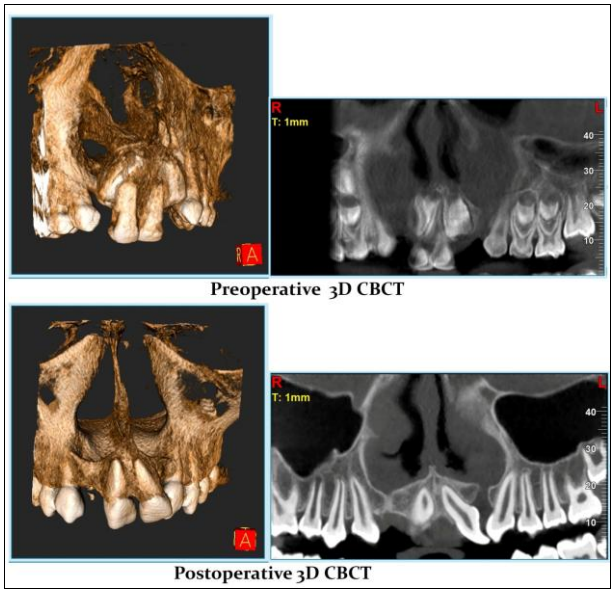

Fig 3: Preoperative and postoperative 3Dimensional CBCT

The comparison of pre, 3 month and 6 month postoperative period with respect to percentage of root coverage by bone at central incisor and canine adjacent to alveolar cleft using IOPA by paired t-test, that pre-operative mean of central incisor was 48.4410±28.5952 which increased to 90.5140±2.8262 at 3 month postoperative and was statistically significant with p-value 0.0012 and paired t-value -4.6569, the 6 month postoperative value as compared with preoperative was highly significant with p-value of 0.0017, paired t-value of -4.4128, with a slight decrease in the value at 6 month postoperatively when compared with 3 month was 88.7040±4.4422 which was statistically significant (p=0.0284). Similarly for canine the mean was 46.2256±24.8812 which increased to 88.9600±6.1242 at 3 month postoperative and was statistically significant with p-value 0.0014 and paired t-value -4.7871. The 6 month postoperative value as compared with preoperative raised to 87.9778±7.7601 which was highly significant with p-value of 0.0013, paired t-value of -4.8188, with a slight decrease in the value at 6 months postoperatively which was 87.9778±7.7601 when compared with 3rd month value of 88.7040±4.4422 which was statistically not significant(p=0.4779).

Discussion

Oyama *et al.* [7] performed alveolar bone grafting with autologous iliac cancellous bone mixed with platelet rich plasma and evaluated its efficacy in osteoregeneration. Quantitative evaluation of regenerated bone was made with 3-D CT and compared with controls preoperatively and at five to six months post- operatively. In their study the average of volume ratio of regenerated bone to alveolar cleft in cases with PRP was higher than in controls. They assessed the volume of bone regenerated by calculating the ratio of values for volume of alveolar cleft to volume of regenerated bone and expressed it as percentage. The highest and lowest ratios expressed as percentage in their series using bone marrow with PRP were 88.16% and 71.27% respectively with mean of 80.19% ± 6.77%. There was no correlation between VAC and VRB. This indicates that even if the cleft was wide the result was not necessarily poor.

In the present study the highest and lowest ratios are 90.82% and 70.17% respectively with mean of 81.18% ± 6.97%. The values obtained in the present study are in close agreement with those of Oyama *et al.* [7].

Luaces- Rey *et al.* [8], conducted a study to evaluate the efficacy of PRP mixed with autogenous bone graft, in regeneration of bone for secondary alveoloplasty. The amount of bone formed has been evaluated by comparing digital orthomopantomographs taken immediately after surgery and again at three and six month postoperative periods. The researchers did not find any significant differences between the operated cases and controls. The researchers however concede that their series is too small to obtain conclusive results and that more prospective randomized controlled studies are required to achieve definitive data against or in favor of PRP use in osteoplasty of cleft patients.

The average amount of height of bone support and percentage coverage of root surface by bone as assumed on 3D CT for lateral incisor was 8.7 mm and 76.5% in a study by Dado[9] while in the present study the average pre-operative and 9 month post-operative amount of height of bone support and percentage coverage of root surface for central incisor adjacent to cleft are 41.56% ± 15.38 and 86.78% ± 5.78 respectively.

The average amount of height of bone support and percentage coverage of root surface as assumed on 3D CT for canine was 14.1mm (82.6%) in the study by Dado [9] while in the present study the average pre-operative and 9 month post-operative amount of height of bone support and percentage coverage of root surface for canine adjacent to cleft are 52.38% ± 21.31 and 88.33% ± 8.08 respectively.

