Evaluation of Intra-Osseous Defects Regeneration Treated by Platelet Rich Plasma Using Silver- Stained Nucleolar Organizer Regions (Agnors)

Mohamed A. Nassan [1], Hany G. Gobran[2], Abdelhamied Y. Saad[2] and Abdelbassit M. Abdelaal[3]

[1]Pathology Department, Fac. Vet. Med., Zagazig University, Egypt
[2]Oral Biology Department, Fac. Dental Med., Al-Azhar University, Egypt
[3]Surgery Department, Fac. Vet. Med., Zagazig University, Egypt
Moh_nassan@yahoo.com

Abstract: The purpose of this research was to evaluate using of silver stained nuclear organizer regions (AgNORs), in mandibular intra–osseous defects regeneration treated by Platelet-rich plasma (PRP) in 15 adult healthy male Mongrel dogs. Under general anaesthesia bilateral circular bony defects were created at the posterior border of the mandible. The bony cavity on the right side filled with autogenous PRP, and the left side was empty and used as a control. The animals were divided into three equal groups that were sacrificed after 1, 5 and 9 weeks postoperatively. Then, all specimens were harvested for AgNORs, histopathologic and densitometric analysis. Successful bone regeneration was observed at bone defect treated with PRP, in marked contrast to control groups. Both qualitative and quantitative AgNORs analysis showed highly significant difference between experimental and control groups. These *in vivo* results support the view that the PRP influence the bone formation within the PRP-treated bone grafts.

1. Introduction

Platelet-rich plasma (PRP) is a new approach in tissue regeneration which is prepared from the patient's own blood that eliminates concerns about immunogenic reactions and disease transmission (**To¨zu¨m and Demirapl, 2003**). Additionally, PRP contains proteins and growth factors which may activate several cell types involved in wound healing and bone regeneration. Furthermore platelets play a key role in homeostasis and wound healing. Platelets are chemotactic and induce proliferation of fibroblasts, endothelial cells and progenitor cells, regulating wound-healing (**Jung *et al.,* 2005 and Pallua *et al.,* 2010**).

The effect of PRP on bone cells may be due to the synergistic effects of many growth factors derived from platelets (**Bolander, 1992**). PRP stimulated cell growth and differentiation of rat bone marrow cells up to 8 days (**Van den Dolder *et al.,* 2006**). Use of PRP has been reported to improve bone healing in rabbits (**Kim *et al.,* 2001**) and to facilitate the incorporation of particulate cancellous bone grafts in goat mandibular reconstructions (**Fennis *et al.,* 2004**).

Nucleolar organizer regions (NORs) are chromosomal segments, which contain a number of acidic proteins that have a high affinity for silver (AgNOR) proteins (**Derenzini and Ploton, 1991**). At light microscopy, AgNOR proteins appear as well-defined black dots, which in interphase cells were exclusively located within nucleoli. Several investigators study the qualitative and quantitative changes during the cell cycle and showed that, the amount of AgNOR proteins can be a marker of proliferation, because this amount is related to cell cycle phases (**Sirri *et al.,* 2000**).

2. Material and Methods

The present study was carried out on 15 adult healthy male Mongrel dogs, aged 12 to 15 months and their weight ranged from12 to 17 kg. The experiment was carried out in the Department of Veterinary Surgery, Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were housed in separate cages, supplied with food and water tanks and allowed to live in optimal conditions according to the hospital housing protocol of the Veterinary Hospital, Zagazig University, Egypt.

Surgical procedures:
1- Preoperative preparation :
Food and water were kept away from the animals 12 hours prior to the operation to prepare them for anesthesia.

Platelet-rich plasma preparation
A 15 mL sample of autologous blood was taken from each animal, by cannulation of the recurrent cephalic tarsal vein, into a syringe (20 mL) containing 1.48 mL of acid citrate dextrose –A as anticoagulant (Sigma Aldrich, Germany). Saline solution 15 mL (Alnasr Company, Egypt) was injected through the tarsal vein to maintain the systemic blood volume of

the animal. The blood sample was then separated into PRP and red blood cells collected in platelet-poor plasma as described by Marx (2004). Cell separation was done by centrifugation at 5600 rpm for 10 minutes then at 2400 rpm, for 10 minutes to finally extract the platelets in a concentrated form in plasma. Furthermore, platelets count was done at Zagazig Common Laboratory for proper assessment. Importantly, the concentrations of thrombin and $CaCl_2$ (Sigma Aldrich,Germany) used are equivalent to those proposed in the study by Marx (2004). However, the PRP was aspirated with 0.5 ml of red blood corpuscles (RBCs) that were newly formed and highly active. Then the aspirated PRP and RBCs were mixed with 10% $CaCl_2$ and thrombin to change the PRP into the gel form.

