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Research Article

Effect of Plasma Rich in Growth Factors on the Healing of Intrabony Defects in Human: A Comparative Clinical Trial

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Abstract

Background and aims. A combination of guided tissue regeneration (GTR), bovine porous bone mineral (BPBM), and platelet rich plasma has been already applied for the regeneration of intrabony defects. The aim of this study was to determine the role of plasma rich in growth factor (PRGF) alone in the periodontal regeneration of intrabony defects in humans as compared with BPBM/GTR Technique.

Materials and methods. In this split-mouth clinical trial, nine advanced periodontal patients with 20 intrabony defects were randomly treated with a combination of either BPBM/GTR (control) or PRGF (test). Periodontal parameters including gingival recession, probing depth (PD), gingival index (GI), plaque index (PI), and relative clinical attachment level (CAL) were evaluated at baseline and 6 months post-operatively.

Results. No difference was observed at baseline between the two groups. At 6-month post-treatment, both techniques demonstrated significant reduction in CAL, PD and GI. The mean pocket depth reduction in control group was 4.1 ± 1.52 mm and 4.5 ± 1.5 mm on buccal and lingual sites, respectively ($P < 0.0001$); and 3.6 ± 0.9 mm in the test group. The changes in CAL in the deepest part of the defect were 3.06 ± 1.79 mm and 2.33 ± 0.78 mm for test and control groups, respectively. No statistically significant difference was detected between the two groups after 6 months.

Conclusion. According to the results, the use of PRGF alone, similar to BPBM/GTR improves clinical parameters and plays a significant role in PD reduction and CAL gain.

Key words: **Bone graft, guided tissue regeneration, intrabony defect, platelet rich plasma.**

Introduction

The important goal in periodontal therapy is the prevention of periodontal disease progress and the regeneration of lost supporting tissues, including cementum, periodontal ligament and the alveolar bone.[1-5] Favorable results have been gained in the treatment of intrabony defects using a combination of graft materi-

als and collagen membranes.[6-9] Recently, the use of polypeptide growth factors (PGFs) for periodontal regeneration has received attention from periodontal researchers and clinicians.[10-15] PGFs are known as biologic mediators regulating cell proliferation, chemotaxis and differentiation.[16-19] Among all known PGFs, platelet derived growth factors (PDGF) and bone morphogenic protein 3 (BMP-3), members of transforming growth factor-β (TGF-β) super family, have shown to exert a favorable effect on periodontal regeneration.[20] PDGF and TGF-β are found abundantly in the alpha granules of platelets.[21] The positive effect on bone healing, associated with platelet-rich plasma (PRP) which contains high concentrations of such granules, may be attributed to angiogenic, proliferative and differentiative effects exerted by these factors on osteoblasts.[22] The use of PRP on wound healing sites enhances platelet concentration up to 338%.[23] It has also yielded successful results in tissue and bone regenerative therapies in dentistry.[24] In recent years, a combination of PRP with bone graft materials, such as bovine porous bone mineral (BPBM), and guided tissue regeneration (GTR) have revealed significant improvements in clinical parameters,[11,12,15,25,26] however; PRP/BPBM/GTR treatment has not yielded superior results to BPBM/GTR without PRP.[27] Due to the fact that most of the studies regarding the influence of PRP on the regeneration of intrabony defects has been performed in combination with bone grafts and GTR, the effect of PRP alone is not yet obvious. Plasma rich in growth factors (PRGF), like PRP, is rich in growth factors and has been presented as a material for use in tissue regenerative techniques.[28] Therefore, the aim of this study was to determine the effect of PRGF alone in the regeneration of intrabony defects.

Materials and Methods

Study Population

Nine patients (5 females and 4 males; mean age, 44 ± 5.6 years old) with moderate to advanced periodontal disease were included in this split-mouth clinical trial. The purpose of the study was explained to the patients and they were required to sign informed consent forms. All patients were treated at the Department of Periodontology, Tehran Azad University, by one experienced surgeon during 2008-2009. The study protocol was approved by the ethical committee of the university.

