ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Evaluation of the Osteogenic Potential of the Platelet-rich plasma and Autologous Cancellous bone grafts on Caudolateral ulna ostectomies of Nigerian local dogs

Nnaji, Theophilus and Kene, Raphael

Department of Veterinary Surgery, Faculty of Veterinary Medicine, University of Nigeria, Nsukka

Manuscript Info

Manuscript History:

Received: 12 December 2014
Final Accepted: 22 January 2015
Published Online: February 2015

Key words:

Platelet-rich plasma; Cancellous bone graft; Bone healing; Ulna defect; Dogs

Corresponding Author

Nnaji

Abstract

The osteogenic effects of the Platelet rich plasma (PRP) and autologous cancellous bone grafts (ACBG) on caudolateral ulna ostectomies were evaluated in 12 Nigerian local dogs. Partial ostectomy of the caudal cortical surface of the left ulna was performed under general anaesthesia. They were grouped into four groups namely; platelet rich plasma group (PRPG), cancellous bone graft group (CBG), platelet rich plasma/cancellous bone graft group (PRP/CBG) and control group (CG).The defects were treated as follows: PRPG, with autologously PRP; CBG with autologous bone graft; PRP/CBG with both autologous PRP and bone graft and the CG with normal saline before closure. Post treatment radiographs were taken at weeks 1, 6 and 8. Callus proliferation, mineralization and the radiographic optical densities (ROD) of the osteoid were compared. The PRP/CBG defects showed superior osteogenic indices. The PRP group however, performed better than the CBG group.

INTRODUCTION

The increasing use of platelet rich plasma (PRP) in surgery presents significant opportunities as well as questions about the appropriate clinical applications for this developing therapy (Boyan *et al*., 2007). Platelet rich plasma has been used in a variety of surgical settings especially in soft tissue surgery and in sports medicine with variable degrees of successes and failures.

Platelets which are the main component of PRP play fundamental role in haemostasis and are a natural source of growth factors. Growth factors are involved in key stages of wound healing and regenerative processes, including chemotaxis, proliferation, differentiation and angiogenesis (Bennett and Schultz, 1993). In addition to growth factors, platelets release numerous other substances (fibronectin, vitronectin, sphingosine 1-phosphate etc) that are important in wound healing.

In soft tissue augmentation, the rationale for using PRP is to accelerate vascularisation of grafts, improve healing, and reduce post-operative morbidity (Anitua *et al.*, 2004).Increased concentrations of these growth factors at the site of injury was likely responsible for the accelerated soft tissue wound healing time (Anitua *et al.*, 2004) It is postulated that for hard tissues (bone), growth factors released from PRP may likely affect local vital cells such as osteoblasts (Hanna *et al.*, 2004).

Preliminary studies with calcium chloride-activated PRP alone or in combination with autologous bone grafts in management of bone defects of Nigeria local dogs yielded unappreciable results. Similar findings were also reported when the PRP was added to xenografts (Bovine HA) (Furst *et al.*, 2003).

With the insignificant effect of calcium chloride-activated PRP on bone healing of Nigerian local dogs, it might interest one to find out the effect of PRP prepared with a standardizes PRP preparation kits on bone defects of Nigerian local dogs. In doing this, the osteogenic effect was compared with that of autologous cancellous bone grafts on caudo-lateral ulna ostectomies of Nigerian local dogs.

Methodology

Ethics statement

This work was carried out in accordance with the guidelines for animal experiments released by the National Institute of Animal Health. This study was approved by the Animal Welfare Committee of the Faculty of Veterinary Medicine University of Nigeria, Nsukka. They were housed in standard animal house, fed standard dog food. They had access to water ad libitum and the surgery was conducted under strict aseptic conditions.

Animals

Twelve healthy Nigerian local dogs, (1year old) were used for the study. They were acclimatized for two weeks prior to the surgery. These dogs were randomly assigned to four groups of 3 dogs each as follows:
Platelet rich plasma-treated group (PRPG); Cancellous bone graft treated-group (CBG) Platelet rich plasma/Cancellous bone graft treated-group (PRP/CBG) and the Normal saline treated-control group- (CG).

