A Prospective Study Involving the Use of Platelet Rich Plasma in Enhancing the Uptake of Bone Grafts in the Oral and Maxillofacial Region

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Abstract Platelet-rich plasma (PRP) is an autologous product that contains highly concentrated number of platelets in a small volume of plasma, derived from whole blood by gradient density centrifugation. It has been speculated that local growth factors in human platelets (insulin-like growth factor, IGF; transforming growth factor, TGF- β ; platelet derived growth factor, PDGF) would enhance healing of grafts and also counteract resorption. The aim of this study was to evaluate efficacy of PRP on early healing after autogenous bone grafting. Of the twenty patients selected ten were treated with autogenous bone graft and PRP (PRP group) and other ten with autogenous bone graft alone (non-PRP group). PRP group consisted of two benign tumor of mandible, one post surgical defect, two unilateral alveolar cleft, one bilateral alveolar cleft with skeletal class III malocclusion, one maxillary hypoplasia, one oronasal fistula, one recurrent tumor of mandible, one multiple impacted mandibular teeth. Non-PRP group consisted of seven benign tumor of jaw, one keratocyst odontogenic tumor, one orbital blow out fracture, one residual traumatic defect. Biopsies were taken in the native bone, PRP treated grafted bone, grafted bone without PRP at 3 months to assess the maturity of bone. Radiographic imaging was performed by panoramic radiography at 3 and 6 months to evaluate bone opacity of grafted bone on comparison with native bone and computerized tomography at 6 months to evaluate grafted bone morphologically and to measure bone density in Hounsfield

units. Microscopic results showed that significantly more matured bone was formed at PRP treated sites as that of native bone and immature bone in controls after 3 months of healing. Bone opacity of PRP treated bone grafts was close to that of native bone than that of non-PRP treated bone grafts on panoramic radiograph at 3 and 6 months. There was graft loss in three cases and graft resorption in one case of non-PRP treated bone grafts at 6 months. In PRP group the compact bone was clearly differentiated from cancellous bone as in native bone and thick in five cases, thin in five cases. In non-PRP group the compact bone was thin as a whole. Comparing native bone group and PRP group the CT value of PRP treated bone graft was more or less close to native bone group and comparing native bone group and non-PRP group CT value was low in non-PRP treated bone graft. Whereas when comparing PRP and non-PRP group CT value was higher in PRP group. Autologous PRP was a safe, biocompatible, effective, source for growth factors and carries no risk of transmissible diseases. It enhances and accelerates bone regeneration of autogenous bone grafts.

Keywords Platelet-rich plasma · Mandibular reconstruction · Nonvascularised bone grafts · Alveolar bone grafting · Hounsfield unit

Introduction

Loss of mandibular continuity due to neoplasm, trauma, or infection results in major esthetic and biologic compromise. Functional disability involving speech, mastication, deglutition, and airway patency varies greatly, but is invariably present. The primary goal of reconstruction is full restitution of function, which secondarily leads to

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normalization of the cosmetic deformity [1]. Cohen and Schultz [2] credited Sykoff [3] as the first to reconstruct the mandible with an autogenous bone graft in 1900. Since then, many graft and graft-stabilizing materials and techniques have been advocated for mandibular reconstruction [2, 4]. Reestablishment of mandibular continuity with an adequate quantity and quality of bone ideally should allow dental rehabilitation with osseointegrated implants and an osseoprosthesis [5]. While there are many methods and modes of reconstruction of mandible, they have their own advantages and disadvantages. Though vascularised grafts are considered as most successful, they require extra expertise and equipment and higher cost make them unaffordable to common man. In the case of non-vascularised bone grafts, apart from graft failure, the resorption of the grafted bone poses serious concern for surgeon. These complications have made the surgeons to look for the adjuvant in order to improve both graft take up and at the same time to avoid graft resorption in patients undergoing reconstructive procedures with autogenous non-vascularised bone grafts. One of the recent advances made towards success of free bone graft is using of platelet rich plasma (PRP) as an adjuvant with bone graft. This study is to assess the efficiency of PRP in enhancing the bone healing and bone regeneration in patients undergoing reconstructive procedures with non-vascularised bone grafts in the maxillofacial region.

Aims and Objectives

The aim of this study is to evaluate the efficacy of PRP on early healing after autogenous bone grafting and to assess new bone formed at PRP treated bone graft sites qualitatively and quantitatively, by means of bone biopsies for histological evaluation after 3 months, bone consolidation by OPG taken at 3rd and 6th months post-operatively, bone density of newly formed bone in grafted area by means of CT scan in Hounsfield units (HU).

