J Periodont Res 2005; 40; 489–495

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2005.00828.x

In vitro cell-type specific biological response of human periodontally related cells to platelet-rich plasma

M. Annunziata[1], A. Oliva[2], C. Buonaiuto[1], A. Di Feo[2], R. Di Pasquale[2], I. Passaro[2], L. Guida[1]

[1]Department of Odontostomatological, Orthodontic and Surgical Disciplines, and [2]Department of Biochemistry and Biophysics 'F. Cedrangolo', Second University of Naples, Naples, Italy

Annunziata M, Oliva A, Buonaiuto C, Di Feo A, Di Pasquale R, Passaro I, Guida L: In vitro *cell-type specific biological response of human periodontally related cells to platelet-rich plasma. J Periodont Res 2005; 40: 489–495. © Blackwell Munksgaard 2005.*

Objectives: Platelet-rich plasma is a blood-derived fraction containing high concentrations of platelets and growth factors. Applied in the form of a gel on surgical wounds, it is able to stimulate hard and soft tissue repair and has been proposed for use in the field of periodontal regeneration. However to date, little is known about the biological interactions between platelet-rich plasma and periodontally related cells. In this study, we investigated the effects between platelet-rich plasma and cell populations involved in periodontal regeneration, namely primary human periodontal ligament cells, gingival fibroblasts and keratinocytes.

Material and methods: The proliferation of human periodontal ligament cells, gingival fibroblasts and keratinocytes by [^{3}H]thymidine incorporation was assessed. The alkaline phosphatase activity and type I collagen levels of human periodontal ligament cells were also evaluated by a spectrophotometric assay and western blot analysis, respectively.

Results: Incubation of human periodontal ligament cells with platelet-rich plasma resulted in time-dependent growth stimulation (up to fourfold of control at 72 h). Likewise, an increase in the specific activity of alkaline phosphatase (fourfold at 6 days) and collagen (twofold at 7 days) was observed. Platelet-rich plasma also enhanced human gingival fibroblasts proliferation by twofold, whereas it inhibited human keratinocytes growth by 40%, with respect to their own controls at 72 h.

Conclusion: Cell populations related to periodontal tissue were differently affected by platelet-rich plasma. In fact, a strong stimulation of human periodontal ligament cells proliferation, a minor increase in the growth rate of human gingival fibroblasts and a marked decrease of human keratinocytes proliferation were evident. In addition, in human periodontal ligament cells increased collagen and alkaline phosphatase activity levels were observed. These findings appear interesting in view of platelet-rich plasma utilization in periodontal regeneration.

Professor Adriana Oliva, Dipartimento di Biochimica e Biofisica 'F. Cedrangolo Facoltà di Medicina e Chirurgia, Seconda Università degli Studi di Napoli, Via L. De Crecchio, 7, 80138 – Napoli, Italy
Tel: +39 (0) 81 5665861
Fax: +39 (0) 81 5667608
e-mail: adriana.oliva@unina2.it

Key words: human gingival fibroblasts; human keratinocytes; human periodontal ligament cells; platelet-rich plasma

Accepted for publication May 5, 2005

A major goal of periodontal therapy is the reconstruction of the injured tissues to their original form and function through the regeneration of all the lost components of periodontium: cementum, periodontal ligament fibres and alveolar bone. Periodontal regeneration is a complex multifactorial process involving intricate interactions between at least four different cell populations within the periodontal wound: periodontal ligament cells, bone cells, gingival fibroblasts and epithelial cells (1). Periodontal ligament cells play a major role in this

process, being able to synthesize the constituents of the periodontal ligament as well as differentiate into cementum and bone-forming cells (2–6). The expression of osteoblast-like properties, such as the production of alkaline phosphatase, has been widely described and suggested to be of pivotal importance for the regeneration of periodontal tissues (6–11). However, even after debridement, root surface appears to favour the attachment of epithelial cells, compared to periodontal ligament cells, interfering with the formation of an organized periodontal tissue (2, 12, 13). Various approaches have been proposed to promote periodontal regeneration, using membranes, in order to avoid the apical migration of epithelium (guided tissue regeneration), or specific substances able to induce the regenerative process, such as growth/differentiation factors or enamel matrix derivatives (induced tissue regeneration). However, so far, complete regeneration of the periodontal components, although possible, is still not entirely predictable (1, 14, 15), and concerns about the high costs of these remedies, as well as the implications of the exogenous origin of some of them, remain.

Platelet-rich plasma consists of a blood-derived fraction containing high concentrations of platelets and growth factors. Applied in the form of a gel on surgical wounds, it is able to stimulate hard and soft tissue repair. Platelet-rich plasma was first used in maxillofacial surgery (16, 17) but it has been proposed also in the field of periodontal regenerative procedures (18–21).

As known, in addition to haemostasis and inflammation, platelets are involved in wound healing and repair of mineralized tissue. Once activated at the site of injury, platelets release a large amount of cytokines that stimulate cellular chemotaxis, proliferation and differentiation (22, 23). Several studies demonstrated the mitogenic effect of platelet-rich plasma on cells such as fibroblasts, osteoblasts, and mesenchymal stem cells (24–28). However, limited information about the interaction of platelet-rich plasma with periodontally related cells and its role in periodontal regeneration is available.

The aim of this study was to investigate the effects of platelet-rich plasma on the biological behaviour of cell populations involved in periodontal wound healing and regeneration, namely primary human periodontal ligament cells, gingival fibroblasts and keratinocytes.