Study Design

The study was designed as a randomized, comparative clinical trial. Patients included in this study were individuals with at least 2 bilateral, although not similar,

intrabony defects with ≥ 3mm depth, visible on periapical radiographs, and a pocket depth of ≥ 6 mm. Patients with systemic disease, compromised immune system, pregnancy and or lactation, taking any drugs known to cause gingival enlargement, allergy or sensitivity to any of the medications to be used in the study were excluded. All patients received initial therapy including oral hygiene instruction, and scaling and root planning. Plaque control was followed up until O'Leary periodontal index (PI) of ≤ 25% was obtained.[29] Occlusion adjustment was performed if trauma from occlusion was diagnosed. Trauma from occlusion was evaluated by examining the obvious presence of fremitus in centric occlusion or working or balancing excursions. Six to eight weeks following phase one therapy, a periodontal re-evaluation was performed to confirm the suitability of the sites for this periodontal surgical study and preoperative periapical radiographs were taken. Two surgical sites were randomly assigned to the test or control groups with toss of a coin.

Clinical Measurements

The following clinical parameters were assessed by the same calibrated examiner (NA) masked to treatment; at baseline and six months after the surgical procedure using the same type of periodontal probe (15-mm University of North Carolina (UNC-15) periodontal probe, Hu-Friedy, Chicago, IL). Plaque Index (PI),[30] and Gingival Index (GI)[31] were recorded. Gingival Recession (GR), measured as the distance of gingival margin from cementoenamel junction (CEJ) was also recorded. Probing Depth (PD), using the gingival margin as reference; Relative Clinical Attachment Level (CAL), using the acrylic stent, as reference, was measured with customized acrylic stent using grooves to ensure a reproducible placement of the periodontal probe. In order to ensure the correct and repetitive insertion of the stent, the distance between CEJ and stent edge was recorded.

An examiner other than the operator performed the clinical measurements without knowledge of the treatment groups. Intraexaminer calibration was achieved by examination of three patients twice, 48 hours apart, prior to the beginning of the study. Calibration was accepted if measurements at baseline and 48 hours were similar to the whole millimeter at a ≥ 90% level.

PRGF Preparation

In this study, the PRGF preparation was performed using a PRGF system (Biotechnology institution, Spain). One hour before surgery, 9 ml blood was

drawn from the patient. The blood was collected in two 5-cc sterile glass tubes that contained 0.5 cc 3.8% sodium citrate as anticoagulant. The glass tubes containing blood were centrifuged by a digital machine at 460 G for 8 minutes. As a result, plasma was separated into different fractions: at the top, 1 cc plasma poor in growth factors, in the middle, 0.5 cc plasma average in growth factors, and in the bottom, 0.5 cc of plasma rich in growth factors (PRGF), exactly over red blood cells. Following the aspiration of the upper and middle fractions with a 500 µl pipette, the third PRGF fraction was separated in 5 times with a 100 µl pipette. PRGF was mixed with 10% calcium chloride (50 µl of 10% calcium chloride were added to sterile tube containing 1 cc PRGF). A thermal block machine was applied for fibrin preparation and acceleration in PRGF coagulation. After 15-20 minutes, PRGF displayed a plastic consistency, the same as a biologic membrane.

Surgical Procedure

After administration of local anesthesia (Lidocaine 2%, with epinephrine 1:80,000), sulcular incisions were performed extending to the inter-proximal line angle of the neighboring tooth; then full thickness flaps were raised. Vertical-releasing incisions were performed if necessary for a better access or to achieve better closure. All granulation tissues were removed from the defects and the roots were thoroughly scaled and planed by means of hand (Gracey, Hu-Friedy, Chicago, IL, USA) and ultrasonic (Cavitron, Dentsply, Tulsa, OK, USA) instruments.

Treatment in the test group was performed as follows: First, PRGF with plastic consistency was applied to the defects. Then, flap was repositioned and sutured in place by using 4-0 non-absorbable black-braided silk surgical suture (Ethicon, Johnson and Johnson Ltd., Somerville, NJ, USA). Interrupted sutures were used.

