INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 21, no. 2, 393-399 (2008)

THE USEFULNESS OF ^{111}INDIUM-OXINE AUTOLOGOUS PLATELET GEL GRAFT IMAGING TO EVALUATE OSTEOINDUCTION IN PATIENTS UNDERGOING SURGERY OF JAW BONE DEFECTS

G. SAMMARTINO, L. CELENTANO[1], M. TIA, E. D'AGOSTINO[2], A. FUSCO, S. TETÈ[3] and M. OTTIERO[4]

Department of Dental and Maxillo-Facial Sciences, University of Naples "Federico II"; [1]Nuclear Medicine of the Department of Biomorphological Science, University of Naples "Federico II"; [2]Department of Immunoematology, University of Naples "Federico II"; [3]Department of Dental Sciences, University of Chieti "Gabriele d'Annunzio", Chieti; [4]Pharmacy Unit, University of Naples "Federico II", Naples, Italy

Received February 14, 2008 – Accepted May 9, 2008

Autologous platelet gel (AGP) is a source of concentrated growth factors contained in the platelet granules used to enhance bone quality and, especially, quicken bone formation in regeneration techniques, and also ameliorate the haemostasis in anti-coagulated patient management. The purpose of this study is to describe a technique to perform labelling of autologous platelet-gel with ^{111}In -Oxine and to evaluate its usefulness, as a marker of bone osteoinduction by means of scintigraphy, after *in vivo* application in patients with jaw bone defects following cystic lesion enucleation and the extraction of deeply impacted lower third molar. All patients included in the study presented mandible bone defects following cyst enucleation or deeply impacted lower third molar extraction. In sterile conditions, ^{111}In-Oxine AGP was added during the bone-milling phase of the graft preparation and then applied to the bone defects. The scintigraphy was performed 2 hours after the application of labelled AGP (early scan) and at 24, 48, 72, 384 hours (delayed scan). At early scan all the patients presented a high concentration of ^{111}In-Oxine AGP, which was easily recognized at the level of jaw defect. Limited diffusion of AGP was seen in the tissue surrounding the bone defect; this activity was attributed to the presence, in the PRP, of a quote of autologous granulocytes, as marker of inflammatory process, which was labelled with ^{111}In-Oxine. In order to demonstrate the persistence and stability of labelling AGP, abdominal scintigraphies were performed to assess the presence of activity in the liver, spleen and bone marrow. None of the patients presented appreciable activity in these organs. The labelled AGP topically applied showed high uptake values, without statistically significant activity in the surrounding tissues or in critical organs during the early phase, as well as in delayed controls, and confirmed a very low grade of loss of ^{111}In-Oxine from the bone defect. The scintigraphy represents a useful method of assessing the success of surgical procedure for jaw bone defects performed with autogenous grafts. It is well accepted by the patients, offering at the same time a sensitive method of studying uptake of topically applied AGP and to follow up kinetics of AGP in order to correlate quantitative data of the platelet gel life span with evolution of the bone remodelling process. Finally, the labelled granulocytes around the bone defect allow to assess the inflammatory process evolution derived from the surgical technique.

Key words: platelet gel, bone defect, bone remodelling, inflammation, scintigraphy

Mailing address: Prof. S. Tetè,
Department of Oral Science,
University of Chieti,
Via dei Vestini, 31
66100 Chieti, Italy
Tel: ++39 0871 3554122 - 4095
e-mail: tete@unich.it

0394-6320 (2008)

Platelet gel is an autologous source of concentrated growth factors contained in the platelet granules (i.e. Platelet Derived Growth Factor and Transforming Growth Factor-β) used to enhance the bone quality and, in particular, the bone formation speed in regeneration techniques and to ameliorate the haemostasis in anti-coagulated patient management (1-3)

Platelet gel has recently attracted the interest of periodontal, oral, maxillofacial, orthopaedic and dermatological surgeons for its useful effects on bone healing and its proven ability to enhance bone regeneration (osteoinduction). Osteoinduction represents the process of new bone formation after the activation of multivalent mesenchymal host cells and their differentiation in chondroblasts and osteoblasts, mainly produced by growth factors, generally named bone morphogenetic proteins (4-11).

