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Review Article

Plasma Growth Factors in Dental Implantology

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Abstract

Maxillofacial surgery and dental implantology are the fields that Platelet Rich Plasma (PRP) has experienced more visible development. However there is a strong controversy and debate as to the usefulness of the PRP in the recovery of dental alveolar bed with lyophilized bone plasty. Some authors objectifying alveolar increased, improving the healing of the soft tissues and facilitating greater cohesiveness particulate graft, which would useful in dental implantology. Others are more pessimistic when it comes to reproduce these results, due to the large differences in growth factors present in the PRP, according to the method for obtaining the final product applied. This led to think that higher concentration of these factors would be more effective regeneration, promoting the use of systems that got higher levels of growth factors. Far from achieving the desired effect, *in vitro* otherwise completely it observed when the concentration factor exceeds a certain level. Hence the strong controversy arose in its use in many cases fueled by the lack of systematic obtaining PRP that may be incorrect. The objective of this review is to define that is called plasma rich in platelet growth factors or more commonly known as Platelet-Rich Plasma (PRP), the most accepted methods of production in scientific literature and those clinical applications in Dental implantology where there has been more scientific evidence to use.

Keywords: Centrifugation; Implantology; Leucocyte Growth Factors (LGF); Plasma Growth Factors (PGF); Platelet Rich Plasma (PRP)

Physiology of Platelets and Plasma Growth Factors

Platelets are enucleated cell fragments derived from the cytoplasm of megakaryocytes in the bone marrow. Traditionally the best known function is in the process of primary hemostasis, as they are essential for clot formation, but also play an important role in inflammation, immunity, tumor progression and course thrombosis. By electron microscopy, it shows that platelets contain various organelles: mitochondria, peroxisomes, ribosomes and glycogen granules, the latter are divided into three types:

1. **Alpha:** containing fibrinogen, Von-Willebrand factor, platelet derived growth factor, ectodermal growth factor, vasculo-endothelial growth factor, insulin-like growth factor 1 and other growth factors, as can be seen in table 1.
2. **Dense delta:** that containing ADP, ATP, serotonin, epinephrine, norepinephrine and dopamine.
3. **Lambda:** they are lysosomes, which help dissolve the clot once it has fulfilled its function. Similar Vesicules have been found in Lysosomes at cytoplasm of leuko-mononuclear cells.

Content	Feature
Chemokines, cytokines Platelet factor 4 B-tromboglobina RANTES* Macrophage inflammatory protein 1-alpha Interleukin 1 and 8	Regulation of inflammation, chemotaxis
Adhesive proteins Thrombospondin 1 and 2 Fibrinogen Fibronectin	Cell interactions and coagulation
Growth Factors Platelet Derived Growth Factor (PDGF) Transforming Growth Factor B (TGF-B) Epidermal Growth Factor (EGF) Vaculo Endothelial Growth Factor (VEGF) Insulin-like Growth Factor-1 (IGF-1) Hepatocyte Growth Factor (HGF) Brain-Derived Neurotrophic Factor (BDNF)	Cell proliferation and differentiation, chemotaxis angigénesis, extracellular matrix synthesis
Immunoglobulin's Ig-A, Ig-E, Ig-M and Ig-G	Immunological
Clotting factors V and VIII	Thrombin production
Von-wilebrand factor	Platelet adhesion to sub endothelial collagen
Plasminogen activator inhibitor	Inhibition of fibrinolysis
P-selectin	Leukocyte-platelet interaction

Table 1: Summary of the proteins in the platelet alpha granules.

* RANTES: Regulated on Activation Normal T-cell Expressed and Secreted

Besides the classic functions described in platelets, recent discoveries in their ability to protein synthesis, mRNA containing copies of almost 1/3 of known proteins in the human genome, despite the lack of core, has totally changed the perception they had of them, recognizing their ability to synthesize proteins to changes in their environment. Also being investigated are also some non-genomic functions of these factors, such as its effect on signaling pathways involving platelet activation and its role in *de novo* synthesis of both pro-and anti-inflammatory factors. The enormous amount of growth factors contained in platelet alpha granules, the ability to *de novo* synthesis of proteins and their microbicidal and modulating inflammation activity, favor the proliferation and cellular immunomodulation and synthesis of extracellular matrix, promoting healing, wound repair and other tissue damage. These functions are precisely those that have led to

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propose the use of autologous platelet-rich plasma for the repair and regeneration of various tissues [1].