In the control group, the same surgical procedure, undertaken for the test group, was performed without using PRGF. First, intrabony defects were filled with bovine porous bone mineral granules (Bio-Oss, Geistlich, Wolhusen, Switzerland; particle size, 0.25-1.0 mm); then a bioresorbable collagen membrane of Porcine origin (Biogide Perio, Geistlich , Wolhusen , Switzerland) was trimmed and adapted over the entire defect so as to cover 2-3 mm of the surrounding alveolar bone and to ensure stability of wound and of the graft material. Both surgical sites were covered with periodontal dressing (Coe-Pak, GC America Inc., Chicago, IL, USA).

Postoperative Care

All patients received antibiotics for a week (3 × 500 mg amoxicillin per day) and, if necessary, analgesics (Ibuprofen, 3 × 800mg per day) . The post-operative care included 0.2% chlorhexidine rinses twice a day for 2 weeks. Periodontal dressing and sutures were removed one week postoperatively, and patients were instructed not to brush or floss the areas where surgery had been performed for 2 weeks. Patients were examined weekly up to 1 month after surgery and then at 3 and 6 months. At 6 months postoperatively, all clinical measurements were re-evaluated and periapical radiographs were also taken.

Statistical Analysis

All clinical parameters were tested for normal distribution by using the Kolmogorov-Smirnov test. To compare the test and control treatments, the intra-group differences between baseline and 6-months postoperatively were evaluated using the paired t-test was used. Between-group comparisons were performed using the unpaired t-test. Due to the high variability of results in GI and PI, these differences for test and control groups were compared using the non-parametric Wilcoxon Signed Ranks Test. Statistical analysis was performed using commercially available software (SPSS version 16, SPSS Inc., Chicago, IL, USA). Statistical significance was set at 0.05.

Results

All patients completed the study. Test and control sites healed uneventfully. No allergic reaction, abscess or infection, dehiscence, collagen membrane or graft particle exposure were detected in any of the surgical sites. At baseline, the mean pocket depth was similar in the two groups on buccal and lingual sites and no statistically significant difference was found. At six months post-treatment, the mean PD reduction was 4.1 ± 1.52 mm on the buccal and 4.5 ± 1.5 mm on the lingual site in the BPBM/GTR group, and 3.6 ± 0.96 mm on both buccal and lingual sites in the PRGF group (P < 0.0001). No statistically significant difference was found between the groups (Table 1).

At baseline, the mean gingival recession was similar between the two groups, with no statistically significant difference (Table 2). At Six months, the mean GR on buccal sites was increased 0.4 ± 1.35 mm in the BPBM/GTR group and 0.9 ± 1.2 mm in the PRGF group; the increase in GR was statistically significant for PRGF group (P < 0.05). The mean GR on lingual sites, at baseline and 6 months postoperatively, were similar for control and test groups.

The mean CAL was similar at baseline for both groups (Table 3). At six months postoperatively, CAL gains were 3.06 ± 1.79 mm and 2.33 ± 0.78 mm for

Table 1. Probing Pocket Depth of Buccal &Lingual (mean ± SD[mm]) at Baseline and 6 months (n=10 for each treatment group)

	PDB				PDL			
Group	Base line	6 months	Difference	P-Value	Base line	6 months	Difference	P-Value
BPBM/GTR	7.30 ± 1.49	3.20 ± 0.79	4.10 ± 1.52	<0.0001[*]	7.30 ± 1.34	2.80 ± 0.42	4.50 ± 1.50	<0.0001[*]
PRGF	7 ± 1.82	3.40 ± 1.50	3.60 ± 0.97	<0.0001[*]	6.90 ± 0.99	3.30 ± 1.42	3.60 ± 0.97	<0.0001[*]
P-Value	NS	NS	NS		NS	NS	NS	

NS = not statistically significant.
*Statistically significant (P <0.05) !