^{111}In-oxine and technetium-99m hexamethylpropylene amine oxime (HMPAO) have been the radiopharmaceuticals predominantly used for platelet radiolabelling. ^{111}In can be used to monitor the distribution of platelets, their kinetics and their survival *in vivo* and to image lesions with abnormal platelet uptake (12-17). Oxine (8-hydroxyquinoline), owing to the simple handling technique and its commercial availability, has gained wide acceptance as an ^{111}In -chelator for routine platelet labelling.

The purpose of this study is to describe a technique for labelling autologous platelet-gel with ^{111}In-Oxine and to evaluate its usefulness, as a marker of bone osteoinduction by means of scintigraphy, after *in vivo* autograft, in patients with jaw bone defects following cystic lesion enucleation and the extraction of deeply impacted lower third molars.

MATERIALS AND METHODS

Patients

Five healthy patients (2 males and 3 females), non-smokers aged 21-35 years, were selected at the Oral and Maxillo-Facial Department of Naples University. All patients included in the study presented mandible bone defects following cyst enucleation (3 cases) or deeply impacted lower third molar extraction (2 cases). All patients underwent routine controls including endoral radiography, and CT Dental Scan (DS). The radiological studies were used to characterize the jaw bone defect (shape, density and location).

AGP preparation

Consent was obtained from the patients to carry out HIV and HBV testing. Briefly, 40cc to 60cc of blood was taken from each patient. The blood was separated by centrifugation into platelet-rich plasma (PRP), platelet-poor plasma (PPP), and red blood cells (RBC). The red blood cells were discarded. The PRP (comprising approximately 10% of the total blood volume tested) had platelet counts of 500,000-3,000,000/mm^3 (18-20).

AGP labelling

All procedures described were performed in laminar flow chamber. The platelet pellet (approx. 1x10^9 platelets/mL) was re-suspended in Tyrodes-HEPES (134 mM NaCl, 0.34 mM Na_2HPO_4, 2.9 mM KCl, 12 mM $NaHCO_3$, 20 mM HEPES, 5 mM glucose, 1 mM $MgCl_2$, pH 7.3) plus EDTA (1 mM); the ^{111}In-Oxine (37 MBq) containing 0.4 mL of TRIS buffer, was then added to the solution and incubated at room temperature for 30 min. After incubation the radioactive PRP was washed with ACD (saline) and centrifuged at 2500 g. The pellet was re-suspended in 5 mL of previously stored PPP. The labelling efficiency of the pellet was evaluated and, finally, the solution containing PRP labelled with ^{111}Indium Oxine (37 MBq) was ready to make the platelet gel.

Immediately after labelling procedures, the 111 Indium Oxine PRP was transferred into the surgery care unit where the activation was carried out by adding calcified thrombin to labelled PRP. Within 3 to 5 seconds the solution assumed a gel-like consistency forming platelet gel.

Surgical Application of ^{111}In-Oxine AGP

In sterile conditions, the ^{111}In-Oxine AGP was added during the bone-milling phase of the graft preparation and then applied topically into the defect. The topical application of AGP into the bone defect was made with primary closure of the wound in order to avoid labelled platelet dispersion in the mouth.

Imaging

The scintigraphy was performed 2 hours after the application of labelled AGP (early scan) and at 24, 48, 72, 384 hours (delayed scan) by means of a GE STARPORT 400 AT gamma camera and with SPET (ECAT Siemens Gamma Camera) equipped with medium energy collimator. The uptake of the platelet into the bone defect (recognized in the planar opposite view of the jaw (right and left lateral scintigraphy) as in trans-axial slice reconstruction of the SPET imaging) was determined by

means of the ROI generation on digital imaging display.

In vitro sampling

The radioactivity of the plasma samples collected at 2, 24 h and at lapse time = 24 h for 7 days, were used for the plasma clearance determinations and for *in vitro* studies of the platelet-gel survival time.

Istomorphometry

In order to quantify the defect area, subjective delineation between new bone and original/ existing bone was determined by two surgeons blinded to the treatment groups and the purpose of the study. After the irregularly shaped (non-rectangular) defect area was identified high resolution images were acquired at a magnification of 20x using a Spot digital and a Pentium IBM-based computer with expanded memory capabilities.