The Major Platelet Growth Factors of the Most Known Function are

PDGF (Platelet-Derived Growth Factor)

Its main function is indirectly to promote angiogenesis via macrophages by a mechanism of chemotaxis. Activates macrophages has a significant mitogenic activity on mesenchymal and osteoblast cells as well facilitates the formation of type 1 collagen.

TGF-alfa/beta (Transforming Growth Factor-alfa/beta)

Its main mission is to chemotaxis. Induces proliferation and differentiation of mesenchymal cells. It promotes collagen synthesis by osteoclasts. It is pro-angiogenic tissue, inhibits osteoclast formation and proliferation of epithelial cells in the presence of other factors. Induces differentiation of neuronal stem cells.

FGF (Fibroblast Growth Factor)

Enables the proliferation and differentiation of osteoclasts, fibroblasts and induction of fibronectin by stem cells; inhibit osteoclast action. It is an important pro-angiogenic action chemotactic activity on endothelial cells.

IGF-1 (Insulin-Like Growth Factor 1)

It induces the proliferation and differentiation of mesenchymal cells and like coating has a potent mitotic effect on stem cellularity. It facilitates the synthesis of osteocalcin, alkaline phosphatase and type 1 collagen by osteoblasts.

VEGF (Vasculo Endothelial Growth Factor)

Enables chemotaxis and differentiation of endothelial cells, it promotes blood vessel permeability.

EGF (Epidermal Growth Factor)

Great proapoptótotical capacity, chemotaxis and differentiation of epithelial cells, renal, neural, glial and fibroblasts.

BDNF (Brain-Derived Neurotrophic Factor)

Induces the proliferation, differentiation and neuronal chemotaxis, microglia and oligodendrocytes cellularity and remyelination thereof.

HGF (Hepatocyte Growth Factor)

Its main function of cell proliferation and differentiation, chemotaxis, angiogenesis and extracellular matrix synthesis found in human blood plasma of peripheral blood compared to average achieved in platelet quality rich plasma.

Table 1 shows concisely the types of growth factors obtainable in platelet rich plasma and its main physiological function of the tissues. Likewise in table 2 reflect normal levels of growth factors that can be found in human blood plasma of peripheral blood compared to average achieved in platelet quality rich plasma.

Definition of Platelet Rich Plasma

The PRP is an autologous concentration of human platelets in a small volume of plasma which represents an increase over normal baseline platelet levels, making it a source of easy access to growth factors contained therein. It has a pH between 6.5 and 6.7. It comes from the patient's own blood, so it is free of communicable disease and cannot cause hypersensitivity reactions. The platelet count of PRP is optimal debatable. According to the competent authority, it must contain a level of platelets higher than the basal serum levels considered normal (between 200,000 and 450,000 platelets/mm^3). But increasingly the authors dedicated to this area considered a PRP quality when platelet counts obtained in the final product exceeds 1,000,000/mm^3. Alcaraz et al., have demonstrated a prevalence of VEGF and TGF-B growth factors in those enriched leukocyte PRPs, while platelet- PRPs, without leucocytes have higher concentrations of growth factors type PDGF-AB and IGF-1.

	Peripheral Blood	PRP
PDGF-AB (10-50 pg/ml)	45 pg/ml	360 pg/ml
TGF-B1 (10-70 pg/ml)	35 pg/ml	320 pg/ml
VEGF (15-85 pg/ml)	55 pg/ml	560 pg/ml
IGF-1 (5-20 pg/ml)	13 pg/ml	175 pg/ml
Platelets (150.000-350.000/mm^3)	265.000/mm^3	1.250.000/mm^3
Leucocytes (3.200-9000/mm^3)	5.600/mm^3	20.000/mm^3
Granulocytes	60% (3.330/mm^3)	24% (480/mm^3)
Mononuclears	35% (1.960/mm^3)	70% (14.000/mm^3)
CD 34+	1-2/mm^3	175/mm^3

Table 2: Levels of growth factors and cell count in peripheral blood and PRP: (Alcaraz, Oliver y col protocol) [2].