Material and Methods

Twenty patients from our out patient department, who need reconstruction in the oral and maxillofacial region were included in the study. Ten patients were planned for autogenous bone grafting with PRP and ten patients were planned for autogenous bone grafting alone (Table 1). All patients were evaluated clinically and radiographically. Patients who needed similar reconstruction procedure were subjected to bone grafting using PRP in one such patient as a study case and another patient as a control without use of PRP. On necessitation of two side bone grafting, one side

was grafted with adjunctive PRP (test) and other side without (control).

Preparation of Platelet-Rich Plasma

Autologous PRP was prepared from freshly drawn venous blood of the patient. All patients were subjected to withdrawal of 500 ml of whole blood from peripheral vein of the arm at the blood bank 1 day before reconstructive surgery. In addition 63 ml of citrate phosphate dextrose was added to achieve anticoagulation. The cell separation was done in two steps, the first at 5,600 rpm and second at 2,400 rpm. By means of cell separator PRP was separated from platelet poor plasma with red blood cells as described by Marx et al. [6] (Fig. 1). PRP (~60 ml) was temporarily saved in blood bag and stored in platelet agitator till it was required next day for surgery. The platelet-poor plasma with red blood cells was transfused back into the patient immediately after the preparation of PRP at blood bank. Intra-operatively harvested bone graft was kept in a tray containing a small amount of PRP. Autogenous thrombin was attained by adding 0.33 ml of CaCl_2 (18 mg/ml) to 10 ml of anticoagulated PRP in a small glass bowl. After approximately 6 min a gel was formed. This was then gently squeezed and the solution slowly extracted from it was used as autogenous thrombin. By means of initiating clot formation, the ratio of 4 ml of PRP to 1 ml of the autogenous thrombin was contained in a bowl. After approximately 2 min the bowl contained a gel that was used together with the bone for grafting [7] (Fig. 2).

Results

Clinical Evaluation after Reconstruction (PRP Group and non-PRP Group) (Table 1)

Post-operative infection was seen in three cases of non-PRP group who presented with pus discharge and exposure of graft intraorally 4th month post-operatively. Bone formation was microscopically observed 3rd month post-operatively (Table 1). In all cases (PRP group) the hardness was equal to that of native bone. In (non-PRP group) hardness was equal to that of native bone in five cases. Less than that of native bone in three cases. $P = 0.034$ (statistically significant). Microscopic observation of bone formation of 3rd month postoperative biopsies of the defects treated with PRP showed more mature bone (Fig. 3) with better organized trabeculae and greater bone regeneration. Biopsies of the defects not treated with PRP showed immature bone in five cases and necrotic bone in three cases (Fig. 4). $P = 0.001$ (statistically significant). Observation of bone formation by panoramic tomography 3rd,

Table 1 Showing PRP and NONPRP group with preoperative and postoperative evaluation