Data acquisition

After ^{111}In-Oxine AGP had been topically implanted, all patients were submitted to scintigraphy by means of an ECAT scan gamma camera equipped with medium energy high resolution collimator. The peak of 111 Indium spectrum gamma was centred on 172 Kev and 245 Kev (window=20%). Planar and tomographic data were collected; the planar frames were acquired on 128 x 128 matrix in preset time (300 seconds / frame). Tomography was performed in "step and shot" mode with circular orbit (180° starting at – 90°) (1 frame/ 6°/20 s/30 frame). The sagittal plane comprised palatal region and gained sub-mandibular plane. All data were stored in digital mode by means of PC.

Data analysis

The scintigraphies were visually inspected by two physicians who are experts in nuclear medicine and who were blinded to the procedure performed. Data from imaging pair of ROI were generated on the area of the graft implanted and recognized on trans axial slice. The counts collected in each ROI (pixel equivalent) were corrected for attenuation. The uptake index was then calculated from geometric mean determination, as previously described. The values of the geometric mean of counts were then time decay corrected and plotted against the time for life span determinations.

RESULTS

We performed the ^{111}In-Oxine-labelled platelet-gel SPET study on 5 patients. All patients presented early high concentration of ^{111}In-Oxine AGP at the level of the surgical graft, which was easily recognized at the level of the jaw defect (Fig. 1). Limited diffusion of AGP was seen - in scintigraphies performed at delayed controls (24, 48, 96, 384 hours) - in the tissue surrounding the bone defect, generally in the joined sub-mandibular compartment; this activity was attributed to the presence, in the PRP, of a quote of Autologous Granulocytes which was labelled with ^{111}In-Oxine; this quote after gel formation was included and after grafting procedure migrated to tissue inflamed from surgery procedures.

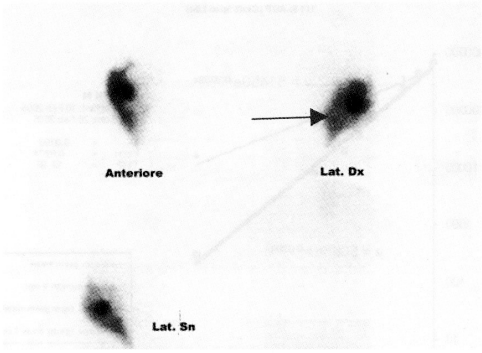

Fig. 1. *^{111}In-AGP Scintigraphy 24 hours after surgical graft into lower jaw bone defect. The labelled gel was applied in the surgically exposed bone defect at the level of lower first molar region. The arrow indicates diffusion of the granolocytes in the inflamed tissue, as scintigraphy showed at 24 hours.*

To demonstrate the persistence and stability of labelling AGP, abdominal scintigraphies were carried out (in anterior and posterior projection) to assess the presence of activity in liver, spleen and bone marrow. None of the patients presented appreciable activity in those organs. The plotted data relative to activity in autograft ROI generated onto images of early and delayed scans showed bicompartimental kinetics in all patients studied.

An example of the curve of ^{111}In-AGP Life Span in the Graft area, delineated on ROI generated onto graft area, is shown in Fig. 2.

All patients in this study gave informed consent. They all showed high concentration of ^{111}In-Oxine AGP (LE% = 86%; range: 82 ± 88%) at the site of application of the Gel, during follow-up all patients were submitted to scintigraphies starting from 2 h after surgery. All patients showed at scintigraphy

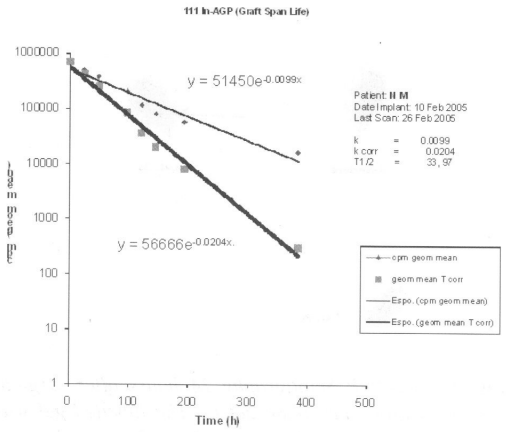

Fig. 2. *The plotted data of values related to the geometric mean uptake of ^{111}In AGP implanted in jaw defect against time in the same patient as illustrated in Fig. 1.*