Preoperative preparation

Each of the dogs was sedated using 2% xylazine HCl (Indian imunobiologicals) at the dose 0.2 mg/kg body weight intramuscularly as a premedicant followed by intravenous administration of pentobarbitone sodium (6%) (Kyron South Africa) at the dose of 25 mg/kg body weight**.**
The left fore-limbs of these animals were generously shaved from the elbow to the distal 1/3 of the ulna. This area was aseptically scrubbed prior to surgery.

Surgical technique:

Each dog was placed in dorsal recumbency and the left forelimb pulled forward to expose the caudal aspect of the antebrachium. A linear incision was then made starting just below the tip of the olecranon and extended to the proximal third of the shaft of the ulna. The fascia was dissected to reach the diaphysis of the ulna. The deep antebrachial fascia was incised between the extensor carpi ulnaris to permit retraction of the muscles laterally and medially. The dissection was continued around the cranial aspect of the bone using Hohmann retractors to maintain retraction. On getting to the ulna a partial ostectomy of about 3mm was done using osteotome. The defects in each group were then treated before wound closure as follows:

Platelet rich plasma treated group (PRPG):

Dogs in this group (PRPG) were treated with autologous PRP prepared with Plateltex^{R} prep and Plateltex^{R} Act following manufacturer's instructions. After the preparation, the autologous PRP was gently collected with thumb forceps and applied onto the defect of the same dog. The wound was closed by suturing the deep fascia in a simple continuous pattern with size 2-0 chromic catgut. The subcuticular stitches were also placed in a simple continuous pattern. The skin incision was closed with simple interrupted sutures with silk.

Cancellous bone graft treated group (CBG):

The dogs in this group were treated by applying an autologously collected cancellous bone grafts from the proximomedial aspect of the tibia of the dog.

Preparation of the grafts

Collection of the bone grafts was done under anaesthesia. The proximomedial region of the contralateral tibia was generously shaved with razor blade and scrubbed with gauze impregnated with hibitane hydrochloride. The dog was then positioned in lateral recumbency with the medial aspect of the donor tibia up. The animal was then properly draped ready for surgery. A skin incision was then made at the medial aspect of the proximal tibia to expose the proximal metaphysis. Cancellous bone graft was then collected using manual bone drill to enter the medullary canal of the metaphyseal region of the bone. Upon removal of the drill bit, a 2-0 bone curette was used in the fashion and motion of an ice cream scoop to collect enough graft to fill the defect. The donor site was then closed routinely. The graft was immediately transferred to the defect on the ulna. The muscles and the skin were then closed with size 2/0 chromic catgut and silk respectively.

PRP/CBG:

The bone defects here were treated by applying both autologous PRP (prepared with Plateltex Prep and Act kits of Czech Republic) and autologous cancellous bone graft onto the defects before closure.

CG:

The dogs in the dogs in this group were treated by applying normal saline on the defects before closure.

Evaluation of the Healing Rate

Radiographs of the affected limbs were taken at weeks 1, 6 and 8. The amount of callus proliferation, mineralization and remodeling of the osteoid were noted. The optical densities of the radiographs were measured using the Black and White Transmission densitometer. Optical density (OD) data were obtained from 4 points within the treated defects and the mean recorded.
The ODs were calculated thus:
$OD=\log (I_i/I_t)$, where

I_i=intensity of incident light,
I_t=intensity of transmitted light (Martinez *et al.*, 1992).