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Table 2. Gingival Recession Buccal &Lingual, (mean ± SD [mm]) at Baseline and 6 months (n=10 for each treatment group)

	GRB				GRL			
Group	Base line	6 months	Difference	P-Value	Base line	6 months	Difference	P-Value
BPBM/ GTR	0.80 ± 0.79	1.20 ± 1.03	0.40 ± 1.35	NS	1.40 ± 1.17	1.20 ± 1.03	0.20 ± 0.42	NS
PRGF	0.90 ± 0.87	1.80 ± 1.55	0.90 ± 1.20	<0.05[*]	1.30 ± 1.16	1.60 ± 1.26	0.30 ± 0.82	NS
P-Value	NS	NS	NS		NS	NS	NS	

NS = not statistically significant.
*Statistically significant (P <0.05) !

BPBM/GTR and PRGF groups, respectively (P < 0.001). In both groups, CAL improved significantly compared to baseline, but no statistically significant difference was observed between the two groups. PI and GI changes are shown in Table 4.

The amount of PI in both groups at baseline and 6 months post operation were similar and showed no significant statistical changes. GI, at baseline and 6 months postoperatively, and its changes were similar between the groups, with a significant reduction in both groups.

Discussion

The results of this study show that the treatment of intrabony defects with both BPBM/GTR and PRGF leads to a significant pocket depth reduction and CAL gain compared to baseline. Pocket depth reduction, on the buccal and lingual sites, were 4.1 ± 1.52 mm and 4.5 ± 1.5 mm in BPBM /GTR group and 3.6 ± 0.97 mm in PRGF group. Gingival index reductions in both

Table 3. Relative clinical attachment level (mean ± SD [mm]) at Baseline and 6 months (n=10 for each treatment group)

	CAL			
Group	Base line	6 months	Difference	P-Value
BPBM/ GTR	11.74 ± 2.43	8.68 ± 1.80	3.06 ± 1.79	<0.0001*
PRGF	11.24 ± 3.47	8.92 ± 3.34	2.33 ± 0.87	<0.0001*
P-Value	NS	NS	NS	

NS = not statistically significant.
*Statistically significant (P <0.05) !

groups were also significant. The increase in gingival recession showed a significant change of 0.9 mm on the buccal site in the PRGF group.

Camargo et al, [9] using BPBM/GTR in the treatment of intrabony defects, showed mean pocket depth reduction and CAL gain of 4.03 ± 0.94 mm and 3.29 ± 1.12 mm on the buccal site, respectively. Another study comparing BPBM/GTR/PRP to open flap debridement in the treatment of intrabony defects found that the mean pocket depth reduction on the buccal site was 2.99 ± 1.42 mm in the control and 5.06 ± 1.15 mm in the case group and this difference was statistically significant for the BPBM/GTR/PRP cases.[15] In the latter study, mean CAL gains were 4.52 ± 1.24 mm and 1.47 ± 1.95 mm in the case and control groups, respectively. [15] Higher pocket depth reduction and CAL gain in the latter study indicate that the growth factors may increase the benefits obtained from bone grafts and GTR. The present study showed pocket depth reductions of 4.1 ± 1.52 mm and 4.5 ± 1.5 mm on the buccal and lingual sites of BPBM/GTR group, respectively, compared to 3.6 ± 0.97 mm on the both sites of the PRGF group. These findings are in line with previous studies.[9,15] The design of the present study, however, allows determining which treatment is responsible for the observed differences. That said, in the present study, the effect of the growth factors alone, as compared to BPBM/GTR, were investigated, and regarding the absence of significant differences in pocket depth reduction and CAL gain, it could be claimed that using PRGF is equally effective to BPBM/GTR combination.

Table 4. Gingival Index, Plaque Index at Baseline and 6 months (n=10 for each treatment group)

	GI[*]				PI[†]			
Group	Base line	6 months	Difference	P-Value	Base line	6 months	Difference	P-Value
BPBM/ GTR	1.45 ± 0.44	0.٧7 ± 0.72	0.67 ± 0.98	<0.05[‡]	0.75 ±0.26	0. 70 ± 0.23	0.05 ± 0.10	NS
PRGF	1.45 ± 0.44	0.90 ± 0.68	0.55 ± 0.86	<0.07[‡]	0.72 ± 0.22	0.72 ± 0.22	0	NS
P-Value	NS	NS	NS	NS	NS	NS	NS	

*Gingival index of Löe (1963)
† Plaque index of Silness & Löe(1964)
NS = not statistically significant
‡Statistically significant (P <0.05)!

