

Original Article

The Use of Platelet Rich Fibrin and Nano Hydroxyapatite as Regenerative Treatment of Furcation Class II Defects (A Histomorphometric Study in Dogs)

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Abstract

Objectives: The aim of the present study was to evaluate the use of platelet rich fibrin and Nano-hydroxyapatite as a regenerative treatment in furcation class II defect.

Material and methods: Eight young adult dogs was selected for the study; in each dog, class II furcation defects were surgically created in 4 premolars and were treated as follow: First was treated with Nano hydroxyapatite alone ; Second with PRF alone ; Third with Nano hydroxyapatite and PRF ; Fourth did not receive any additional treatment than debridement (controls).

Results: histomorphometric analysis revealed that, the greatest amount of newly formed bone was observed in the (PRF+ NHA bone) group, followed by the PRF group, then the NHA group, with the least recorded in the control group. The difference was not statistically significant except in comparing (PRF+ NHA group) with control group (p=0.0035).

Conclusion: The combined use of both PRF and Nano hydroxyapatite in treatment of furcation class II defects could accelerate healing processes, getting use of the synergic effect of the powerful inherent regenerative potential of PRF and Nano hydroxyapatite.

Key words: Platelet rich fibrin; PRF ; Nano-hydroxyapatite; furcation class II defect.

Introduction

Periodontitis is one of the most common diseases affecting the periodontium. Its main cause is bacterial plaque, that can result in destruction of the gingival tissues and

periodontal supporting apparatus (Wang et al., 2005). The difficulty of controlling plaque in furcations is responsible for the presence of extensive lesions in this area (Waerhaug, 1980). Different treatment modalities are used in

management of furcation depending upon degree of furcation involvement including scaling and root planning, furca plasty, tunnel procedure, root resection, bicuspidization and regenerative approach as guided tissue regeneration and bone grafts (**Kornman and Robertson, 2000**).

Hydroxyapatites (HAs) is considered as a family of grafting materials that have a high level of biocompatibility. It has decreased osteoconductivity and poor degradation properties. To improve such shortcomings, nanocrystalline hydroxyapatite (nano-HA) paste containing 65% water and 35% of nanostructured apatite particles has been used for augmentation procedures in intrabony defects (**Thorwarth et al., 2005**).

The preparation of platelet rich fibrin from a patient's blood is a very simple process as it requires neither anticoagulants nor bovine thrombin. Blood is immediately centrifuged at 3,000 rpm for 10 min as claimed by (**Huang FM et al., 2010**). The process must start immediately after drawing the blood to prevent the polymerization of fibrin in diffuse way which may result in a small poorly formed clot (**Jyotsna et al. , 2013**). Doing it quickly, large PRF clot (approximately 40-60% of the product) will be obtained in the middle of the test tube inbetween the lighter clear platelet poor plasma and condensed red blood corpuscles (**Dohan Ehrenfest et al., 2009**).

Material and Method

Study Design

Sample size was determined using sample size calculator* in relation to previous study with mean 64.95 and standard deviation 15.75(**Tatiana et al., 2006**). At study power of 80%; the required sample was 6 and increased in the present study to 8 in each group to avoid any attrition during follow-up.

Nano-HA has a stimulating effect on cultured PDL cells resulted in increased proliferation rate of these cells which is linked to the activation of epidermal growth factor receptor (**Kasaj et al., 2008**)

Heinz et al., 2010 reported that nanocrystalline hydroxyapatite bone graft substitute in treatment of intrabony periodontal defects, showed more reduction in the probing pocket depth (PPD) in the study group compared with the control group.

Platelet-rich fibrin (PRF) was developed in France by **Choukroun et al (2001)** and considered as a second generation of platelet concentrates, it generally accelerates soft and hard tissue healing.

Eight healthy, mature (about 10-12 months old) male Mongrel dogs, weighting about 12-15 kg each with intact maxillary and mandibular teeth were selected for the study. Female dogs were excluded to avoid any hormonal disturbance masking the effect of regeneration.