Methods of Production

Methods of obtaining and preparing PRP are very diverse, depending on whether a single or double centrifugation procedure is used, the same time and the type of filter used and which has more than 40 currently in the market.

Regarding the temperature, according to most experts consulted for the production of a proper PRP, optimal temperature during the process should be in the range between 16 and 22°C. This temperature range is the greater capacity of concentration of platelet and growth factors, as is maintaining a greater platelet survival regardless of the type of procedure and filter used with a mean platelet count of 1,150,000/mm^3 range (750,000 to 1,500,000/mm^3), as well as levels of platelet and plasma factors growth between 5 and 7 times higher than normal levels found in peripheral blood [3].

Depending mainly on the type of filter or pipetting and centrifugation procedure used, can obtain different plasma components, e.g., platelet-rich in growth factors, platelet-rich plasma and poor in growth factors, plasma rich in growth factors and poor in platelets or plasma rich in platelets and leukocytes.

It has not found a clear correlation between the ability of higher platelet concentration levels and platelet growth factors determined in the final product regardless of the filter type and process used in its manufacture. Neither difference was observed in her final product regardless of the type of procedure and filter used in relation to age and gender. It seems certain according to recent studies that these plasmas rich in leukocytes contain higher levels of growth factors VEGF and TGF-B, while the platelet rich plasma without use of the buffy coat

concentrate would be achieved as many factors PDGF growth rate and IGF-1 [3].

From the above, it would be possible to prepare what is called a PRP on demand, depending on the plasma and cellular fraction which want to strengthen depending on the clinical application that you want to give [4].

As for the cell part contained in the PRP, I must say that in those PRPs rich in leukocytes with use of the Buffy-Coat the final spin fraction leukocyte concentration increases between 3 and 5 times more than in peripheral blood predominance mononuclear (90% of total leukocyte, up to 15% of which have positive staining for CD 34+).

Of all the methods of obtaining, 5 procedures stand on the other, since they are the most standardized and used by most authors. 2 which use a dual system of centrifugation, whereas in the other 3 the centrifugation process is unique. Table 3 shows these four methods and the centrifugation used in each end and the average platelet concentration obtained [4-6] are specified.

Comparing these five methods of obtaining PRP, we can see that the largest number of platelets obtained equivalent to an average of 342,80% from baseline whole blood count corresponds to the protocol for obtaining Alcaraz y col (275%) [1,2], followed by Garcia et al. (195%) [7]. Anitua and Andía standardized technique [8] with an average baseline platelet count in peripheral blood of 90.31%. Forth centrifugation procedure Okuda and Kawase et al. [9], found 31.74% final platelet count from baseline obtained and finally around 5.29% would be the protocol for obtaining PRP designed by de Obarrio et al. and Camargo et al. [10,11].

Activation of the PRP requires replacement of calcium and initiation of the blood coagulation cascade. For Anitua authors as this is achieved by adding calcium chloride at 1% (1cc), others as Marx used in conjunction with this bovine thrombin solution (1.5cc); unlike Anitua not describe its use, there is some controversy as to the use thereof as thrombin antibodies have been detected in patients who have been treated by activating the PRP with this procedure [8].

Using a larger amount of activator solution, far from being beneficial, is counterproductive, because a larger volume of this solution does not accelerate the process of coagulation activation, but its rate of formation reduced or completely inhibited by diluting the fibrinogen, an important factor clot formation [4,6,8,12,13].

Finally the use of systemic or intravenous PRP does not require prior activation of the end product, since its entry in to the bloodstream produces natural activation through own serum ionized calc.

Legal Framework in Application of PRP

Currently the end of PRP concentrate lacks sheet for your application.

Basically prescribing this autologous concentrate should be performed by physicians, dentists or podiatrists within their field of clinical action. Although preparation is by a third party ultimately responsible to ensure the characteristics of the person will be prescriptive.

Prior to obtaining the product, the patient must undergo a pre-analytical control serological, biochemical and hematological to verify the suitability or otherwise of treatment. With respect to the latter it must be said that countries like Argentina have specific rules for obtaining PRP plasma and the various fractions exclusive legal hematologist, applying the same rules as any type of blood product for autotransfusion.