In another study, GTR did not yield any clinical effects on PD reduction and CAL gain, compared to BPBM /PRP.[12] In the present study, pocket depth reductions in both groups were also not significant; however, CAL gain showed statistical significance, although to a lower amount. However, the differences in sample size and BPBM application do not allow a comparison of these studies. In one study, PRP was not shown to be superior to combination of GTR and natural bone mineral, failing to achieve significant PD reduction and CAL gain.[27] However, the use of BPBM/GTR/PRP, as compared to GTR alone lead to better results in the regeneration of intrabony defects.[11]

Pocket depth reduction and CAL gain of the PRGF group in the present study were similar to those of GTR group in the study of Camargo et al (3.62 ± 0.81 mm and 2.62 ± 1.23 mm, respectively). Although in the present study the number of patients was not equal in the two groups, it could be concluded that the use of PRGF, could be at least as effective as the use of GTR in the regeneration of intrabony defects. Hanna et al[25] demonstrated that a combination of bone graft and PRP, as compared to bone graft alone, may lead to more desirable results.[25]

Okuda et al,[26] comparing PRP/hydroxyapatite and salin/hydroxyapatite , showed improvements in both clinical and radiographic parameters using both combinations, with higher pocket depth reduction and CAL gain in PRP/hydroxyapatite group. [26] Considering the use of PRGF alone in the present study, significant pocket depth reduction and CAL gain may be attributed to this substance.

Comparison of bioabsorbable barrier membrane (MEM) with autologous platelet concentrate (APC) has shown 3 ± 1.41 mm and 3.6 ± 1.67 mm pocket depth reductions and 2.2 ± 1.79 mm and 3 ± 1 mm those of CAL gains, in APC and MEM groups respectively.[32] In the group with PRGF only in the present study, mean pocket depth reduction and CAL gain were 3.6 ± 0.97 mm and 2.33 ± 0.78 mm, respectively, indicating similar amounts of CAL gain and higher amounts of pocket depth reduction compared to the latter study. Considering the similar results obtained

using growth factors alone in the present study, as compared to controls, a positive effect of these materials on pocket depth reduction and CAL gain can be inferred.

Considering the results of the present study and previous investigations,[11,26] it can be concluded that the use of each material, effective in regeneration, alone cannot provide CAL gain of more than 2.5 mm, probably because regeneration is a multifactorial process and if space providing materials are needed to prevent the immigration of epithelial cells, there would be a simultaneous need for osteoconductive and osteoinductive materials. Pocket depth reduction is not only a favorable consequence of periodontal regenerative therapy, but it also is the most important parameter during the maintenance period, since it directly influences the cleansing of the involved site.[2] The final amount of mean pocket depth in both groups of the present study was 3 mm , which is suitable for the maintenance period. Although CAL gain does not necessarily imply connective tissue attachment promotion and the presence of real regeneration, it still can indicate clinical improvement, as much as reentry or radiographic evaluations do.[33]

The positive effects exerted by PRGF alone on PD reduction and CAL gain, showing no significant difference with those of GTR/BPBM group, can be attributed to biological characteristics of PRGF, one of which is its abundant growth factors, including TGF and PDGF. Large amounts of PDGF results in osteoblastic proliferation, which usually happens at the first stages of healing. TGF-β family has an important role in differentiation of osteoblasts and cementoblasts. TGF-β also stimulates growth and differentiation in cells of periodontal ligament and alveolar bone, while at the same time, inhibits epithelial proliferation.[34,35] Fibronectin stimulation causes the adherence of fibroblast to the root surface and angiogenesis. Due to its high amounts of fibrin, TGF-β also has a sticky property. The fibrin component works as an hemostatic agent and aids in stabilization of the graft material and the blood clot.[9,34] Fibrin adherence to the root surface may impede the apical migration of the epithelial and connective tissue cells exerting an effect similar to that