The animals were housed in separate cages, supplied with food and a bucket for water and allowed to live in optimal conditions at the animal house, Faculty of Medicine, Cairo University where all the study procedures were done.

Induction of Anesthesia

The anesthetic agent used for induction of anesthesia was a mixture of Xylazine-HCl; 1mg/kg body weight and ketamine HCl; 5mg/kg body weight, via a 23 gauge intravenous cannula through the cephalic vein and local infiltration anesthesia with vasoconstrictor 1/80,000 adrenaline to decrease bleeding. Anesthesia were maintained during surgery by venous drip of 500mg Thiopental-Sodium/500 ml Dextrose 5%.

Flap Technique

Two mandibular premolars of both sides in each dog were selected for the study. Before starting the surgical procedure, the surgical sites were rubbed by Betadine 10% then with Bard Parker blade no. 15 sulcular incision was made extending from the mesial of the first molars to distal of the canine.

* Kane SP. Sample size calculator. ClinCalc: <https://clincalc.com/stats/samplesize.aspx>.

mucoperiosteal flap was elevated and using a small rose head bur, class II furcation defects, measured 5 mm apicocronal and 2 mm deep inter-radicular were created surgically on the buccal side of these premolars.

All periodontal ligament fibers on the roots were removed using periodontal cures. Rubber base impression material was used to fill the defects and induce an inflammatory response and prevent spontaneous repair(**fig1**).



Figure (1): The defects filled with a rubber base impression material to inhibit spontaneous healing

The flaps were carefully adapted, and closed with bioresorbable sutures. The rubber base material was left in place for 3 weeks. After that, it was removed with cures under general anesthesia. Periodontal debridement was performed. Daily application of topical 0.2% chlorhexidine was maintained for one week.

Four weeks later, after induction of Anesthesia; mucoperiosteal flaps were raised to gain access to furcation areas, all granulation tissues were removed, and root surfaces were planed. A reference notch was done on root surfaces at the bone crest level with a small 0.5 rose head bur to facilitate histomorphometric analysis. Sterile saline was used to clean the surgical site.

Preparation of platelet rich fibrin

PRF was prepared by collecting 10 ml of venous blood drawn from the arteria femoralis on the hind leg of each dog and were immediately centrifuged at 3000 rpm for 10 min prior the beginning of the surgery.

In each dog the four surgically created furcation defects (2 in each side) were treated as follow:

First (Group **A**) was treated with Nano hydroxyapatite alone (**Fig, 2a**)

Second (Group **B**) was treated with PRF alone (**Fig, 2b**)

Third (Group **C**) was treated with Nano hydroxyapatite and PRF (**Fig, 2c**)

Fourth (Group **D**) debridement only (controls). (**Fig, 2d**)