During the production process, although there are numerous protocols as mentioned earlier, the current regulations, distinguishes on open procurement procedure, where no direct exposure to blood or any of its components during the process of handling the environment, in which case and following the rules of each region, the entire procedure should be performed under a sterile laminar flow hood or closed procedure, using specific filters with CE marking required, in which case, the rules must be followed manufacturer specific [1-3,5].

Dental Implantology

In the field of Implantology, we report the use of PRP in the preparation of maxillary bone for placement of implants; thus it is described that in the alveoli to which PRP are placed they show a greater buccolingual/palatal bone width, accompanied by a higher bone density and faster tissue coverage compared with patients in whom this compound was not used [1,14].

Probably, the benefits of PRP on implants may be related to the type of bone on which they act, most of the studies shown better clinical index corresponding to sites without previous grafts where growth factors could play an important role in vascularization. One radiological series clinical studies in 11 patients in whom were implanted at posterior mandibular area without grafting, no implant failure was observed, and it was shown that the use of PRP could lead an early bone apposition around the implant improving soft tissue healing [15].

Previously, *in vitro* studies have demonstrated a stimulatory effect of PRP on osteoblast proliferation that appears at the second week follow-up, increasing from the third week, and is maintained during the fourth week follow-up; so local application of PRP would increase the amount of newly formed bone around the implant increasing bone density [16]. In several studies on canines, it has been observed that application of PRP increases significantly the contact between bone and implant (P = 0.028).

Procedure	Authors	Centrifugation	Plasma Recentrifugation	Average Platelet Count
1	Garcia y col [7]	1800 rpm for 8 minutes uninterruptedly	1800 rpm	191,31%
2	Anitua y Andia [8]	1800 rpm for 8 minutes uninterruptedly	No	90,31%
3	Okuda y col [9]	2400 rpm for 10 minutes	3600 rpm for 15 minutes	31,74%
4	De Obarrio y col [10]	5600 rpm for 6 minutes uninterruptedly	No	5,29%
5	Alcaraz y col [2]	3500 rpm for 30 minutes uninterruptedly	No	342,80%

Table 3: Methods of obtaining PRP and average platelet count compared to baseline normal values.

In the dental area, most studies have focused on bone regeneration. Use PRP in sinus elevation and increased alveolar ridge, Kassolis et al. [17], published a study that determined the advantages of use in conjunction with lyophilized bone, but also indicate that it is necessary to have more studies that support this method. With regard to repair bone defects, Kaushick et al. [18], shown in a study with 10 patients diagnosed of periodontitis, in whom were used bone graft associated with PRP, comparing the results with the use of bone graft and serum in a control group. Comparatively a greater reduction of the sacks and better quality of bone was obtained in the patients of the PRP group with respect to the control one. Saini et al. [19], evaluated the use of PRP with tri calcium phosphate compared with the only use of alloplastic material in intraosseous defects. They concluded that associated use of the materials presents better clinical and radiographic results. Marukawa et al. [20], conducted a study in which they evaluated the ability to reduce bone resorption in fractured alveoli, in a case group of 14 patients in whom used PRP compared to 6 people in whom it was not used. It was concluded that the use of PRP decreases bone resorption.

Several clinical procedures have used the PRP observing their qualities in dental area. Whitman et al. [21], presented platelet gel for use as an adhesive in bone grafts that aided consolidation. The platelet gel is obtained and processed immediately in the operating room. Marx et al. [22], observed that a platelet concentrate obtained by blood centrifugation caused a high concentration of platelets in the graft and, as soon as the presence of growth factors captured by specific receptors from spongy bone cells.

It was also described that use of plasma growth factors offers a new and useful therapeutic tool in the acceleration of healing and bone maturation in maxillofacial and reconstructive surgery. In this regard, Marx et al. [22] and Fennis et al. [23]. Demonstrated that PRP improves bone regeneration and that platelets can act as local regulators of the healing process; in turn, the application of the PRP, increases the microcirculation of the gingival mucosa surrounding the wound. Other studies have shown that with a single 20pM application of a recombinant factor PDGF-B type, a significant effect can be achieved in increasing capillary density. A similar effect could be achieved in patients treated with PRP [24,25].