Results

At one week post treatment, the ulna defects were all clearly visible in all the dogs in all the groups; (figs.1, 2, 3 and 4 for PRPG, PRP/CBG, CBG and CG respectively). No periosteal reaction was visible radiographically in all of them. At week six post treatment, partial bridging of the ulna defects with soft callus was evident in all the groups (figs 5, 6, 7 and 8) but the PPRP/CBG group (fig.6) had callus of least optical density (0.95±0.03), (Table 1) indicating greater bone density (sclerosis) compared to other groups. The OD of the PRP treated ulna defects (1.01±0.01) varied significantly ($p < 0.05$) with that of the CBG treated defects (1.28±0.02) at week six (Table 1) In all the four groups, extensive periosteal callus proliferation was not exhibited but filling of the defects with endosteal callus was evident in all the groups leading to obliteration of the marrow cavities at the defective sites in most of the animals.

At week eight post treatment, all the ulna defects in the PRPG (fig 9); PRPG/CBG (fig 10) and CBG (fig. 11) had been leveled up with calluses of varying densities. In the PRPG the callus density was almost equal to density of the normal adjacent cortex. This indicated the presence of calcified callus. In the PRPG/CBG, 100% of the defects had been filled with dense mineralized callus. This group exhibited the least OD (0.78±0.02) among the three groups with the callus density almost equal to the normal cortex density (Table 1). At week eight, there was no significant difference ($p > 0.05$) in the ODs of the PRPG (0.91±0.03) and the CBG (0.89±0.01) (Table 1). The level of mineralization (sclerosis) of the PRPG defects was comparable to those of the CBG.

For the control group (fig.12), the defects were still faintly visible with partial bridging of the defects with callus of minimal density compared to those of the experimental groups. This was indicative of poor mineralization of the osteoid.

Radiographs of the defects at various stages of healing, post treatment.

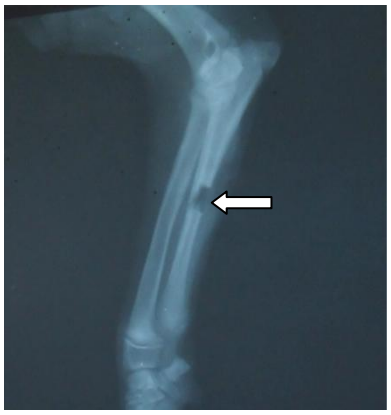


Figure 1

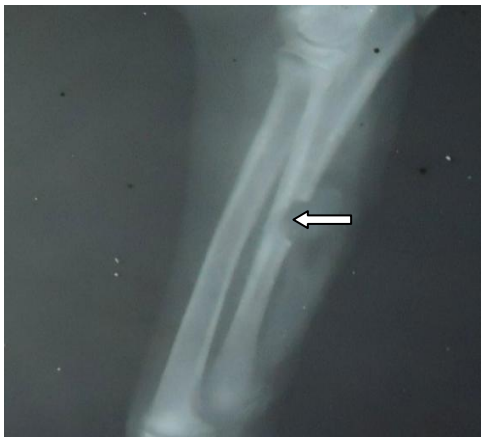
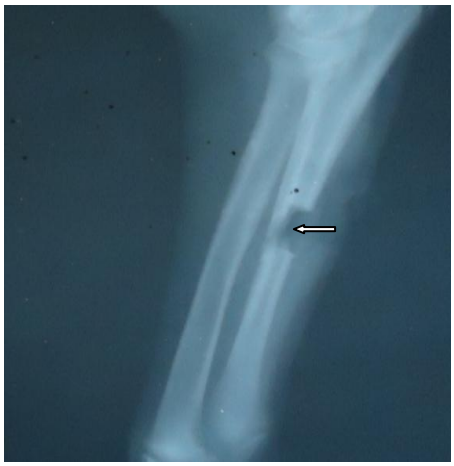