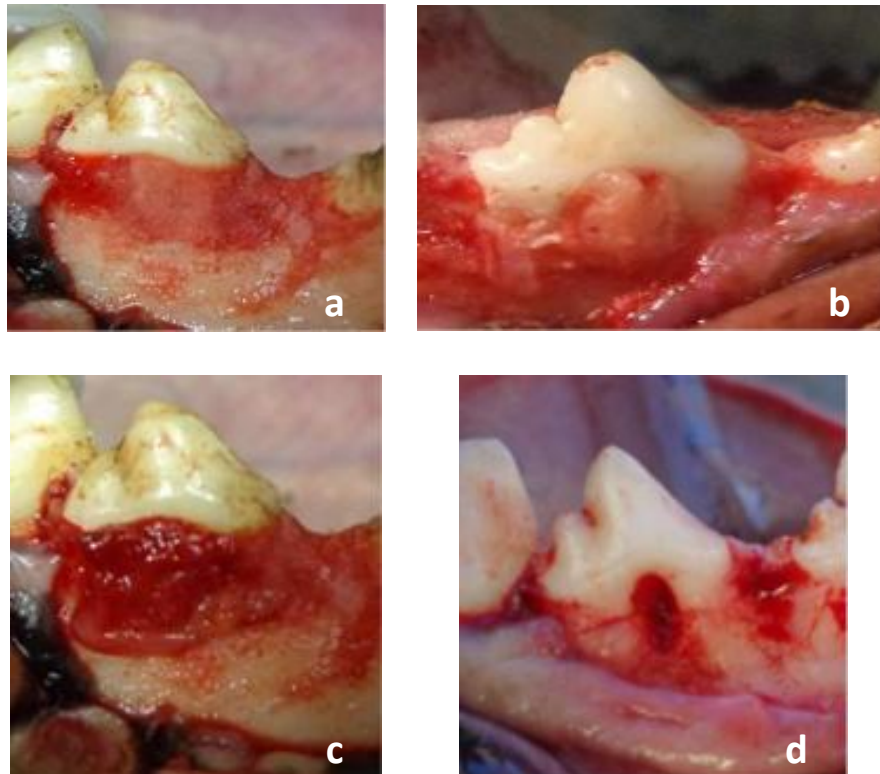


Figure (2): (a) showing defect treated with Nano hydroxyapatite alone (b) showing defect treated with PRF alone (c) defect treated with Nano hydroxyapatite and PRF (d) defect treated with debridement only (controls)

The flaps were repositioned and secured by 4-0 bioresorbable sutures. After finishing the surgical procedure, all dogs were injected with antibiotics and pain killer as a part of post-operative care. The animals were followed for 90 days healing period. During the first 28 days, all dogs fed on soft diet in order to protect the wound from any mechanical trauma. Topical application of 0.2% chlorhexidine solution were maintained to fight against plaque accumulation.

Post-operative Evaluation and Sacrifice

Dogs were sacrificed by excess anesthesia and the mandibles were dissected and biopsies were collected for histopathological examination. The biopsies were preserved in jar containing formalin 10% and sent to the pathology lab. For histopathological examination.

I- Microscopic examination

The mandible was carefully dissected, immediately fixed in 10% formalin, after that decalcification was done using ethylenediaminetetraacetic acid (EDTA) for 4 weeks. Then, tissue blocks were processed and embedded in paraffin. Four μm sections were cut perpendicular to the long axis of the tooth and mounted on glass slides. The collagen and new bone formation were detected by Masson's Trichrome stain (fig , 3) (fig , 4) (fig , 5) (fig , 6)

II- Measuring the newly formed bone

The area of newly deposited bone was estimated using Leica Quin 500 analyzer computer system, (Leica Microsystems, Switzerland). The cursor was used to define the areas of newly deposited bone trabeculae or collagen, that were marked by a binary color which was measured by the

computer. The image analyzer was calibrated automatically to transform pixels produced into actual micrometer units.

The percentage of newly-formed bone was evaluated in five different fields in each group

with magnification (x100). Both mean values and standard deviation (SD) were calculated for all groups

Results

histomorphometric analysis:

I- Area percent of collagen

The greatest area percent of collagen was recorded in Group D, whereas the lowest mean value was

recorded in group A. Analysis of variance (ANOVA) test revealed that the difference was statistically significant ($p < 0.0001$). Tukey's post hoc test revealed a significant difference between each two groups, except between groups B, C (**Table 1**) (**Table 2**).

II- Area percent of bone

The greatest area percent of bone was recorded in Group A, whereas the lowest mean value was recorded in group D. Analysis of variance (ANOVA) test revealed that the difference was statistically significant ($p = 0.047$). Tukey's post hoc test revealed a significant difference between groups A and D only (**Table 3**) (**Table 4**).

Table (1): Detailed results of Tukey's post hoc test for difference between groups regarding the area percent of collagen

Groups	Significance
Group A versus Group B	0.0132*
Group A versus Group C	0.0008*
Group A versus Group D	<0.0001*
Group B versus Group C	0.0668 ^{ns}
Group B versus Group D	<0.0001*
Group C versus Group D	0.0007*

*significant at $p < 0.05$, ns=non significant

Table (2): Area percent of collagen in different groups and significance of the difference using ANOVA test

	Group A	Group B	Group C	Group D
Mean	17.566	28.702	40.359	64.774
Std Dev	1.75	7.658	9.617	3.394
Max	19.813	38.245	52.224	69.995
Min	14.556	20.598	27.486	59.489
F value	44.23			
P value	<0.0001*			