Case	Patient name	Age/ sex	Group	Primary disease	Treatment done	Approach	Type of bone graft	Postop infection	Macroscopic
1	Guruvaiah	60/ M	Non PRP	Central ossifying fibroma	Segmental resection and reconstruction with NVBG.	Extra oral	Cortico cancellous block	+	—
2	Srinu	28/ M	Non PRP	Central ossifying fibroma	Segmental resection and reconstruction with NVBG	Extra oral	Cortico cancellous block	+	—
3	Pochamma	35/F	Non PRP	Keratocyst odontogenic tumor	Right hemimandibulectomy and reconstruction with NVBG	Extra oral	Cortico Cancellous block	+	—
4	Vijaya lakshmi	19/ M	Non PRP	Ameloblastoma	Segmental resection and reconstruction with NVBG	Extra oral	cancellous block	—	+
5	Seetamma	40/F	Non PRP	Ameloblastoma	Segmental resection and reconstruction with NVBG	Extra oral	Cortico cancellous block	—	+
6	Neela	14/F	Non PRP	Ameloblastoma	Segmental resection and reconstruction with NVBG	Extra oral	Cortico cancellous block	—	+
7	Sangappa	38/ M	Non PRP	Orbital blow out fracture	Orbital floor reconstruction	Subciliary incision	Cortical	—	Not assessed
8	Ravinder Reddy	29/ M	Non PRP	Complex odontoma	Segmental resection and reconstruction with NVBG	Intra oral	Cortical	—	+
9	Ramulamma	60/F	NON PRP	Ameloblastoma	Segmental resection and reconstruction with NVBG	Extra oral	Cortico cancellous block	—	+
10	Amitha	22/F	NON PRP	Residual traumatic defect	Orbital floor reconstruction, malar augmentation	Coronal incision	Cortical	—	Not assessed
11	Rehmatunisa	25/F	PRP	Central ossifying fibroma	Segmental resection and reconstruction with NVBG	Extra oral	Cortico cancellous block	—	+
12	Saba Sultana	22/F	PRP	Post surgical defect of anterior mandible	Reconstruction with NVBG	Extra oral	Cortico cancellous Block	—	+
13	Edukondalu	21/ M	PRP	Alveolar cleft	Secondary alveolar bone grafting	Intra oral	Cancellous bone marrow	—	+
14	Shivaram	22/ M	PRP	Alveolar cleft	Secondary alveolar bone grafting	Intra oral	Cancellous bone marrow		+
15	Samatha	23/F	PRP	Bilateral alveolar cleft	Secondary alveolar bone grafting	Intra oral	Cancellous bone marrow	—	+
16	Dhana shekar	35/ M	PRP	Maxillary hypoplasia	Onlay bone grafting	Intra oral	Onlay	—	+
17	Chandramma	40/F	PRP	Oro nasal fistula	Bone grafting	Intra oral	Cancellous bone marrow	—	+
18	Laxmi narayana	53/ M	PRP	Recurrent ameloblastoma	Segmental resection and reconstruction with NVBG	Extra oral	Cortico cancellous block	—	+
19	Srinivas	23/ M	PRP	Ameloblastoma	Segmental resection and reconstruction with NVBG	Extra oral	Cortico cancellous block	—	+
20	Shravan	22/ M	PRP	Marfan syndrome,	Extract of multiple impacted mandibular teeth, sockets packed with cancellous bone marrow, chips	Intra oral	Cancellous bone marrow	—	+

Table 1 continued

Case	Microscopic	OPG 3rd month	OPG 6th month	Compact bone	CT value in HU			
					Native bone		Grafted bone	
					Mean	SD	Mean	SD
1	–	+		Absent	–	–	–	–
2	–	+		Absent	–	–	–	–
3	–	+		Absent	–	–	–	–
4	+	+	+	Thin	799.8	565.5	857.9	378.8
5	+	+	+	Thin	596	848	117.4	310.6
6	+	+	+	Thin	562.8	822.4	241.5	386.3
7	Not assessed	Not assessed	Not assessed	Not assessed	–	–	–	–
8	+	+	+	Thin	611.4	417.9	525.9	788.8
9	+	+	+	Absent	–	–	–	–
10	Not assessed	Not assessed	Not assessed	Not assessed	–	–	–	–
11	++	++	++	Thick	560.8	739	1,131	354.2
12	++	++	++	Thick	367.1	293.6	604.2	394
13	++	++	++	Thin	706.6	328.3	967.9	273.5
14	++	++	++	Thin	650.6	404	467.1	250.9
15	++	++	++	Thin	806.6	400	992.9	292.5
16	++	++	++	Thick	1162.2	303.3	848.1	848.1
17	++	++	++	Thick	397.1	297.6	304.2	184
18	++	++	++	Thick	370.4	713.1	47.3	221
19	++	++	++	Thick	902	344.2	504.2	324
20	++	++		Thick	798	420	604.2	394

++ Double positive, + positive, – negative

Fig. 1 Cell separator

6th month postoperatively in PRP group showed that bone opacity was close to that of native bone but still lower on panoramic tomography in two cases, while it was equal or nearly equal in eight cases at 3rd and 6th month postoperatively. In non-PRP group bone opacity was close to

Fig. 2 PRP Gel

and lower than that of native bone in eight cases at 3rd month. At 6th month it was far lower than that of native bone in three cases and was close to and lower than that of native bone in five cases. $P = 0.001$ (bone opacity on 3rd month OPG was statistically significant) (Table 1).

Fig. 3 Section $\times 10$ magnification shows native or mature bone with uniform staining, lacunae with viable osteocytes. Osteoblastic or osteoclastic activity seen at peripheries. Resting and reversal lines are seen

Fig. 4 Section $\times 10$ magnification shows immature bone with pale eosinophilic uniform staining of newly deposited young bone. Extensive osteoblastic rimming, larger vascular spaces, canals and haphazardly arranged osteocytes within lacunae are seen

$P = 0.02$ (Bone opacity on 6th month OPG was statistically significant).