high Target/non-Target ratio (T/nT) = 3.5. Limited diffusion of ^{111}In-AGP in surrounding tissues was detected, in delayed scan performed at 24 h, 48 h, 96 h and until 15 days after implant (Fig. 2). The data of disappearance curve of radioactivity (T ½ corrected) were employed to the span life calculation of ^{111}In-AGP (Fig. 3); in the patients studied, the life span of AGP resulted = 25 ± 9.2 h (mean±SD). The imaging of the abdomen performed at 24 h and 48 h showed lack of activity in the liver and spleen; no activity was seen in the bone marrow. This data suggests stability of the labelling in the gel until 15 days after surgery. All patients were enrolled in the present study after radiological study of the bone lesions of the jaw performed with endoral radiographies and CT (Dental Scan).

The follow-up showed active bone remodelling in the bone defect after 3 months.

The results of ^{111}In-AGP scintigraphy carried out on the patients studied is reported in Table I. Table I shows the value of life span, labelling efficiency calculated before AGP application in the jaw defect, the diameter of bone defect (as resulted from radiological evaluation for measuring the lesion during surgery), and shape change of the same lesion (as resulted from radiology performed at 6 months follow up).

DISCUSSION

Autologous platelet gel (AGP) was developed in the early 1990s as a product of multicomponent aphaeresis (21-23). When platelet concentrate is combined with thrombin and calcium gluconate, a viscous coagulate (gel) is rapidly formed.

Several studies have also shown that *in vitro* tissue cultures are enhanced by the addition of platelets (24). Platelet derived wound healing factor (PDWHF), an extract of activated platelets, has been shown to enhance healing of cutaneous ulcers.(25-27). Platelet derived growth factor as an isolated cytokine has been shown to enhance wound healing in several animal models and non-healing wounds in humans (28-33).

The gel possesses tensile strength and adequate adhesive ability due to platelet/fibrin interaction (34).

Native concentrations of fibrinogen lead to effective fibrinolysis and resorption of the platelet gel.

Autologous platelet gel contains supraphysiologic amounts of platelet derived growth factors (50-60 ng/mL) in a sustained medium at the site of wound healing.

Benefits of multiple growth factors acting synergistically on platelet derived tissue growth factors: enhanced wound healing by autocrine and paracrine mechanisms. Human platelets contain platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-beta) in their alpha granules. PDGF has been shown to play a role in periodontal regeneration. It has been demonstrated that TGF-beta has a very potent effect

Table I. *Values of ^{111}-Indium Oxine AGP lifespan (time in hours), Labelling Efficiency Percentage(LE %), Diameter of Bone Defect (D mm), Shape Change of defect (SC mm) in patients.*

Patients	AGP LifeSpan (h)	LE %	D mm	SC mm
1	17	88	20	4
2	18	89	24	10
3	13	92	24	9
4	34	95	40	10
5	10	89	26	8

on cells associated with bone (35-36).

Recently Garcia et al. (37), using narrow pI range two-dimensional electrophoresis (2-DE) for protein separation followed by high-throughput tandem mass spectrometry (MS/MS) for protein identification, identified 760 protein features, corresponding to 311 different genes, resulting in the annotation of 54% of the pI 5-11 range 2-DE proteome map. The author evaluated the physicochemical properties and functions of the identified platelet proteome and concluded that the main group of proteins identified is involved in intracellular signalling and regulation of the cytoskeleton.

No studies have been carried out on AGP kinetics *in vivo* after grafting. In the present study we first developed and described a labelling technique of AGP with ^{111}In-Oxine, according to Thakur's method and to the procedures suggested by the International Society of Radioabelled Blood Elements (ISORBE) in 1999 (16, 38).

Platelet concentrate in our patients caused a marked increase in proliferative activity and the continued differentiated activities, including matrix formation and mineral deposition, of osteoblast-like cells.

In the present study, we describe a new approach to labelling the autologous platelets and gel forming with ^{111}In-Oxine (^{111}In-Oxine AGP). The first results showed a high efficiency of the labelling procedure; in our group of patients mean value of Labelling Efficiency (LE%) = 86%. The labelled AGP topically applied by surgeons showed high uptake values, without statistically significant activity (T/nT ratio = 3.5) in the surrounding tissues or in critical organs in the early phase and also in the delayed controls, and confirms a very low grade of loss of ^{111}In-Oxine from the bone defect.