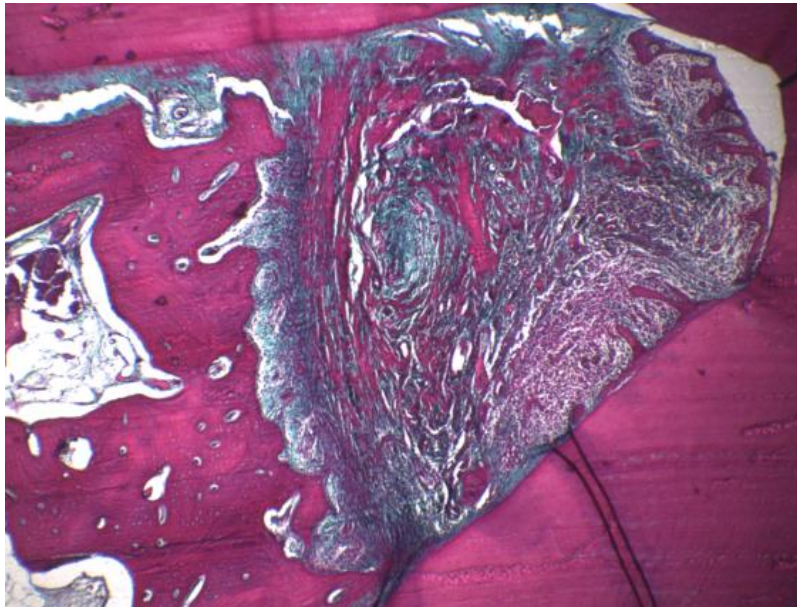
Table (3) Detailed results of Tukey's post hoc test for difference between groups regarding the area percent of bone

Groups	Significance
Group A versus Group B	0.1376 ^{ns}
Group A versus Group C	0.4924 ^{ns}
Group A versus Group D	0.0035*
Group B versus Group C	0.5108 ^{ns}
Group B versus Group D	0.1894 ^{ns}
Group C versus Group D	0.0631 ^{ns}

*significant at $p < 0.05$, ns=non significant

Table (4) Area percent of bone in different groups and significance of the difference using ANOVA test:

	Group A	Group B	Group C	Group D
Mean	36.442	29.08	32.811	23.147
Std Dev	5.784	8.13	8.993	4.412
Max	41.23	44.457	45.683	28.279
Min	25.169	19.987	21.966	16.534
F value		3.321		
P value		0.047*		

**Figure (3):** A photomicrograph of group A experimental specimen showing a mixture of woven bone and the more regular concentric lamellar bone (masson trichrome)

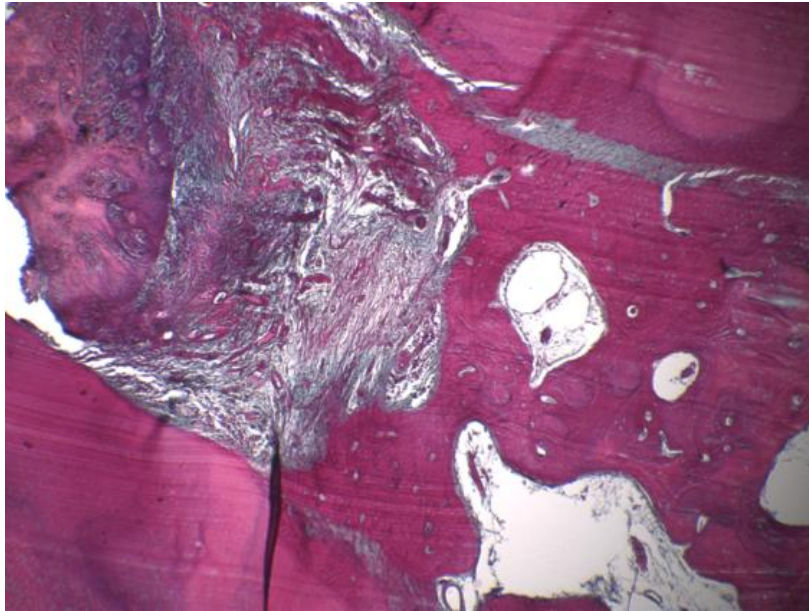


Figure (4): A photomicrograph of group B showing woven bone with wide osteocytes forming thickened irregular trabeculae. Note the widespread osteoblastic rimming and the dense fibrous background.(masson trichrome)