Morphological analysis (Figs. 5, 6). In PRP Group the compact bone was clearly differentiated from cancellous bone as in native bone. It was thick in five cases and thin in five cases. In non-PRP group the compact bone was thin as a whole in four cases and was not clearly differentiated from the cancellous bone. $P = 0.011$ (statistically significant). CT value measurement (The CT value of the native bone (PRP group) was 2,700 HU maximum, -291 HU minimum, 672.14 HU on average (SD: 258.35 HU) compact + cancellous bone was included. The CT value of the grafted bone (PRP Group) was 1,997 HU maximum, -104 HU minimum, 819.25 HU on average (SD: 219.40 HU) compact + cancellous bone was included. $P = 0.20$ (not

Fig. 5 PRP group CT findings

statistically significant) (Fig. 5). The CT value of the native bone (non-PRP group) was 2,230 HU maximum, -312 HU minimum, 642.5 HU on average (SD: 106.81 HU) compact + cancellous bone was included. The CT value of the grafted bone (non-PRP group) was 1420 HU maximum, -332 HU minimum, 409.22 HU on average (SD: 73.65 HU) compact + cancellous bone was included. $P = 0.04$ (statistically significant). Comparing native bone group and PRP group the CT value in PRP group was close to that of native bone group; whereas when comparing PRP and non-PRP group CT value was higher in PRP group. $P = 0.001$ (statistically significant) (Fig. 6).

Discussion

Platelet-rich plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma. Therefore, the term PRP is preferred to autologous platelet gel, plasma-rich growth factors (PRGFs), or a mere autologous platelet concentrate [8]. PRP is normal autogenous blood clot that contains a highly concentrated number of platelets. Because it is the patients own blood, it is free of transmissible diseases and cannot cause hypersensitivity reactions. The minimum platelet count required for a blood clot to qualify as PRP may be arguable, but concentration of about 1 million platelets/l, or about 4–7 times the usual baseline platelet count (200,000 platelets/l) has been shown to provide clinical benefits.

Fig. 6 Non-PRP group CT findings

The scientific rationale behind the use of these preparations lies in the fact that growth factors (GFs) are known to play a crucial role in hard and soft tissue repair mechanisms. These GFs exhibit chemotactic and mitogenic properties that promote and modulate cellular functions involved in tissue healing and regeneration and cell proliferation. PRP works via the degranulation of the α granules in platelets, which contain the synthesized and prepackaged GFs. The active secretion of these GFs is initiated by the clotting process of blood and begins within 10 min after clotting. More than 95 % of the presynthesized GFs are secreted within 1 h [9]. Therefore, PRP must be developed in an anticoagulated state and should be used on the graft, flap, or wound, within 10 min of clot initiation.

Ideally a bone graft for ridge reconstruction will have osteogenic, osteoconductive, and osteoinductive properties which are all available in autogenous bone, which offers mineralized structures and pluripotential undifferentiated mesenchymal cells with osteogenic potential. Because PRP enhances osteoprogenitor cells in the host bone and in bone grafts [10], it has found clinical applications in fully autogenous bone grafts and composites of autogenous bone grafts with a variety of bone substitutes with as little as 20 % autogenous bone [11]. In the present study the findings are significant. On visual inspection and palpation the hardness of the newly formed bone was more or less similar to that of the native group in both control and test groups. Only three of ten cases in control group had less hardness mainly because of graft rejection. But

radiologically and histologically the difference between the two groups were more evident. Radiographically on a panarogram the bone opacity was close or some times more than that of the native bone in test group. The opacity in the grafted area was evident after 3 months and progressively increased to the maximum by 6th month in those cases treated by PRP group. Whereas in non-PRP group virtually there was no bone opacity in three cases. Naturally these are the cases which ultimately had graft rejection. But in other four cases of control group the bone opacity was obviously less than that of the native bone. Only in two cases in control group no assessment was carried out due to technical difficulties as the reconstruction was carried out for orbital floor. The microscopic examination of the grafted bone had ratified the above findings. Grafted areas were biopsied on 3rd month post operatively. The biopsies of the defects treated with PRP showed more mature bone with better organized trabeculae and greater bone regeneration with uniform staining, lacunae with viable osteocytes. Osteoblastic or osteoclastic activity were seen at peripheries. Resting and reversal lines were present. These findings are more or less similar to the findings as reported by Anitua [12]. Biopsies of the defects treated without PRP showed immature bone with less organized trabeculae and less regenerative bone than native bone. Also in three cases of the control group there was complete dead bone. In cases treated without PRP the bone was immature 3 months following grafting. This shows that the PRP enhances wound healing and bone maturation.