2- General anesthesia:
Animals were anesthetized using intravenous injection of Thiopental sodium (E.I.P.I.Co 10th of Ramadan Egypt) in the recurrent cephalic tarsal vein in a dose of 25mg/kg b. wt.. Bilateral semilunar mucoperiosteal flap were created at both sides of the mandible at the buccal surface of the posterior border of the mandible using Bard Parker blade No 12. A blunt and sharp dissection was done to expose the bone of the mandibular angle.
Bilateral circular bony defects of 10mm diameter and 5mm depth were created using carbide rose head surgical bur number 6 mounted in a dental hand piece connected to a micro- motor with speed of 18.000 rpm. Bleeding was controlled and the created defect was irrigated with warm normal saline before grafting. The bony cavity on the right side was filled with autogenous PRP, and the left side was left empty as a control. The soft tissue incisions were closed with interrupted sutures using 3/0 silk. The wounds were inspected for any changes, cleaned with antiseptic solution (Betadine). Sutures were removed after 7 days.

Postoperative care
The animals received Dolphen–K® ampoule (Sodium Diclofenac) (Delta Pharm Company), 3ml intramuscularly every 12 hours for 3 days to control of pain. Animals were injected with Flumox (1000mg) every 24hours for seven postoperative days. Daily examination was carried out for the presence of signs of infection.

The animals grouping and scarification
In this study 15 animals were used and divided into 3equal groups, 5 animals each. The animals were sacrificed after 1, 5 and 9 weeks alternatively post operatively.

Assessment of bone regeneration

After euthanization of the animals by overdose intravenous injection of pentobarbital, the mandible were surgically removed and divided at the midline into two halves right and left one. Bone regeneration at both sides at the area of bone defect was assessed by densitometric analysis (DEXA) and AgNORs evaluation as a proliferative marker.

1- Densitometric analysis (DEXA)
The two mandibular halves were referred freshly for examination, by using dual-energy X-ray absorptiometry (DEXA) examinations after 1, 5 and 9 weeks. Bone mineral content (BMC) representing the weight of minerals in each region of interest (ROI) was measured in grams. Bone mineral density (BMD) represents the value of BMD in each ROI in units of gm/cm^3. These measures were done using a three sites axial scanner, bone mineral analyzer (Norland) at Al-Bahr Radiology center in Zagazig city. BMD of each bone defect was measured at the 5×5 mm^2 region of interest by DEXA. This instrument was calibrated with a phantom of known mineral content. Each scan was performed at a speed of 20 mm/s and the scanning length was 1 mm, then each specimen was then scanned. On the monitor, partial acquisition of images was shown while the scanner proceeds to analysis mode. When the scan finished the densitometric images saved and printed. All data were collected, tabulated, and statistically analyzed.

2- AgNORs analysis
Tissue specimens were retrieved using orthopedic electrical saw then fixed in 10% neutral buffered formalin for at least 24 hrs Decalcification was performed by immersion of the bony specimens in 5% formic acid for two months (Van der Lubbe *et al.,* 1988) and then routinely processed and paraffin embedded. Specimens were sectioned at 4-6μ thickness, and stained with AgNORs as a proliferative marker as the following (Trerè, 2000):
1. All the Sections were deparaffinized in xylene, and hydrated through 100% and 95% ethanol Reduction of sections was done with 1% potassium iodide for 60 min.
2. Silver staining solution was prepared by dissolving 2% gelatin in 1% formic acid at room temperature and filtered through syringe filter (Sigma Chemical Co., St. Louis, MO, USA).
3. One part of the solution was mixed with two parts of 50% silver nitrate immediately before use. Staining was done in dark at room temperature for 30 min.
4. The sections were immersed in 5% Sodium Thiosulphate for 5 min. Sections were washed in water, dehydrated in 95% and 100% ethanol, and left for 2 - 3 minutes in isopropanol. The sections were cleared in xylene and mounted in

permanent mounting media (Corbit-Balsam) (Eukitt, Hecht, Kiel-Hassee, Germany).