The only appreciable activity was seen in tissues surrounding bone jaw lesions and it was probably due to the small number of autologous granulocytes in the PRP and included in the labelled AGP– during GEL forming phase. The autologous granulocytes, in fact showed high affinity to ^{111}Indium-Oxine. Already proven as a sensitive inflammatory seeking agent, the labelled granulocytes around the bone defect allow to assess the inflammatory process evolution derived from surgical technique.

Scintigraphy represents, in our opinion, a useful method for assessing the success of surgical procedure for jaw bone defects performed with autogenous grafts. It is well accepted by patients, and, at the same time, offers a sensitive method for studying the uptake of topically applied AGP and the follow-up kinetics of AGP allowing to correlate quantitative data of the life span of platelet gel with the evolution of the bone remodelling process.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Maria Ottiero, for her valuable suggestions, quality control and guidance for the labelling technique of AGP with ^{111}Indium-Oxine, and for help given in the compartmental analysis of the radio compounds employed.

The authors also wish to thank Maria Triassi, PhD for Prophylactic engineering which was aimed at minimizing employee exposure by prompt and correct removal or disposal of hazardous materials. This ensured proper employee isolation from a hazardous environment. Sharp instruments and breakable materials were disposed of in puncture-resistant containers; self-sheathing needles were used when available. All other contaminated materials were disposed of in yellow double bags labeled contaminated hazardous waste.

REFERENCES

1. Sammartino G, Tia M, Marenzi G, di Lauro AE, Claudio PP. Use of autologous platelet-rich plasma (PRP) in periodontal defect treatment after extraction of impacted mandibular third molars. J Oral Maxillofac Surg 2005; 63(6):766-70.
2. Della Valle A, Sammartino G, Marenzi G, Tia M, di Lauro AE, Ferrari F, Lo Muzio L. Prevention of postoperative bleeding in anticoagulated patients undergoing oral surgery: use of Platelet-Rich Plasma. J Oral Maxillofac Surg 2003; 61:1275-1278.
3. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss GE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85:638-46.
4. Kale AA, Di Cesare PE. Osteoinductive agents:

Basic science and clinical applications. Am J Orthop 1995; 24:752-61.
5. Martin P, Hopkinson-Woolley J, McCluskey J. Growth factors and cutaneous wound repair. Prog Growth Factor Res 1992; 4:25-44.
6. Rabie AB, Chay SH. Clinical applications of composite intramembranous bone grafts. Am J Orthod Dentofacial Orthop 2000; 4:375-83.
7. Rose LF, Rosenberg E. Bone grafts and growth and differentiation factors for regenerative therapy: a review. Pract Proced Aesthet Dent 2001; 19:725-34.
8. Stevenson S. Biology of bone grafts. Orthop Clin North Am 1999; 4:543-52.
9. Urist M, Strates B. Bone morphogenetic protein. J Dent Res 1971; 50:1392.
10. Urist MR, Mikulsky A, Boyd SD. A chemosterilized, antigen extracted autodigested alloimplant for bone banks. Archives of Surgery 1975; 110:416-29.
11. Urist MR. Bone Morphogenetic Protein. In: *Bone Grafts & Bone Substitutes.* Eds. MB Habal and AH Reddi), WB Saunders Co. 1992; 7:80-93.
12. Thakur ML, Welch MJ, Malech HL. Indium-111 labelled human platelets: studies on preparation and evaluation of *in vitro* and *in vivo* functions. Thromb Res 1976; 9:345–357.
13. De Vries RA, De Bruin M, Marx JJM, van de Wiel A. Radioisotopic labels for blood cell survival studies: a review. Nucl Med Biol 1993; 20:809–817.
14. Najean Y. The choice of tracer for platelet kinetics and scintigraphic studies. Nucl Med Biol 1986; 13: 159–164.
15. Sinzinger H, Virgolini I. Nuclear medicine and atherosclerosis. Eur J Nucl Med 1990; 17:160–178.
16. Thakur ML, Welch MJ, Malech HL. Indium-111 labelled human platelets: improved method, efficacy and evaluation. J Nucl Med 1984; 22:381–385.
17. Tsan MF. Kinetics and distribution of platelets in man. Am J Hematol 1984; 17:97–104.
18. Marx RE. University of Miami School of Medicine, Miami, FL, Kevy SV, Jacobson MS. Center for Blood Research Laboratories, Boston, MA, Platelet Concentrate Preparation in the Office Setting: A Comparison of Manual and Automated Devices, 2001; p. 1.
19. Kevy SV, Jacobson MS. Platelet Concentrate Preparation in the Office Setting: A Comparison of Manual and Automated Devices 2001; p. 1.
20. Marx RE. Platelet-Rich Plasma (PRP): What Is PRP and What Is Not PRP?; Implant Dent 2001; 10:225-8..
21. Marx RE., Garg AK. Bone structure, metabolism, and physiology: its impact on dental implantology. Implant Dent 1998; 7:267-276.
22. Garg AK. Grafting Materials in Repair and Restoration in Tissue Engineering - Applications in Maxillofacial Surgery and Periodontics 5; 83-101.
23. Stefani CM, Machado MA, Sallum EA, Sallum AW, Toledo S, Nociti FH Jr. Platelet-derived growth factor/insulin-like growth factor-1 combination and bone regeneration around implants placed into extraction sockets: a histometric study in dogs. Implant Dent 2000; 126-130.
24. Abernathy W et al. Non-metallic fixation in elective maxillofacial surgery, AORN J 2000; 71:193-198.
25. Marx RE: Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent 2001; 10(4):225-8.
26. Lekovic V et al. Preservation of alveolar bone in extraction sockets using bioabsorbable membranes. J Periodontol 1998; 69:1044-1049.
27. Bell D. Particles versus solid forms of hydroxyapatite as a treatment modality to preserve residual alveolar ridges. J Prosthetic Dent. 1986; 56:323-326.
28. Bodo M, Muzi G, Bellucci C, Lilli C, Calvitti M, Lumare A, Dell'Omo M, Gambelunghe A, Baroni T, Murgia N. Comparative *in vitro* studies on the fibrogenic effects of two samples of silica on epithelial bronchial cells. J Biol Regul Homeost Agents 2007; 21:97-104.
29. Roberts AB et al. Physiological actions and clinical applications of transforming Growth Factor-beta (TGF-beta).Growth Factors 1993; 8:1-9.
30. Mori G, Brunetti G, Colucci S, Ciccolella F, Coricciati M, Pignataro P, Oranger A, Ballini A, Farronato D, Mastrangelo F, Tetè S, Grassi FR, Grano M. Alteration of activity and survival of osteoblasts obtained from human periodontitis patients: role of TRAIL.J Biol Regul Homeost Agents 2007; 21:105-14.
31. Kassolis J, Rosen PS, Reynolds MA. Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: Case Series. J Periodontol 2000; 1654-1661.
32. Marx RE, Carlson ER et al. Platelet-rich plasma - growth factor enhancement for bone grafts, Oral

Radiol Endod 1998; 85:638-646.
33. Mastrangelo F, Scioletti AP, Tranasi M, Tecco S, Sberna MT, Vinci R, Grilli A, Stuppia L, Gherlone E, Tete S. Dentin sialophosphoprotein expression during human matrix development. J Biol Regul Homeost Agents 2007; 21:33-9.
34. Seibert JS, Salama H. Alveolar ridge preservation and reconstruction, Periodontol 2000 1996; 11:69-84.
35. de Obarrio JJ, Arauz-Dutari JI, Chamberlain TM, Croston A. The use of autologous growth factors in periodontal surgical therapy: platelet gel biotechnology--case reports. Int J Periodont Restorative Dent 2000; 20(5):486-97.
36. Jensen TB, Rahbek O, Overgaard S, Soballe K. Platelet rich plasma and fresh frozen bone allograft as enhancement of implant fixation. An experimental study in dogs. J Orthop Res 2004; 22(3):653-8.
37. Garcia A, Prabhakar S, Brock CJ, Pearce AC, Dwek RA, Watson SP, Hebestreit HF, Zitzmann N. Extensive analysis of the human platelet proteome by two-dimensional gel electrophoresis and mass spectrometry. Proteomics 2004; 4(3):656-68.
38. Rodrigues M, Sinzinger H, Thakur M et al. On behalf of the International Society of Radiolabelled Blood Elements (ISORBE). Labelling of platelets with indium-111 oxine and technetium-99m hexamethylpropylene amine oxime: suggested methods Eur J Nucl Med 1999; 26:1614–1616.