The CT findings were more specific. All cases whether control or test was subjected to CT scan 6th month post-operatively. In all cases treated by the PRP group hard thick compact bone could be easily and clearly differentiated from cancellous bone as in native bone. In addition it was thick in eight cases and thin in ten cases. The difference was because of cortico cancellous block bone grafting in eight cases and secondary alveolar bone grafting with cancellous bone marrow in two cases. In non-PRP group the compact bone was thin as a whole in four cases and was not clearly differentiated from the cancellous bone. Two cases which were of orbital reconstruction could not be assessed due to technical reasons, three cases (non-PRP group) were of graft rejection, one case (non-PRP group) of graft failure.

The bone density was measured in terms of Hounsfield Units [13]. The CT value of Graft treated with PRP was more or less close to that of native bone and CT value of native bone was higher in non-PRP group. When comparing PRP group and non-PRP group, CT value was higher in PRP group as reported by Iwamoto et al. [14], thus signifying the value of PRP in regeneration of the grafted bone with compact and viable bone. These gross, histopathological and radiological findings indicate that

PRP definitely plays a major role in enhancing the osteogenic potential of the grafted bone. The PRP with its inherent GFs enhances the uptake of bone thereby increasing the chances of bone take up in nonvascularised bone grafts. The grafting of the continuity deformities is always an uphill task for every maxillofacial surgeon particularly when it is nonvascularised bone grafts, as we have done in the present study. The most challenging situation for a surgeon is graft rejection or graft failure.

Kazanjian [15] listed the cardinal pre-requisites of successful bone grafting of mandibular defects: bone transplantation into healthy tissues, recipient area with adequate blood supply, wide contact between adjacent bone and graft and positive fixation. All these measures were meticulously followed by us but yet there was graft rejection in three cases in non PRP group. Biopsies revealed total dead bone and ultimately the rejected bone had to be completely removed by means of a secondary surgery so that the patients could be relieved of their agony. These patients were then managed by placing training flange appliance to bring the rest of the mandible into correct occlusion. Significantly there were no signs of post-operative infections or graft loss in the test group treated by PRP, once again reemphasizing the fact that the PRP plays a major role in graft acceptance and graft regeneration. Three cases of alveolar clefts and one case of oro nasal fistula secondary to trauma was included in the test group with PRP. These cases have been grafted with corticocancellous bone harvested from ilium. All these patients had fistulas prior to surgeries. Grafting by PRP enhanced the growth and the bone regenerated and successfully closed all alveolar clefts in addition to eliminating all fistulas. A well planned surgical technique combined with grafting with PRP yielded excellent results. One case of Marfan syndrome (PRP group) had multiple impacted teeth in the mandible. Surgical extraction of all impacted teeth made mandible weak and hence cancellous bone marrow with cortical chips were placed in the defect with PRP. The bone formation was excellent with good compact bone to enable us to place implants and rehabilitate the patient with endosseous implant supported complete denture prosthesis. In two cases (non-PRP group) proper analysis by means of clinical, radiological, histopathological assessment could not be carried out because of technical reasons. These were the cases where orbital floor was reconstructed with iliac crest bone graft, zygoma, nasal augmentation done by calvarial bone graft. Obviously analyzing the success of graft by means of clinical, radiological and by biopsy is just next to impossible for the reasons well-known to us. Therefore these two cases have been included in the study but without assessment. The success of these two cases were merely on the basis of clinical outcome which was good in both cases.