Counting of silver stained nucleolar organizer regions (AgNORs)

NORs appear as brown black dots inside the nucleus using silver stain. AgNORs counts were according to the recommendations standard protocol using the morphometeric method.

Statistical analysis:

Numerical data were presented as means (M) and standard deviation (SD) values. Analysis Of Variance (ANOVA) was used to compare between several means. Kruskal-wallis test was used when using median for non parametric results. Furthermore, t-test was used for comparison of control versus

experimental sides. Statistical analysis was performed by software programs, SPSS 14.0 (Statistical Package for Social Sciences) at level of significance $P \leq 0.05$. (Trere` *et al.,* 1995).

3. Results
STATISTICAL ANALYSIS) -Densitometric analysis (DEXA
Bone Mineral Content (BMC) & Bone Mineral Density (BMD)

No significant difference between control and experimental group was detected in group I while groups II & III showed highly significant difference ($P < 0.001$) between control and experimental group (Tables 1, 2).

Table (1): BMC in the different groups

Group	Type	Mean ±SD	(Range)	T	p
Group I (1 week postoperatively)	C	1.52 ± 0.08	1.41 – 1.61	1.5	< 0.7
	EXP	1.6 ± 0.08	1.49 – 1.7		
Group II (5 weeks postoperatively)	C	1.96 ± 0.05	1.87 – 2.01	11.62	< 0.001**
	EXP	2.286±0.03	2.25 – 2.32		
Group III (9 weeks postoperatively)	C	2.15 ± 0.05	(2.09 –2.23)	8.71	< 0.001**
	EXP	2.65 ± 0.12	(2.51– 2.81)		

Table (2): BMD in the different groups

Group	Type	Mean ± SD	(Range)	T	p
Group I (1week postoperatively)	C	0.604 ± 0.07	(0.5 – 0.67)	1.33	< 0.21
	EXP	0.65 ± 0.05	(0.59 –0.71)		
Group II (5 weeks postoperatively)	C	0.768 ± 0.06	(0.69 –0.82)	3.63	< 0.006**
	EXP	0.91 ± 0.06	(0.81– 0.97)		
Group III (9 weeks postoperatively)	C	0.926 ± 0.05	(0.88 –1.02)	4.7	< 0.0015**
	EXP	1.188 ± 0.11	(1.01– 1.31)		

Histopathological evaluation
Histopathological features of group I (After one week):
1- Control side

There were fine granulation tissue, dense inflammatory cells and congested blood vessels in the bone defects. In some specimens there were very small areas containing early stages of osteoid formation, which were very thin and short with very wide bone marrow spaces (Fig 1A).

2- Experimental side

Osteoid were formed in a large distribution rather than present in some specimens of control .These osteoid were very thin and surrounded by osteoblasts, large numbers of fibroblasts with open face nucleus. Furthermore, there were very wide bone

marrow spaces containing huge numbers of blood vessels (Fig 1B).

Histopathological features of group II (After five weeks):
1- Control side

There were thin osteoid with variable distribution, wide bone marrow spaces filled with granulation tissues and blood vessels (Fig 1C).

2- Experimental side

Characterized by the presence of osteoblast cells, woven bone, osteoid, variable reduction of the bone marrow spaces containing osteoblast cells, blood vessel, dense fibrous connective tissues (Fig 1D).

Histopathological features of group III (After nine week):

1- Control side

There were osteoid and markedly thick bone trabeculae, osteoblast cells, osteocyte, woven bone and thin bone marrow spaces containing blood vessels and fibrous tissues (Fig 1E).