of GTR in defect regeneration.[33] PRGF can modulate the regeneration process and significantly reduce pocket depth and improve CAL gain as well. However, considering the gingival recession on buccal sites in PRGF group in the 6-month evaluation period of the present study, it seems PRGF, due to its inability to increase tissue tonicity, should be used with bone graft.[12,25,26] Further histologic investigations need to be carried out to determine the exact effects of PRGF on tissue regeneration. The use of bone graft alone could enhance tissue consistency and prevent probe insertion even in the lack of any new attachment gain. Therefore, considering pocket depth reduction and gingival recession in PRGF group, such a reduction, should not be definitely attributed to attachment level improvement.

The limited number of cases evaluated does not allow for extrapolation of the obtained results. The device which produces PRGF is expensive and necessitates the involvement of especially-trained personnel. In addition to these limitations, processing of PRGF elongates the surgical time for about half an hour, which may be a limiting factor for its use in private clinics.

Conclusions

Within the limitations of the present study, it can be concluded that PRGF application would be beneficial for the treatment of intrabony defects as well as pocket depth reduction and clinical attachment gain.

Acknowledgments

References

1. 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. *J Periodontol* 2005;76:1601-22.
2. The American Academy of Periodontology. *Glossary of Periodontal Terms*, 4th ed. Chicago: The American Academy of Periodontology; 2001:44.
3. Garrett S. Periodontal regeneration around natural teeth. *Ann Periodontol* 1996;1:621-66.
4. Bowers GM, Chadroff B, Carnevale R etal. Histologic evaluation of new attachment apparatus formation in humans. Part I. *J Periodontol* 1989;60:664-74.
5. Karring T, Lindhe J, Cortellini P. Regenerative periodontal therapy. In: Lindhe J, Karring T, Lang NP, eds. *Clinical Periodontology and Implant Dentistry*, 4th ed. Copenhagen: Black well-Munksgaard; 2003:650-704.
6. Tonetti MS, Cortellini P, Lang NP etal. Clinical outcomes following treatment of human intrabony defects with GTR/bone replacement material or access flap alone. A multicenter randomized controlled clinical trial. *J Clin Periodontol* 2004;31:770-6.
7. Sculean A, Berakdar M, Chiantella GC, Donos N, Arweiler NB, Brecx M. Healing of intrabony defects following treatment with a bovine-derived xenograft and collagen membrane. A controlled clinical study. *J Clin Periodontol* 2003;30:73-80.
8. Paolantonio M. Combined periodontal regenerative technique in human intrabony defects by collagen membranes and anorganic bovine bone. A controlled clinical study. *J Periodontol* 2002;73:158-66.
9. Camargo PM, Lekovic V, Weinlaender M, Nedic M, Vasilic N, Wolinsky LE, et al. A controlled re-entry study on the effectiveness of bovine porous bone mineral used in combination with a collagen membrane of porcine origin in the treatment of intrabony defects in humans. *J Clin Periodontol* 2000; 27:889-96.
10. deObarrio JJ, Araúz-Dutari JI, Chamberlain TM, Croston A. The use of autologous growthfactors in periodontal surgical therapy: platelet gel biotechnology-case reports. *Int J Periodontics Restorative Dent* 2000;20:486-97.
11. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB. Platelet-rich plasma and bovine porous bone mineral combined with guided tissue regeneration in the treatment of intrabony defects in humans. *J Periodontal Res* 2002;37:300-6.
12. Lekovic V, Camargo PM, Weinlaender M, Vasilic N, Kenney EB. Comparison of platelet-rich plasma, bovine porous bone mineral, and guided tissue regeneration versus platelet-rich plasma and bovine porous bone mineral in the treatment of intrabony defects: a reentry study. *J Periodontol* 2002;73:198-205.
13. Lekovic V, Camargo PM, Weinlaender M, Vasilic N, Aleksic Z, Kenney EB. Effectiveness of a combination of platelet-rich plasma, bovine porous bone mineral and guided tissue regeneration in the treatment of mandibular grade II molar furcations in humans. *J Clin Periodontol* 2003;30:746-51.
14. Camelo M, Nevins ML, Schenk RK, Lynch SE, Nevins M. Periodontal regeneration in human Class II furcations using purified recombinant human platelet-derived growth factor-BB (rhPDGF-BB) with bone allograft. *Int J Periodontics Restorative Dent* 2003;23:213-25.
15. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB. A reentry study on the use of bovine porous bone mineral, GTR, and platelet-rich plasma in the regenerative treatment of intrabony defects in humans. Int *J Periodontics Restorative Dent* 2005;25:49-59.
16. Graves DT, Cochran DL. Mesenchymal cell growth factors. *Crit Rev Oral Biol Med* 1990;1:17-36.
17. Lynch SE, Giannobil WV. Polypeptide growth factors: Molecular mediators of tissue repair. In: Genco RJ , Hamada S , Lehner T , Mergenhagen S, editors. Molecular Pathogenesis of periodontal disease . Washington , DC: *American Society for Microbiology Press;* 1994:415-25
18. Kiritsy CP, Lynch AB, Lynch SE. Role of growth factors in cutaneous wound healing: a review. *Crit Rev Oral Biol Med* 1993;4:729-60.
19. Giannobile WV. The potential role of growth and differentiation factors in periodontal regeneration . *J Periodontol.* 1996;67:545-53.
20. Carranza FA Jr. Reconstructive osseous surgery .in: Carranza FA Jr, Newman MG, eds. *Glickman's Clinical Periodontology.* Philadelphia: Saunders; 1996:269-78.
21. Assoian RK, Grotendorst GR, Miller DM, Sporn MB. Cellular transformation by coordinated action of three peptide growth factors from human platelets. *Nature* 1984; 309:804-6.
22. Marx RE. Platelet-rich plasma: evidence to support its use. *J*