The results of the quantitative analysis of the graft sites show that the mean bone loss was seen in four cases in non-PRP group and was absent in PRP group at 6 months after surgery. It appears that the resorption of regenerated bone that occurs post-operatively may be reduced significantly by using PRP. It has been believed that the fibrin networks of PRP might aid the decrease in post-operative bone resorption. The function of fibrin networks is an osteoconductive scaffold [16, 17] and thus the fibrin gel would have provided a matrix for cell growth and differentiation by enhancing three-dimensional intercellular interactions or cell adhesion, both of which are thought to be good environments for the maturation of osteoblasts [18]. With respect to the biologic effect of PRP on bone regeneration in a graft, the present results support the findings of Marx et al. [6], who found that a combination of PRP and autogenous bone graft can increase the rate of osteogenesis and enhance bone formation qualitatively. In the present study we have preferred autologous PRP to readymade blood bank PRP as autologous PRP is safe, not associated with any transmissible diseases. To avoid possible development of coagulopathies associated with bovine thrombin, the use of an alternative method for PRP activation should be considered. Most of the evidence on the clinical potential of PRP comes from case series and case reports. While it has been recognized that these types of studies represent the starting point, they are not definitive. Despite the evident clinical advantages of PRP, evidence of their beneficial actions is still lacking—a requirement for the justification of their widespread use. Additional randomized, controlled clinical trials are warranted to test the long term benefits and ultimate surgical outcomes associated with PRP.

Conclusion

To conclude PRP with various GFs appears to have positive synergistic effects both on vascular ingrowth and bone healing. PRP is an autologous preparation; thus it eliminates concerns about disease transmission or immunogenic reaction. These GFs exhibit chemotactic and mitogenic properties that promote and modulate cellular functions involved in tissue healing and regeneration and cell proliferation. Because of this the use of PRP in combination with non-vascularised bone grafts is believed to be advantageous in skeletal reconstruction. PRP improves the handling characteristics of grafts, the rate of bone formation and the quality of bone formed. To avoid possible development of coagulopathies associated with bovine thrombin, the use of an alternative method for PRP activation should be considered. The need for evidence-based clinical decisions and for well-controlled randomized

clinical studies to assess the ideal concentration of the different GFs and to determine whether PRP application to different bone graft substitutes and different barrier membranes provide a beneficial effect is required.

References

1. Kyle D, Keller EE (1990) Reconstruction of mandibular discontinuity with autogenous Iliac bone graft. *J Oral Maxillofac Surg* 48:336–346
2. Cohen M, Schultz RC (1985) Mandibular reconstruction. *Clin Plast Surg* (3):411
3. Sykoff W (1900) Zur Frage der Knochenplastik am Unterkiefer. *Zentralbl Chir* (35):881
4. Branemark P-I, Lindstrom J, Hallen O (1975) Reconstruction of the defective mandible. *Scand J Plast Reconstr Surg* 9:116
5. Keller EE, Desjardins RP, Eckert SE (1988) Composite bone grafts and titanium implants in mandibular discontinuity reconstruction. *Int J Oral Maxillofac Implants* 3:261
6. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR (1998) Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 85:638–646
7. Thor A, Franke-Stenport V, Johansson CB, Rasmusson L (2007) Early bone formation in human bone grafts treated with platelet-rich plasma: preliminary histomorphometric results. *Int J Oral Maxillofac Surg* 36:1164–1171
8. Marx RE (2004) Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg* 62:489–496
9. Kevy S, Jacobson M (2001) Preparation of growth factors enriched autologous platelet gel. In: *Proceedings of the 27th Annual Meeting of Service Biomaterials*, Minnesota, April 2001
10. Whitman DH, Berry RL (1998) A technique for improving the handling of particulate cancellous bone and marrow grafts using platelet gel. *J Oral Maxillofac Surg* 56:1217–1218
11. Garg A (2000) The use of platelet rich plasma to enhance the success of bone grafts around dental implants. *Dent Implantol Update* 11:17–21
12. Anitua E (1999) Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofac Implant* 14:529–535
13. Hounsfield GN (1973) Computerized transverse axial scanning (tomography). *Br J Radiol* 46:1016–1022
14. Iwamoto M, Matsuo A, Kato N, Takeuchi S, Takahashi H, Hojo S, Chiba H (2009) Computed tomographic evaluation of bone quality of the mandible reconstructed by particulate cellular bone and marrow combined with platelet rich plasma. *Oral Sci Int* 6:63–72
15. Kazanjian VH (1952) Bone transplanting of the mandible. *Am J Surg* 83:633
16. Breine U, Johanson B, Rockert H (1965) Human autogenous bone grafts. Studies of the fate of grafts to the hard palate in clefts by using tetracycline ultra-violet fluorescence technique and X-ray microscopy. *Acta Chir Scand* 129:250
17. Lozano AJ, Cestero HJ Jr, Salyer KE (1976) The early vascularization of onlay bone grafts. *Plast Reconstr Surg* 58:302
18. Keller EE, Triplett WW (1987) Iliac bone grafting: review of 160 consecutive cases. *J Oral Maxillofac Surg* 45:11