Figure 2



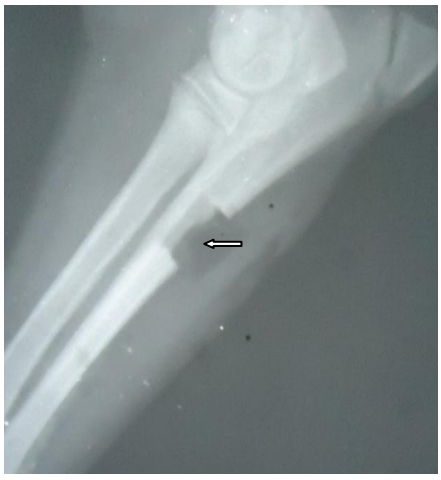


Figure 3 **Figure 4**

Figures 1, 2, 3 and 4 are radiographs of one of the defects (arrows) at week 1 in the PRPG, PRPG/CBG, CBG and CG animals respectively. (Note the absence of periosteal reaction in all)

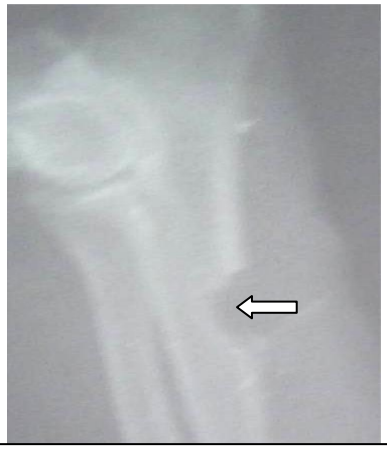


Figure 5: Post-treatment radiograph of one of PRPG defects at week 6. Note the partial bridging of the defect with callus of soft tissue density (arrow)

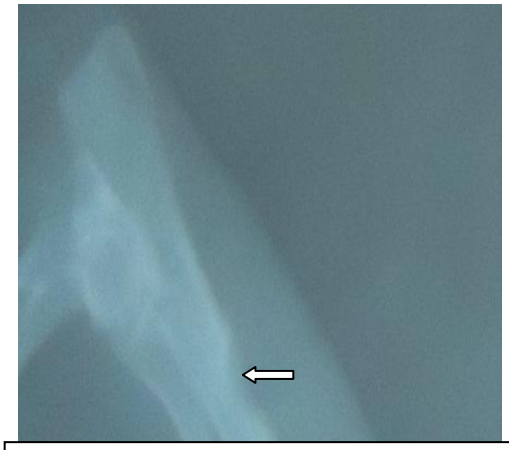


Figure 6: Post-treatment radiograph of one of PRPG/CBG defects at week 6. Note the extensive proliferation of bridging callus of greater sclerosis here (arrow)

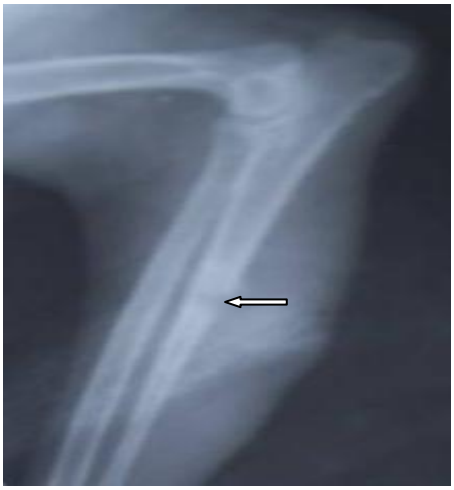


Figure 7: Post-treatment radiograph of one of CBG defects at week 6
Note the partial bridging of the defects with callus of soft tissue density (arrow)

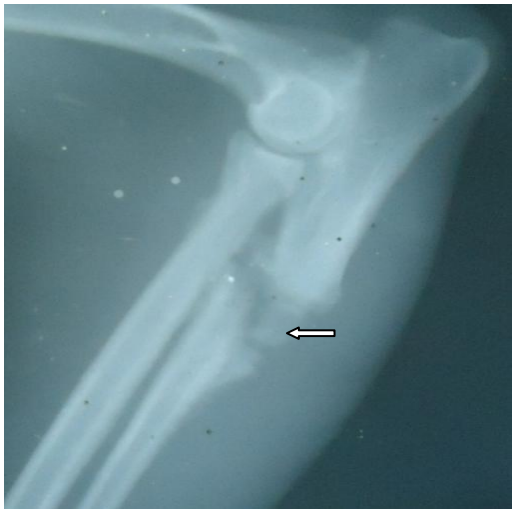


Figure 8: Post-treatment radiograph of one of Control (CG) defects at week 6.Note the partial bridging of the defects with callus of soft tissue density (arrow)