2- Experimental side

The application of PRP as an autologous bone graft had beneficial effects on the bone development which appeared clearly in this group. The improvement in this group was reported by presence of normal haversian system including interstitial lamella, haversian canal and osteocytes, very narrow bone marrow spaces. These interesting features seen in this group mainly and previous groups treated with PRP was confirmed with the densitometric analysis and AgNORs (Fig 1F).
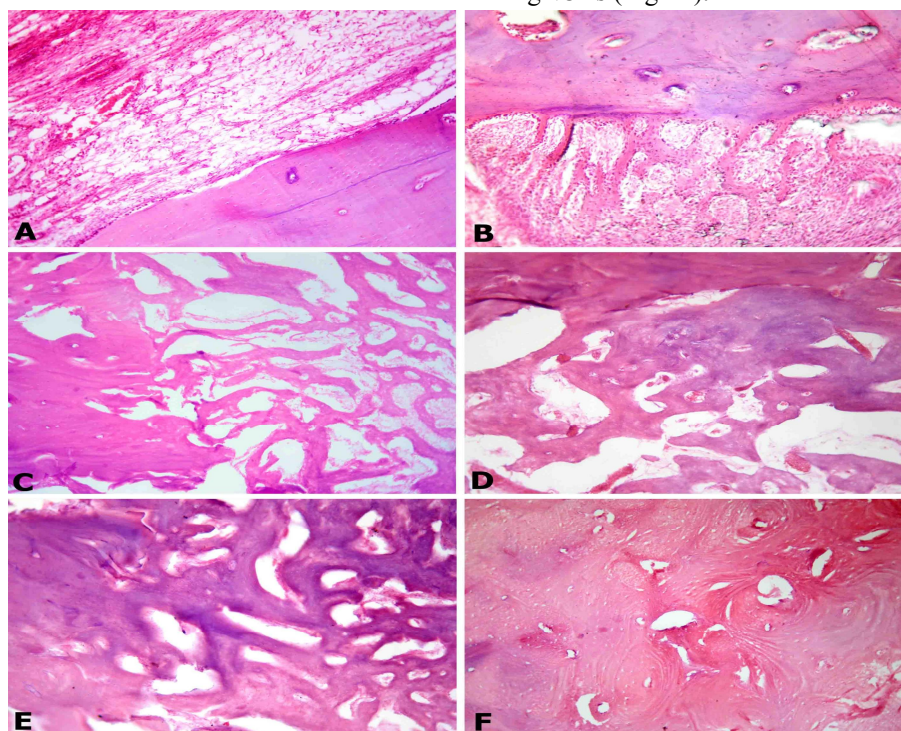

Figure (1):

Group I (control) showing inflammatory cells, fine granulation tissue, congested blood vessels in the upper left and normal bone in the lower right part (HE ×100) **(A).**

Group I (experimental side) showing, very wide marrow spaces, osteoid, fine granulation tissue in the lower portion of the picture and normal bone in the upper part (HE ×100) **(B).**

Group II (control) showing osteoid tissues, wide bone marrow spaces filed with blood vessels and normal bone (left side of the picture) (HE ×100) **(C).**

Group II (experimental side) showing, woven bone, wide bone marrow spaces , osteoid ,blood vessels and normal bone (upper part of the picture) (HE ×100) **(D).**

Group III (control) showing, thick bone trabeculae, narrow bone marrow spaces ,osteoid ,woven bone, blood vessels and normal bone (HE ×100) **(E).**

Group III (experimental side) showing haversion system, Volkmans canal, interstitial lamella and very narrow bone marrow spaces (HE ×100) **(F).**

AgNORs statistical analysis
Quantitative statistical analysis

Table (3) and Fig (2) showing the means± standard deviation (±SD) values and range results of

ANOVA and t- test revealed that there was statistically highly significant differences between numbers of dots/unite area in control versus to experimental group in the three groups.

Table (3): BMC in the different groups

Group	Type	Mean ± SD	(Range)	t	P
Group I (1 week postoperatively)	C	1.017 ± 0.009	(1.01 – 1.03)	8.67	< 0.001**
	EXP	1.075 ± 0.012	(1.067 – 1.097)		
Group II (5 weeks postoperatively)	C	1.055± 0.03	(1.01 –1.091)	6.86	0.008**
	EXP	1.182± 0.18	(1.097– 1.51)		
Group III (9 weeks postoperatively)	C	1.154 ± 0.14	(1.085 –1.41)	4.81	0.02*
	EXP	1.447 ± 0.3	(1.99– 1.734)		

Qualitative statistical analysis

Table (4) showed that there was statistically no significant differences between darkness (density) of dots in control versus to experimental group in group I meanwhile a highly significant differences were detected in group II& III.