Nonetheless, there is no agreement in the literature about the success in bone integration of an implant. Our observations have shown lower rates of failure in the use of PRP compared with conventional techniques without PRP; although this difference is not statistically significant

Side Effects of Implementation of PRP

Using this technique provided, it is done according to current regulations regarding quality control and traceability is virtually free of side effects because it is an autologous product. It has several advantages such as non-transmission of infectious diseases: HIV, hepatitis or Creutzfeldt-Jakob disease; and is convenient for the patient, since the blood is collected in the immediate preoperative. Reported effects in most cases are banal: small hematoma or erythema at the puncture site or infiltration and mild fever for 24-48 hours due to inflammatory mediators, controllable with conventional antipyretics.

A fact that has traditionally been attributed to the PRP is its ability to produce an oncogenic effect through the activity of growth factors to activate certain antiapoptotic pathways of tumor clones in patients predetermined for it. This has been borne only explained by *in vitro* stimulation antiporter Na+ /K+ which could theoretically accelerate malignancy locally present, and even induce *de novo*; although there is some to suggest that the procedures of application of growth factors, could constitute a risk of neoplastic degeneration or influence tumor progression or metastatic spread in patients previously diagnosed with malignant cancer process evidence in medical practice [1,2].

Contraindication in Use of PRP

According to most authors consulted, the use of PRP is not recommended in patients with bleeding disorders, hemostasis, or treatment with oral anticoagulants or antiplatelet drugs, whole blood platelet counts below 100,000/mm^3, pregnancy, active infection or tumors by the effect of progression of the inflammatory process mediated by the infection as well as tumor dissemination theoretical in already diagnosed patients, which produce growth factors.

Conclusion

Surely we are facing a new era of treatment in the new field that is called regenerative medicine", with an extraordinary range of possibilities for increassed clinical applications; but requires a process of scientific and medical systematization that allows channel it safely and effectively in applications where really there is a enough scientific evidence weight for applying. To do this it is necessary two things: first the consensus of the authors engaged in the production and application of this therapy in order to standardize procedures for obtaining those more effective and allow adequate traceability and monitoring of the end product, depending of clinical application given them and secondly the design of clinical trials which management and establish appropriate guidelines to this purpose.

Today we are still so far from achieve, given that all existing clinical applications, the scientific evidence is weak, based on case series or case-control studies in the most positive assumptions. The growing presence of several protocols for obtaining, low control on the final product component and variety of clinical applications, difficult consensus on more reliable procedures and adequate collection and development of appropriate clinical trials to test them in different pathologies susceptible to it.

Revised everything published about the conclusion is that the PRP is well tolerated technique, restricted its use to prescribing by physicians, dentists and podiatrists, lacking tab Currently technique, and can not be considered standard treatment for any medical condition where intended to be used, if it is accepted that it could be used as adjunctive therapy with conventional ones to implement clinical and functional improvement of the patient.

They are necessary in the future of basic research and translational medicine to better understand the pathophysiological mechanisms underlying its regenerative effects.

Similarly sheet to establish a sound scientific studies are necessary in the form of clinical trials to standardize the techniques for obtaining both depending on the cellular composition of the final product as a protein obtained and is reproducible by all authors and specific management guidelines for each clinical application where feasible use in regenerative medicine.

Competing Interests

The authors declare that they have not competing interests.