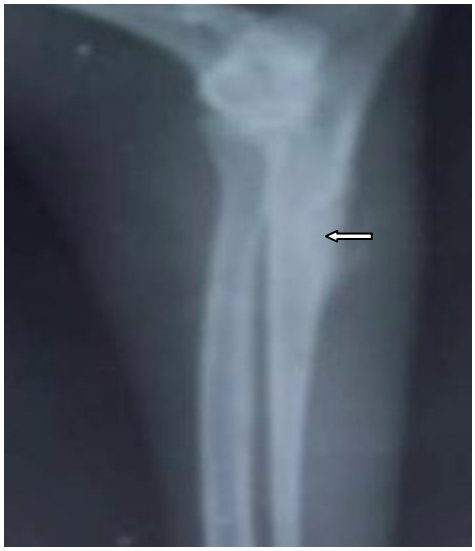


Figure 9: Post-treatment radiograph of one of PRPG defects at week 8. All the defects in this group have been filled up with callus. (Note the greater sclerosis in fig. 9)

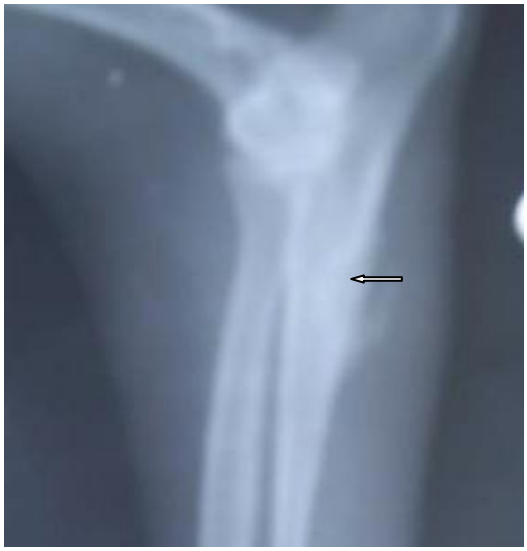


Figure 10: Post-treatment radiograph of one of PRPG/CBG defects at week 8. All the defects in this group have been filled up with dense mineralized callus (arrow)

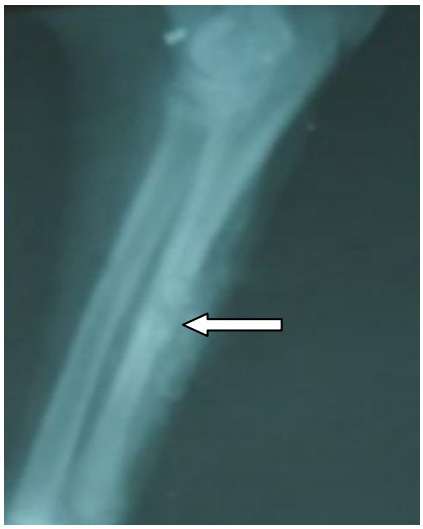


Figure 11: Post-treatment radiograph of one of CBG defects at week 8.
The defect has been filled up with dense callus (arrow).The bone density is however less than those of the PRP/CBG