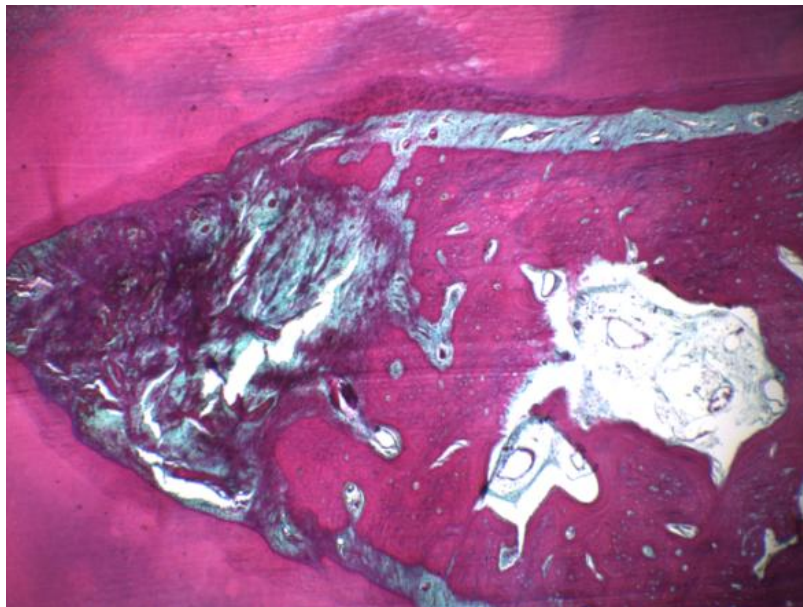


Figure (5) : A photomicrograph of group C experimental specimen showing mature lamellar bone (RED) surrounding marrow cavities and less organized woven bone (BLUE). (masson trichrome)

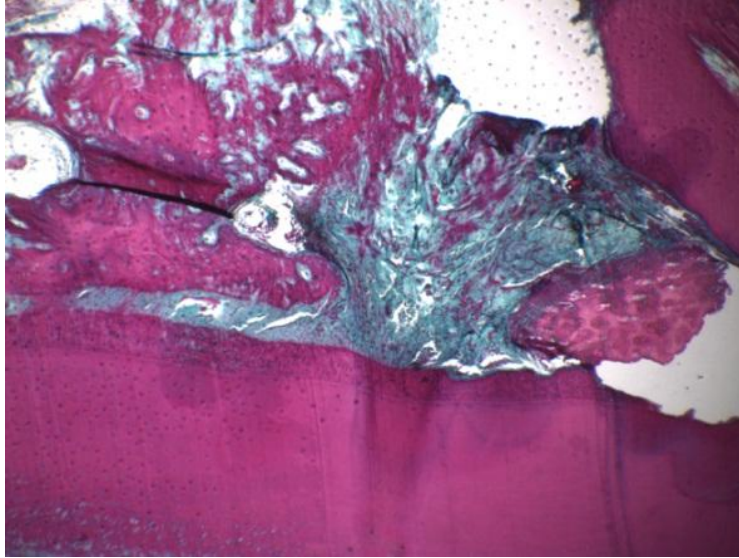


Figure (6): A photomicrograph of group D control spaceman showing irregular woven bone spicules rimmed by osteoblasts as well as densely packed Collagen fibers (masson trichrome).

Discussion

Animal studies are efficient complementary to *in vitro* studies prior to apply new clinical treatments because biopsy harvesting for histology is inapplicable in humans. Thus, animal experimentation is a necessary bridge prior to clinic application (**Struillou et al, 2010; Seol et al, 2010**). Many animals have been used in evaluation of periodontal regeneration however; dogs and nonhuman primates are considered the most devilishly used in literature (**Bogle et al., 1997**). A chronic class II furcation defect was used to hinder spontaneous regeneration that may reduce the sensitivity of testing any regenerative power in acute defects (**Soares et al, 2005**).