Table (4): BMC in the different groups

Group	Type	Mean ± SD	(Range)	t	P
Group I (1 week postoperatively)	C	1.37 ± 0.19	(1.039 – 1.54)	0.78	< 0.45
	EXP	1.455 ± 0.15	(1.214 – 1.591)		
Group II (5 weeks postoperatively)	C	1.54 ± 0.09	(1.399 – 1.625)	4.3	0.0025**
	EXP	1.73 ± 0.04	(1.698 – 1.782)		
Group III (9 weeks postoperatively)	C	1.63± 0.1	(1.519 – 1.791)	3.2	< 0.012*
	EXP	1.825± .08	(1.709 – 1.93)		

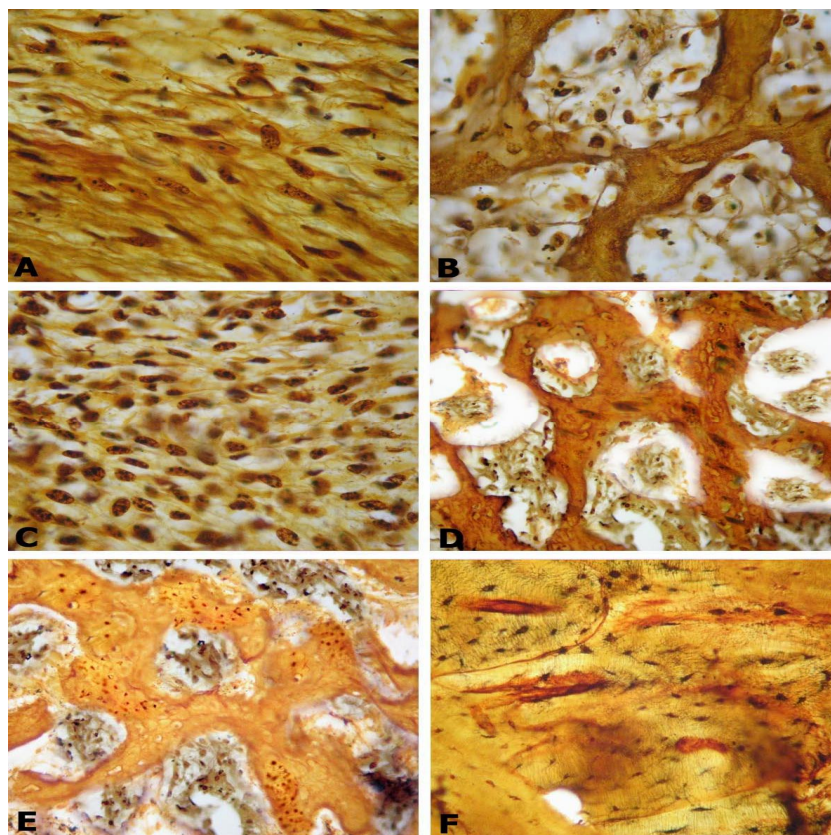**Figure (2):** Photomicrograph showing brown black dots for AgNORs quantification and qualitification (original magnification ×1000). Group I (control) **(A),** group I (experimental side) **(B)**, group II (control) **(C),** group II (experimental side) **(D)**, group III (control) **(E)**, group III (experimental side) **(F).**

4. Discussion

Many researchers have tried to develop technology and methodology to repair soft and bony tissues more strongly and at a faster rate. One possible way is to use growth factors that function to accelerate the wound healing of soft and bony tissues. Several growth factors are present in platelets (Castelnovo *et al.,* 2000), as angiogenic, mitogenic, and osteogenic growth factors in their α granules which can be secreted when activated by substance contact and stimulus actions.

PRP has been thought to promote bone healing for several years but there are contradictory reports about its clinical efficacy (Nazaroglou *et al.,* 2009).

The histological findings of the present experiment corroborate the findings of earlier studies and confirm the positive effect of PRP preparations on bone healing (Freymiller and Aghaloo, 2004). In regard to the densitometric statistical analysis, there was a beneficial effect of PRP on bone density (74% ± 11% with PRP vs. 55% ± 8% without PRP) (Marx *et al.,* 1998). These finding support the results of densitometric analysis obtained in the present study as demonstrated by the highly difference significance of both BMC and BMD in the experimental versus to the control groups.

Additionally, in the present study, AgNORs evaluations as a proliferative markers indicated that, the qualitative and quantitative AgNORs statistical analysis of the proliferation of the bone cells showed that there was statistically highly significant difference in the experimental side vs. to the control and assessed the proper effect of the PRP as autogenous bone graft. These finding were strongly supported by Marx (2001) who assessed that PRP is a proven source of growth factors. These act on bony cells (osteoblasts, osteocytes and preosteoblasts), increase the mitosis rate and stimulate angiogenesis.