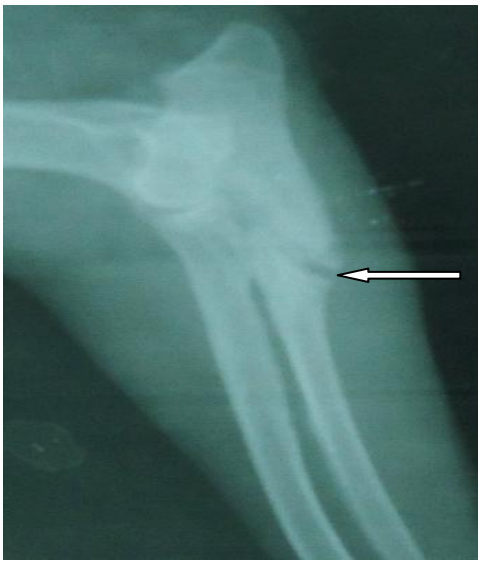


Fig 12: Post-treatment radiograph of one of control defects at week 8
Note the poor mineralization of the osteoid here with the defect still faintly visible (arrow)

TABLE 1: MEAN PERIODIC OPTICAL DENSITY (±SEM) OF THE BONE DEFECTS OF DIFFERENT TREATMENT GROUPS

	PRP	PRP/CBG	CBG	CG
WEEK1	1.50 ± 0.00^{a}	1.46 ± 0.02^{ab}	1.45 ± 0.02^{b}	1.43 ± 0.02^{b}
WEEK6	1.01 ± 0.01^{a}	0.95 ± 0.03^{b}	1.28 ± 0.02^{c}	1.34 ± 0.02^{d}
WEEK8	0.91 ± 0.03^{a}	0.78 ± 0.02^{b}	0.89 ± 0.01^{a}	1.19 ± 0.02^{c}

a, b, c=mean in same row with different superscript differ significantly ($p < 0.05$)

DISCUSSION

At week one post treatment, hematoma/granulation tissues of the ulna defects in dogs in PRP, PRP/CBG, CBG and control groups had not been mineralized and therefore had soft tissue density, radiographically (figs 1, 2, 3 and 4 respectively). This explains why the ODs of the PRP/CBG, CBG and the control groups did not vary significantly ($p > 0.05$) at this period. However at week six post treatment, the variation in the ODs of the ulna defects of dogs in the experimental groups (PRPG, PRP/CBG, and CBG) varied significantly ($P < 0.05$). This could probably be due to the fact that the PRP applications in addition to enhancing bone proliferation in the PRP and PRP/CBG groups also significantly improved the handling of the particulate grafts at the site of the defects in the PRP/CBG group and hence faster rate of graft incorporation and mineralization of the osteoids (Jakse *et al.*, 2003). The presence of soft tissue callus on the ulna defects of all the groups at week six was as a result of the granulation tissues that were gradually maturing into fibrocartilageneous matrix which is always seen in early stage of bone healing (Slatter, 2003). The greater density of the osteoid at week six in the PRP/CBG treated defects signified increased bone mass as a result of the formation of hard callus following mineralization. This mineralized fibrocartillage had acceptable level of structural strength and stiffness that could allow bone formation to begin (Brown, 2009). This explains the

result at week eight in PRP, PRPG/CBG and CBG defects which, in addition to mineralization, had undergone appreciable level of endochondral ossification. The callus to cortex density was equal at week eight in 100% of the defects of the PRPG/CBG probably because the mineralized fibrocartilages were gradually being replaced with cancellous bones which were gradually being rearranged (remodeled) into lamellar bones (Slatter, 2003).The high optical densities seen in the control group means decreased bone mass which signified poor osteogenesis.