The lowest and highest amount of root surface coverage in

study by Dado *et al* [9] were 42% and 100% respectively for lateral incisors, as assessed by CT. while corresponding values for central incisors in the present study are 21.42% and 63.86% pre-operatively. At 9 month post-operative evaluation the lowest amount of root surface coverage for central incisor in present study is 77.74% and the highest is 93.71%. The lowest and highest amount of root surface coverage in study by Dado *et al* for canine was 78% and 85%. In present study the lowest and highest amount of root surface coverage for canine tooth at pre-operative evaluation are 20.00% and 82.11%, and the corresponding values at 9 month post-operative are 77.74% and 93.71% respectively.

Rosenstein *et al*. [10] assessed the ratio of root surface covered by bone on CT scan and radiographs and they have expressed the value directly as a ratio, while Dado *et al* [9] using the same series of subjects have expressed the values in percentage. In the present study the values are expressed as percentages.

The mean ratio of root surface covered by bone on radiographs as assessed by Rosenstein *et al*. [10] was 0.792 ± 0.175. In the present study the mean percentage of root support on IOPA radiograph expressed as percentage for central incisor is 48.44% ± 28.60 preoperatively, 90.51% ± 2.82 at three month postoperative evaluation and 88.70% ± 4.44 at six month postoperative evaluation. In the present study the mean percentage of root surface covered by bone on IOPA radiograph expressed as percentage for canine is 46.23% ± 24.90 pre operatively, 88.96% ± 6.12 at three month postoperative evaluation and 87.97% ± 7.76 at six month postoperative evaluation.

In the present study the mean percentage of root surface covered by bone on 3D radiograph expressed as percentage for central incisor is 43.58%±19.94 pre- operatively, 90.70%±5.40 at three month postoperative evaluation and 90.64%±6.70 at six month postoperative evaluation. And the mean percentage of root surface covered by bone on 3D radiograph expressed as percentage for canine is 54.27%±15.05 preoperatively, 92.30%±4.92 at three month postoperative evaluation and 91.03%±6.60 at six month post-op evaluation.

The highest and lowest ratios of root surface covered by bone in the study by Rosenstein *et al*[12] were 1.00 and 0.401 respectively while in the present study the corresponding values for central incisor on Occlusal radiographs are 71.42% and 8.33% respectively on pre-operative, 100.00% and 82.22% at 3 month post-operative & 100.00% and 83.33% at 6 month post-operative respectively. The corresponding highest and lowest values for canine on occlusal radiographs are 66.66% and 26.66% respectively on preoperative, 100.00% and 85.71% at 3 month postoperative & 100.00% and 81.81% at 6 month postoperative respectively.

Platelet rich plasma (PRP) is a rich source of growth factors like PDGF, TGF-β, IGF, VEGF etc, which help in faster recovery from various injuries [11]. This property is utilized in Secondary alveolar bone grafting for repair of cleft alveolus. Platelet rich plasma mixed with corticocancellous bone harvested from iliac crest, ribs, cranial bones etc has been used by various researchers [12]. PRP has also been shown to significantly reduce bone resorption at one year post surgery [13] in the grafted site as compared to controls. PRP may preserve the width and the height of the graft better than only cancellous bone graft, making autogenous cancellous bone grafting with PRP useful for alveolar bone grafting in cleft patients [11-13].

The present study is an attempt to determine the amount of bone growth occurring after secondary alveolar bone grafting using a combination of PRP with cortico-cancellous bone.

Conclusion

Platelet rich plasma (PRP) is a rich source of growth factors like PDGF, TGF-β, VEGF etc which help in faster initiation of healing process and this property has applications in treatment of musculo-tendinous injuries, cardiothoracic surgeries, neurosurgery, cosmetic surgery etc. The use of PRP combined with autologous cancellous bone grafts in the repair of alveolar clefts by SABG is a relatively new choice for oral surgeons and has proponents, both for and against its use.

PRP is an easily available and safe source of growth factors and when mixed with pluripotent cells of iliac leads to faster initiation of healing process, earlier formation of bone-bridge across cleft sites and also enhances the osteoneogenesis of alveolar bone in cleft patients. It may also be useful in subsequent orthodontic treatment. It may be considered as a valid protocol for the reconstruction in patients with alveolar clefts. The low price and low morbidity associated with its use make it more attractive for its employment in cleft patients. We do recommend its use in repair of alveolar clefts by SABG by using meticulous surgical techniques specially tension free closure of the defect which is critical for the success of surgery.

The limitations of the present study are its small sample size which has been limited by factors of economy. Accurate and accepted methods of evaluating three dimensional structures on two dimensional radiographs are yet to be established beyond doubt. We have not assessed the bone bridge formed qualitatively.

Future studies on larger samples may further refine the results and reduce errors in estimates.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article
Kothari M, Patil SG, Shah A, Joshi U, Kothari C. PRP Augmented Iliac Corticocancellous Graft: Enhanced volume retention and stability in Bilateral Alveolar Cleft Reconstruction. International Journal of Applied Dental Sciences. 2026;12(1): 09-13.