In the present study no adverse effects of PRP were observed. Our data were in agreement with the results of Marx (2001) who revealed that, one of the advantages of PRP is that it is autologous (Man *et al.,* 2001).

Other studies could not confirm the positive effect of PRP preparations on bone formation in bone grafts. No beneficial effect of PRP on wound healing and bone remodelling 3 months after implantation of autologous bone grafts for augmentation of the floor of the maxillary sinus (Raghoebar *et al.,* 2005). These studies used a small sample size and bone from the iliac crest. No beneficial effect of autologous PRP on the healing of non-critical sized cranial defects in rabbits after 4 weeks of implantation (Aghaloo *et al.,* 2002). These finding were in disagreement with the result of the present study. The variation in the beneficial effect of PRP in different studies might be attributable to variations in composition of the various PRP formulations.

Additionally, several variables should be carefully monitored during PRP preparation. Special care must be placed on blood extraction tools, machines for blood centrifugation and $CaCl_2$ solutions to prevent contamination of the compound. The process must be kept sterile and precisely suited to separate platelets from red blood cells. Unless platelet sequestration is done carefully and without causing any damage, platelets will no longer be able to secrete growth factors actively. PRP failure in previous studies may have resulted from non-adherence to these recommendations (Weibrich *et al.,* 2004).

However, the numbers of platelets in PRP according to the laboratory finding in the present study was 1500×10^6 platelets /μL was in agreement with those obtained by Marx (2001) who stated that PRP should be more than 1000×10^6 platelet /μL. Another studies concluded that, commercially available PRP systems can provide an enrichment of platelets two to five times higher than that in whole blood (Plachokova *et al.,* 2007), or even up to 10 times higher (Wagner and Foitzik, 2000). There are probably two major reasons for the conflicting results in various experimental studies using PRP in animals: the average concentrations used in the various studies did not take into account the great variation in the baseline values of the animals and the variation in the concentration of platelets in the PRP used. Moreover, it cannot be ruled out that there is a discrepancy in the concentration of platelets needed for acceleration of bone formation between different animal species (Yazawa *et al.,* 2003).

In the present study, administration of PRP in the form of platelet gel provides an adhesive support that can confine secretion to a chosen site. This was concurrent with, Nikolidakis and Jansen (2008). Some arguments may have been raised with regard to the gel form application of the PRP. The effect of PRP gel on bone regeneration around self tapping titanium screw implants inserted in the femoral bone of rabbits was evaluated (Weibrich *et al.,* 2004). After 4-weeks healing period, no advantageous effect on bone-implant contact rate was seen. An explanation for the difference in result between PRP liquid and PRP gel might be the trauma caused during the installation of the PRP gel. After putting the PRP gel in place, the implant is forced into the implant cavity. PRP gel has a viscous, jelly-like consistency, which can result in additional pressure on the drill walls of the already tightly fitting implant. As a consequence, the healing process at the bone-implant interface might become disturbed (Yoichiro *et al.,* 2006). Furthermore, a higher percentage of bone contact 6 weeks after implant placement was found when using PRP gel, because of using a peri-implant defect model and not a

press-fit technique for implant insertion (Kim *et al.,* 2001).

However our data strongly suggest potential improvements in the routine practices of PRP therapy.

Conclusion

1- PRP as autologous bone graft is the best to avoid cross infections and any immune reactions.
2- PRP is the simplest in its manipulation, more economically and the safest.
3- The platelets contents within PRP are in proportional to the growth factors which considered the main agent for the proliferation of the bone cells.
4- PRP is one of the best means as antihaemorrhagic.
5- AgNORs is one of the best means as a proliferative marker.