To create such chronic defect a rubber base impression material was used to prevent spontaneous repair and induce an inflammatory response as reported by (**Burcak Simsek et al ., 2012**). The critical-sized defect is the minimal size of a defect that can not heal spontaneously (**Liu et al, 2010**). A surgically created furcation defect is better than natural plaque-induced

chronic defect, as the surgical preparation saves more time and permits standardization of the defect as reported by **Jung et al, 2011**. In the present study, the defect size measured five mm in the apicoocclusal direction and two mm deep in horizontal direction and was performed on the buccal side of the mandibular second and third premolars as this defect size was reported in many studies to work as class II furcation defects (**Plínio Regazzini et al ., 2004 ; Suaid et al., 2011 and Burcak Simsek et al ., 2012**). Histologic analysis was necessary in this study to evaluate the effectiveness of the regenerative techniques and to predict the presence and the extent of periodontal regeneration as done by **Burcak Simsek et al., 2012**.

The usage of PRF in regenerative periodontal procedures is based on the presence of many growth factors within a homogenous, 3-dimensional organization of fibrin meshwork which contains circulating cytokines (**Dohan Ehrenfest et al ., 2009**). Growth factors release from the PRF membrane could further augment the regenerative power of the bone cells, leading to considerable amount of bone formation

(Sambhav Jain *et al.*, 2014). The combined use of both PRF and Nano-HA could accelerate healing processes getting use of the synergic effect of the powerful inherent regenerative potential of PRF and the osteoconductive property of Nano-HA. In the present study, Nano-HA was used based on the hypothesis that the Nano structured materials have a huge number of molecules on their surface when compared to bulk materials. Although Nano-HA showed close contact with surrounding tissues and quick resorption characteristics, It was found that undisturbed osseous-integration and complete resorption occurred within 12 weeks (Schwarz *et al.*, 2006).

The Nano-HA as bone graft substitute could result in rapid healing of critical sized defect in animal's experiments and human trails. Schnettler *et al.*, (2004) reported that Nano-HA stimulates bone healing by activation of osteoblast. Nano-hydroxyapatite has been used successfully to fill in bone defects. It provides an ideal scaffold for new bone formation in animal studies, this bone substitute with small granules that allow more bone substitution. It has been widely used in bone regeneration, implant tooth replacement, and intra-bony defects as reported by Lekovic *et al.*, 2012.

The present study showed that, the largest area percent of newly regenerated bone was observed in the (PRF+ NHA bone) group, followed by the PRF group, then the NHA group, with the least value recorded in the control group. The difference was not statistically significant except in comparing (PRF+ NHA group) with control group ($p < 0.05$). The maximum regenerative potential was noticed in combined PRF and NHA group than other groups. PRF has angiogenic properties that might lead to an increased probability of revascularization and enhances consolidation of the graft when used in combination with an osteoconductive medium. NHA application showed non-significant

improvements compared with the control group. Non statistically significant improvement was observed in PRF group compared with bone group regarding % of newly formed bone in the defect, this was in accordance with Wang and Boyapati 2006 who reported that angiogenesis is a critical factor for success in furcation regeneration and as PRF play an important role in angiogenesis and contain growth factors such as PDGF and TGF β that cause better induction of osteoblastic differentiation as well, PRF clot provide a scaffold for recruitment of tissue cells to injured site (Dohan *et al.*, 2006; Dohan *et al.*, 2009). In the control group (group D) extensive fibrous and mesenchymal activity was found with densely packed granulation tissue that formed of fibroblasts, new collagen fibers, and blood vessels. The histomorphometric results within this group showed the least area percent of the bone and this was in agreement with many authors who had reported the difficulty of periodontal regeneration in furcation defects (Bowers *et al.*, 2003 ; Verma *et al.*, 2013). The present study revealed no inflammatory or foreign body reaction and no root resorption or ankylosis in all included four groups. Clinically, no allergic or toxic reaction recorded during the study period indicating biocompatibility of Nano hydroxyapatite and the safety of platelet rich fibrin.