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References

1. Alcaraz J, Oliver A, Sánchez-Juana M, Lajara J (2015) Clinical use of Platelet-Rich Plasma: A new dimension in Regenerative Medicine. Medical Science Review 2: 111-120.
2. Alcaraz J, Oliver A, Sánchez-Juana M, Lajara J (2015) Concentrations of growth factors in plasma enriched with platelets, leukocytes or erythrocytes. Descriptive study in 75 patients. Rev Hematol Mex 16: 294-305.
3. Klinger MH (1996) The storage lesion of platelets: ultrastructural and functional aspects. Ann Hematol 73: 103-112.
4. Weibrich G, Kleiss WK, Hafner G, Hitzler WE (2002) Growth factors level in platelets-rich plasma and correlations with donor age, sex and platelets count. J Craniomaxillofac Surg 30: 97-102.
5. Beca T, Hernández G, Morante S, Bascones A (2007) Plasma rico en plaquetas. Una revisón bibliográfica. Av Periodon Implantol 19.
6. Alsousou J, Thompson M, Hulley P, Noble A, Willett K (2009) The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery: a review of the literature. J Bone Joint Surg Br 91: 987-996.
7. García LA, Luces G (2006) Uso del plasma rico en plaquetas para la regeneración tisular en la terapia periodontal. Tésis monográficas, Caracas, USA.
8. Anutua E, Prado R, Sánchez M, Orive G (2012) Platelet-rich plasma: preparation and formulation. Oper Tech Orthop 22: 25-32.
9. Okuda K, Kawase T, Momose M, Murata M, Saito Y, et al. (2003) Platelet-rich plasma contains high levels of platelet-derived growth factor and transforming growth factor-beta and modulates the proliferation of periodontally related cells *in vitro*. J Periodontol 74: 849-857.
10. de Obarrio JJ, Araúz Dutari I, Chamberlain TM (2000) The use of autologous growth factors in periodontal surgical therapy: platelet gel biotechnology--case reports. Int J Periodontics Restorative Dent 20: 486-497.
11. Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, et al. (2007) YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol 17: 2054-2060.
12. Everts PA, Brown Mahoney C, Hoffmann JJ, Schonberger JP, Box HA, et al. (2006) Platelet-rich plasma preparation using three devices: Implications for platelets activation and platelet growth factors release. Growth Factors 24: 165-171.
13. Scherer S, Tobalem M, Vigato E, Heit Y, Modarressi A, et al. (2012) Nonactivated versus thrombin-activated platelets on wound healing and fibroblast-to-myofibroblast differentiation *in vivo* and *in vitro*. Plast Reconstr Surg 129: 46-54.
14. Birang R, Torabi A, Shahabooei M, Rismanchian M (2012) Effect of plasma-rich in platelet-derived growth factors on peri-implant bone healing: an experimental study in canines. Dental Research Journal (Isfahan) 9: 93-99.
15. Anand U, Mehta DS (2012) Evaluation of immediately loaded dental implants bioactivated with platelet-rich plasma placed in the mandibular posterior region: A clinico-radiographic study. J Indian Soc Periodontol 16: 89-95.
16. Altin N, Ergun S, Katz J, Sancakli E, Koray M, et al. (2013) Implant-supported oral rehabilitation of a patient with pemphigus vulgaris: a clinical report. J Prosthodont 22: 581-586.
17. Kassolis JD, Rosen PS, Reynolds MA (2000) Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: case series. J Periodontol 71: 1654-1661.
18. Kaushick BT, Jayakumar ND, Padmalatha O, Varghese S (2011) Treatment of human periodontal infrabony defects with hydroxyapatite + β tricalcium phosphate bone graft alone and in combination with platelet rich plasma: a randomized clinical trial. Indian J Dent Res 22: 505-510.
19. Saini N, Sikri P, Gupta H (2011) Evaluation of the relative efficacy of autologous platelet-rich plasma in combination with β-tricalcium phosphate alloplast versus an alloplast alone in the treatment of human periodontal infrabony defects: a clinical and radiological study. Indian J Dent Res 22: 107-115.
20. Marukawa E, Oshina H, Iino G, Morita K, Omura K (2011) Reduction of bone resorption by the application of Platelet-Rich Plasma (PRP) in bone grafting of the alveolar cleft. J Craniomaxillofac Surg 39: 278-283.
21. Whitman DH, Berry RL, Green DM (1997) Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg 55: 1294-1299.
22. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, et al. (1998) Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 85: 638-646.
23. Fennis JP, Stoelinga PJ, Jansen JA (2004) Mandibular reconstruction: a histological and histomorphometric study on the use of autogenous scaffolds, particulate cortico-cancellous bone grafts and platelet-rich plasma in goats. Int J Oral Maxillofac Surg 33: 48-55.
24. Spector M (1999) Basic principles of tissue Engeneering. Tissue engeneering: applications in maxillofacial surgery and periodontics. Quintessense Publishing, Illinois, USA.
25. Marx RE (1999) Platelet-rich plasma: A source of multiple autologous growth factors for bone grafts. Tissue engeneering: applications in maxillofacial surgery and periodontics. Quintessense Publishing, Illinois USA.