Corresponding author
Mohamed A. Nassan
Pathology Department, Fac. Vet. Med., Zagazig University, Egypt
Moh_nassan@yahoo.com

References

1. Aghaloo T, Moy P and Freymiller E (2002): Investigation of platelet-rich plasma in rabbit cranial defects: a pilot study. J Oral Maxillofac Surg., 60: 1176–1181.
2. Bolander, M (1992): Regulation of fracture repair by growth factors. Proc. Soc Exp Biol Med., 200: 165-170.
3. Castelnovo L, Dosquet C, Gaudric A, Sahel J and Hicks D (2000): Human platelet suspension stimulates porcine retinal glial proliferation and migration *in vitro*. Invest Ophthalmol Vis Sci., 41: 601-609.
4. Derenzini M and Ploton D (1991): Interphase nucleolar organizer regions in cancer cells. Int. Rev. Exp. Pathol., 32: 149-192.
5. Fennis J, Stoelinga P and Jansen A (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 OralMaxillofac Surg., 33: 48-55.
6. Freymiller E and Aghaloo T (2004): Platelet-rich plasma: ready or not? J Oral Maxillofac Surg., 62: 484–488.
7. Jung R, Schmoekel H, Zwahlen R, Kokovic V, Hammerle C and Weber F (2005): Platelet-rich plasma and fibrin as delivery systems for recombinant human bone morfogenetic protein-2. Clin Oral Implants Res., 16: 676–682.
8. Kim E, Park E and Choung P (2001): Platelet concentration and its effect on bone formation in calvarial defects: an experimental study in rabbits. J Prosthet Dent., 86: 428-433.
9. Man D, Plosket H and Winl-Brown J (2001): The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery. Plast Reconstr Surg., 107: 229-237.
10. Marx R (2001): Platelet-Rich Plasma (PRP): What Is PRP and what is not PRP? Implant Dent., 10: 225–228.
11. Marx R (2004): Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg., 62: 489-496.
12. Marx R, Carlson E, Eichstaedt R, Schimmele S, Strauss J and Georgeff K (1998): Platelet rich plasma. Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod., 85: 638-646.
13. Nazaroglou E, Matoulas E, Kafas P and Dabarakis N (2009): The effect of PRP (platelet rich plasma) on reconstruction of mandibular and maxillary bone defects: A review of recent experimental studies on humans and animals. Surgery Journal. 4: 1-7.
14. Nikolidakis D and Jansen R (2008): The biology of platelet-rich plasma and its application in oral surgery: literature review. Tissue Eng Part B Rev., 14: 249-258.
15. Pallua N, Wolter T and Markowicz M (2010): Platelet-rich plasma in burns. Burns., 36: 4– 8.
16. Plachokova A, van den Dolder J, Stoelinga P and Jansen J (2007): Early effect of platelet-rich plasma on bone healing in combination with an osteoconductive material in rat cranial defects. Clin Oral Implants Res., 18: 244–251
17. Raghoebar G, Schortinghuis J, Liem R, Ruben J, van der Wal J and Vissink A (2005): Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor? Clin Oral Implants Res., 16: 349–356.
18. Sirri V, Roussel P and Hernandez-Verdum D (2000): The AgNOR proteins: qualitative and quantitative changes during the cell cycle. Micron., 31: 121– 126.
19. To¨zu¨m T and Demirapl B (2003): Platelet-rich plasma: a promising innovation in dentistry. J Can Dent Assoc., 69: 664.
20. Trerè D (2000): AgNOR staining and quantification. Micron., 31: 127-31.
21. Trere` D, Migaldi M and Trentin G (1995): Higher reproducibility of morphometric analysis over the counting method for interphase AgNOR quantification. Anal Cell Pathol., 8: 57–65.
22. Van den Dolder J, Mooren R, Vloon A, Stoelinga P and Jansen J (2006): Platelet-rich plasma: quantification of growth factor levels and the effect on growth and differentiation of rat bone marrow cells .Tissue Eng., 12: 3067-3073.
23. Van der Lubbe H, Klein C and de Groot K (1988): A simple method for preparing thin (10 mM) histological sections of undecalcified plastic embedded bone with implants. Stain Technol., 63: 171– 176.
24. Wagner B and Foitzik C (2000): Praxistaugliches Verfahren zur Gewinnung von Platelet-rich Plasma. Z Zahnärztl Impl., 16: 146-150.
25. Weibrich G, Hansen T, Kleis W, Buch R and Hitzler W (2004): Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone. 34: 665–671.
26. Yazawa M, Ogata H, Nakajima T, Mori T, Watanabe N and Handa M (2003): Basic studies on the clinical applications of platelet-richplasma. Cell Transplant., 12: 509-518.
27. Yoichiro O, Yasunori A, Toshio K and Kiyoshi K (2006): The contribution of platelet-derived growth factor, transforming growth factor-b1, and insulin-like growth factor-I in platelet-rich plasma to the proliferation of osteoblast-like cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod., 101:724-729.

2/20/12