Conclusions

The use of platelet rich fibrin in treatment of furcation class II defects could result in a new bone formation in the furcation area .

Combining PRF with Nano hydroxyapatite bone graft in treatment of furcation class II defects could significantly accelerate healing processes.

References:

1. Bogle G, Garrett S, Stoller NH, Swanbom DD, Fulfs JC, Rodgers , PW, Whitman S, Dunn RL, Southard GL,

- Polson AM (1997):** Periodontal regeneration in naturally occurring class II furcation defects in beagle dogs after guided tissue regeneration with bioabsorbable barriers. *J Periodontol* 68:536–544
2. **Bowers GM , Schallhorn RG , McClain PK , Morrison GM , Morgan, R & Reynolds MA :** factors influencing the outcome of regenerative therapy in mandibular class furcation : part 1 *j periodontal* 2003 ; 74 1255- 1268
 3. **Burcak Simsek & Gonca Cayir Keles & Sancar Baris & Burcu Ozkan Cetinkaya :** Comparison of mesenchymal stem cells and autogenous cortical bone graft in the treatment of class II furcation defects in dogs *Clin Oral Invest* (2012) 16:251–258
 4. **Choukroun J, Adda F, Schoeffler C, Vervelle A :** Une opportunité en parodontologie: le PRF. *Implantodontie* 2001; 42:55-62
 5. **Dohan Ehrenfest DM, Rasmusson L, Albrektsson T :** Classification of platelet concentrates : From pure platelet-rich plasma (P-PRP) to leucocyte-and platelet-rich fibrin (L-PRF). *Trends Biotechnol* 2009;27:158-67.
 6. **Dohan Ehrenfest DM, Rasmusson L, Albrektsson T :** **Classification of platelet concentrates :** From pure platelet-rich plasma (P-PRP) to leucocyte-and platelet-rich fibrin (L-PRF). *Trends Biotechnol* 2009;27:158-67.
 7. **Heinz B , Kasaj A , Teach M , Jepsen S :** clinical effects of nanocrystalline hydroxyapatite paste in the treatment of intrabony periodontal defects : a randomized controlled clinical study . *clin oral invest* ; 14:525-531, 2010
 8. **Huang FM, Yang SF, Zhao JH, Chang Y :** Platelet-rich Fibrin increases proliferation and differentiation of human dental pulp cells. *J Endod* 2010;36:1628-32.
 9. **Jung U-W, Chang Y-Y, Um Y-J, Kim C-S, Cho K-S, Choi S-H:** Interproximal periodontal defect model in dogs: a pilot study. *Oral Diseases* 2011; 17, 26–32
 10. **Jyotsna S. Patel, Sandeep G. Patel , Chandrashekhar Kadam :** Choukroun's Platelet Rich Fibrin in Regenerative Dentistry *Universal Research Journal of Dentistry* • January-April 2013 • Vol 3 • Issue 1
 11. **Kasaj A , Willershausen B , Reichert C , Rohrig B , Smeets R, Schmidt M :** ability of nanocrystalline hydroxyapatite paste to promote human periodontal ligament cell proliferation . *j oral science* ; 50 ,3,279-285,2008
 12. **Kornman KS & Robertson PB :** fundamental principles affecting the leonardis outcome of therapy of osseous lesions . *periodontal* 2000 2000;22:22-43
 13. **Lekovic V, Milinkovic I, Aleksic Z, Jankovic S, Stankovic P, Kenney EB, Camargo PM:** Platelet-rich fibrin and bovine porous bone mineral vs. platelet-rich fibrin in the treatment of intrabony periodontal defects. *J Periodontal Res.* 2012Aug;47(4):409-17
 14. **Liu X, Ma L, Mao Z, and Gao C:** Chitosan-Based Biomaterials for Tissue Repair and Regeneration *Adv Polym Sci* 2011; 244: 81–128.
 15. **Plínio Regazzini, Arthur B. Novaes, Paulo T. de Oliveira, Daniela B. Palioto, Mário Taba, Jr, Sergio L. S. de Souza, Márcio F. M. Grisi,** Comparative Study of Enamel Matrix Derivative with or without GTR in the Treatment of Class II Furcation Lesions in Dogs *Int J Periodontics Restorative Dent* 2004;24:476–487.)
 16. **Pushendra Kumar Verma, Ruchi Srivastava, K K Gupta, and T P**