Oral Maxillofac Surg 2004; 62:489-96.

23.   Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma. Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; 85:638-46.

24.   Tözüm TF, Demiralp B. Platelet-rich plasma: a promising innovation in dentistry. *J Can Den Assoc* 2003;69:664.

25.   Hanna R, Trejo PM, Weltman RL. Treatment of intrabony defects with bovine-derived xenograft alone and in combination with platelet-rich plasma: a randomized clinical trial *J Periodontol* 2004;75:1668-77.

26.   Okuda K, Tai H, Tanabe K et al. Platelet-rich plasma combined with a porous hydroxyapatite graft for the treatment of intrabony periodontal defects in humans: a comparative controlled clinical study. *J Periodontol* 2005;76:890-8.

27.   Döri F, Huszár T, Nikolidakis D, Arweiler NB, Gera I, Sculean A. Effect of platelet-rich plasma on the healing of intra-bony defects treated with a natural bone mineral and a collagen membrane. *J Clin Periodontol* 2007 Mar;34:254-61.

28.   Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofac Implants* 1999;14:529-35.

29.   O'Leary TJ, Drake RB, Naylor JE. The plaque control record.

!

30.   Silness J, Loe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;22:121-35.

31.   Loe H. The gingival index, the plaque index & the retention index system. *J Periodontol* 1967; 38:610.

32.   Papli R,Chen S. Surgical treatment of infrabony defects with autologous platelet concentrate or bioabsorbable barrier membrane: a prospective case series. *J Periodontol* 2007; 78:185-93.

33.   Zybutz M, Rapoport D, Laurell L, Persson GR. Comparisons of clinical and radiographic measurements of inter-proximal vertical defects before and 1 year after surgical treatments. *J Clin Periodontol* 2000;27:179-86.

34.   Okuda K, Kawase T, Momose M, Murata M, Saito Y, Suzuki H, et al. 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* 2003;74:849-57.

35.   Kawase T, Okuda K, Wolff LF, Yoshie H. Platelet-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro. *J Periodontol* 2003; 74:858-64.

J Periodontol 1972;43:38.