At week eight, the defects in the PRPG/CBG had OD that was significantly ($p < 0.05$) different from that of the dogs in the CBG group. Also the PRPG/CBG defects had slightly lower OD compared to PRPG indicating that the PRPG/CBG defects had higher bone mass. This could be ascribed to the fact that the microenvironments of the PRPG/CBG defects in addition to being enriched with multiple exogeneous growth factors also had a good source of osteoprogenitor cells, osteoinductive and osteoconductive scaffold for bone proliferation from the autologous PRP and the cancellous bone graft applied (Everts *et al*., 2006). The control group exhibited the highest OD at week eight because the biochemical environment of the defects in this group was not enriched with as much bioactive material as the PRP, PRP/CBG and the CBG treated defects. Normally, the repair of musculoskeletal tissues generally starts with the formation of blood clots and degranulation of platelets, which releases growth factors and cytokines at the sites. This microenvironment results in chemotaxis of inflammatory cells as well as the activation and proliferation of local progenitor cells (Tabata, 2003). Enriching this microenvironment with exogeneous PRP and PRP/CBG could have contributed immensely to the greater osteogenic activities and enhanced healing of the defects seen in the PRP and PRP/CBG groups. This was possible since PRP has been proven to contain the following growth factors: transforming growth factor, platelet derived growth factor, insulin-like growth factor, vascular endothelial growth factor, epidermal growth factor, fibroblast growth factor and other useful peptides (Boyan et al., 2007). Many of these growth factors have been shown to enhance one or more phases of osteogenesis in man (Boyan et al., 2007). For instance, PDGF, EGF, and FGF-2 have been shown to stimulate the proliferation of osteoblastic progenators. TGF also increases matrix synthesis (Boyan *et al*., 2007). In this study, PRP demonstrated positive osteogenic activity comparable to that of the autologous bone grafts but for optimal performance, concurrent application of both PRP and autologous bone grafts is highly advocated.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Nnaji and Kene, participated in literature review, drafting of the manuscript, designed and carried out all the works. All authors have read and approved the final manuscript.

Acknowledgements: I wish to thank Plateltex of Czech Republic for giving me the Plateltex Prep and Act kits used for this work. My thanks also go to Dr. Chinwe Chukwudi who collected and shipped the kits to me via London.

References

Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT (2004) Autologous platelets as a source of proteins for healing and tissue regeneration. *Thrombosis and Haemostasis* **91**: 4– 15

Bennett NT, Schultz GS (1993).Growth factors and wound healing: biochemical properties of growth factors and their receptors. *American Journal of Surgery* **165**:728–737.

Boyan, BD; Schwartz,Z; Patterson,TE and Muschler,G (2007) Clinical use of platelet-rich plasma in orthopedics.

Brown, S.E (2009) How to speed fracture healing; Center for Better Bones, *www.betterbones.com* (accessed January 2, 2012),

Everts PA, Brown Mahoney C, Hoffmann JJ (2006) Platelet-rich plasma preparation using three devices: implications for platelet activation and platelet growth factor release. *Growth Factors*; **24(3)**:165–171.

Furst G, Gruber R, Tangl S, Zechner W, Haas R, Mailath G (2003) Sinus grafting with autogenous platelet-rich plasma and bovine hydroxyapatite: A histomorphometric study in minipigs, *Clinical and Oral Implants Research.* **14**:500–508.

Hanna R, Trejo PM, Weltman RL (2004). Treatment of intrabony defects with bovine-derived xenograft alone and in combination with platelet-rich plasma: a randomized clinical trial. *Journal of Periodontology* **75**:1668–1677.

Jakse, N, Tangl, S, Gilli, R, Berghold, A, Lorenzoni, M (2003) Influence of PRP on autologous sinus grafts; An experimental studyon sheep. *Clinical and Oral Implants Research* **14**: 578-583

Martinez, SA; Probst, CW; Hauptman, JG and Weisbrode, SE (1992) Effects of a fixed compression load on the osteogenic effect of autologous cancellous bone grafts in dogs. *American Journal of Vet Research*, **53 (12)** 2381-2385

Nilsson, OS; Urist, MR; Dawson, EG; Schmalzried, TP and Finerman, GAM (1986) Bone Repair induced by Bone Morphogenic Protein in ulna defect in Dogs. *Journal of Bone and Joint Surgery* **68B (4)**;635-642

Slatter, D (2003) Fracture Biology and Biomechanics *In Textbook of Small Animal Surgery 3rd edn.* Saunders Co. p1792

Tabata, Y (2003) Tissue regeneration based on growth factor release, *Tissue Engineering* **9**:5-15.