- Chaturvedi.** Treatment strategy for guided tissue regeneration in various class II furcation defect: Case series. *Dent Res J (Isfahan)*. 2013 Sep-Oct; 10(5): 689–694.
17. **Sambhav Jain, Ranjana Mohan, Karthik Krishna M., Janardhana Amarnath BJ TMU J.:** Platelet Rich Fibrin and b-Tricalcium Phosphate for the Treatment of Grade-II Furcation Defect A Case Report *Dent Vol. – 1; Issue 1 Jan – Mar 2014*
 18. **Schnettler R, Stahl JP, Alt V, Pavlidis T, Dingeldein E, Wenisch S :** Calcium phosphate-based bone substitutes. *Eur J Trauma* 2004;4:219-29
 19. **Schwarz F , Bieling K , Latz T , Nuersy E , Becker J :** healing of intrabony peri-implantitis defects following application of a nanocrystalline hydroxyapatite (ostim) or a bovine – derived xenograft (bio-oss) in combination with collagen membrane (bio-guide). A case series . *j clin periodontal*:33:491-499,2006a
 20. **Seol YJ, Pellegrini G, Franco LM, Chang PC, Park C and GiannobileWG:** Preclinical Methods for the Evaluation of Periodontal Regeneration In Vivo. G.J. Seymour et al. (eds.), *Oral Biology, Methods in Molecular Biology*, © Springer Science+Business Media, LLC 20. 2010.
 21. **Soares FP, Hayashi F, Yorioka CW, Pannuti CM, Gioso MA, de Lima LA, Romito GA, Pustiglioni FE (2005) :** Repair of class II furcation defects after a reparative tissue graft obtained from extraction sockets treated with growth factors: a histologic and histometric study in dogs. *J Periodontol* 76:1681–1689
 22. **Struillou X, Boutigny H, Soueidan A, and Layrolle P:** Experimental Animal Models in Periodontology: A Review. *The Open Dentistry Journal*, 2010; 4: 37–47
 23. **Suaid FF, Ribeiro FV, Rodrigues TL, Silve´rio KG, Carvalho MD, Nociti Jr. FH, Casati MZ, Sallum EA:** Autologous periodontal ligament cells in the treatment of class II furcation defects: a study in dogs. *J Clin Periodontol* 2011; 38: 491–498.
 24. **Tatiana M. Deliberador, Maria J.H. Nagata, Fla´via A.C. Furlaneto, Luiz G.N. Melo, Tetuo Okamoto, Maria L.M.M. Sundefeld, and Stephen E. Fucini.** Autogenous Bone Graft With or Without a Calcium Sulfate Barrier in the Treatment of Class II Furcation Defects: A Histologic and Histometric Study in Dogs. *J Periodontol • Volume 77 • Number 5, May 2006.*
 25. **Thorwarth M , Schultze-Mosgau , Kessler P , Wiltfang J , Schlegel KA :** bone regeneration in osseous defects using a resorbable nanoparticulate hydroxyapatite. *J oral maxillofacial surg*; 63 : 1626-1633,2005
 26. **Waerhaug J : the furcation problem :** etiology, pathogenesis , diagnosis , therapy and prognosis . *j clin periodontal* 1980; 7:73-95
 27. **Wang HL, Greenwell H, Fiorellini J, Giannobile W, Offenbacher S, Salkin L, Townsend C, Sheridan P, Genco RJ; Research, Science and Therapy Committee.:** periodontal regeneration , position paper . *j periodontal* ;76:1601-1622 , 2005.
 28. **Wang HL, Boyapati L:** “PASS” principles for predictable bone regeneration. *Implant Dent* 2006;15:8-17