Material and methods

Tissue culture biochemicals were obtained from Gibco-Invitrogen (Grand Island, NY, USA), plasticware from Falcon BD Labware (Franklin Lakes, NJ, USA) and chemicals from Sigma Chemical Co. (St. Louis, MO, USA), if not otherwise specified.

Preparation of the cellular models

Human periodontal ligament cells and gingival fibroblasts were obtained by using an enzymatic method. Periodontal ligaments were aseptically scraped out from the middle part of roots of periodontally healthy teeth extracted from adult patients. Human gingival tissue was obtained from periodontal operations (e.g. frenulectomies, flap operations). In all cases, patients were duly informed of the nature and extent of the study, and their informed consent was obtained according to the Helsinki Declaration. Both collected tissues were washed two times with phosphate-buffered saline (150 mM NaCl, 20 mM sodium phosphate pH 7.2) supplemented with antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin) and cut into small pieces with a sterile surgical blade. Tissue fragments were digested in 1 ml of Dulbecco's modified Eagle's medium-F12 (DMEM-F12) containing antibiotics and 1 mg/ml type IV collagenase (Worthington Biochemical, Freehold, NJ, USA) at 37°C for 3 h. Released cells were harvested, plated in complete DMEM-F12 containing antibiotics and 10% fetal bovine serum, and incubated at 37°C in a 5% CO_2 humidified atmosphere. First cell islets were visible after 3–4 days and confluence was reached in about 2–3 weeks. During this period, fresh medium was added two times per week, never removing the entire conditioned medium. After the confluence was reached, cells were trypsinized and cultures expanded.

Human keratinocytes were purchased from Gibco-Invitrogen and cultured in serum-free keratinocyte growth medium.

Cells between the second and fifth passage were used in our experiments.

Collection and preparation of platelet-rich plasma

Platelet-rich plasma was prepared following a simple protocol. Venous blood samples from healthy volunteers were withdrawn into sterile 4.5-ml glass tubes (Vacutainer, Becton & Dickinson, Rutherford, NJ, USA) containing citric acid–citrate–dextrose anticoagulant (ACD-A) at ratio of 1:10. After blood was centrifuged at 300 *g* for 10 min at room temperature, two phases were obtained: the upper (plasma) and the lower phase (red and white blood cells). The upper millilitre was collected as platelet-poor plasma. The remaining plasma, platelet-rich plasma, was aspirated and immediately used. Platelet-rich plasma was added to complete medium at 1:10 ratio, and incubated at 37°C for 30 min: the presence of fetal bovine serum allowed platelet aggregation and degranulation. Conversely, when serum-free keratinocyte growth medium was used, platelet activation was induced adding 1 U/ml of human thrombin (Immuno AG, Vienna, Austria). In any case, the incubation resulted in the formation of a gel that was successively disaggregated and removed before adding the medium, enriched in platelet-derived growth factors, to the cells.

Mitogenic assay

Cell proliferation was measured by radiolabelled thymidine incorporation. In brief, cells were plated at low density in 12-well plates (10^4 human periodontal ligament cells/cm^2, 6×10^3 human gingival fibroblasts/cm^2 and 2×10^4 human keratinocytes/cm^2) with appropriate medium. The following day, fresh medium with platelet-rich plasma or

without (control) was added to each cell type. The effect of platelet-rich plasma on human periodontal ligament cells proliferation was measured over time at 24, 48 and 72 h. Furthermore, in order to compare the effect of platelet-rich plasma on different cell types, human periodontal ligament cells, gingival fibroblasts and keratinocytes growth rate at 72 h was also assessed. In all cases, cells were pulse-labelled with 1 μCi/ml [^{3}H]thymidine (Amersham Life Science, Buckinghamshire, UK) for the last 16 h of culture, washed two times with phosphate-buffered saline and lysed with 2 ml/well of 1% sodium dodecyl sulfate−0.3 M NaOH. The incorporated thymidine was measured with a liquid scintillation counter (Wallac 1409 DSA, Perkin-Elmer, MA, USA).

Collagen extraction and analysis

Equivalent numbers of human periodontal ligament cells (10^6), derived from cultures grown in the absence or presence of platelet-rich plasma for 7 days, were rinsed with phosphate-buffered saline and resuspended in 0.5 ml 0.5 M acetic acid containing 350 μg/ml pepsin. After 16 h of gentle shaking at 4°C, the extract was centrifuged at 20,000 *g* for 20 min. The supernatant was discarded and the pellet was dissolved in lysis buffer (50 mM Tris/HCl pH 7.4, 150 mM NaCl, 0.1% NP-40, 100 μg/ml phenylmethylsulfonyl fluoride, 100 μg/ml N-tosyl-L-phenylanine chloromethyl-ketone, 1 μg/ml leupeptin, 0.83 μg/ml chemostatin, 10 μg/ml soybean trypsin inhibitor, 0.5 mM dithiothreitol, 7.4 mg/ml *p*-nitrophenyl phosphate, 1 mM sodium ortho-vanadate, 40 mM sodium fluoride and 1 mM sodium pyrophosphate). Cell extracts were separated by 6% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane (Amersham). Membrane was incubated with specific monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. The immunocomplexes were detected by the enhanced chemoluminescent technique (ECL: Amersham). Images were processed and analysed by the ImageJ 1.33 software (NIH, Bethesda, MD, USA).

Alkaline phosphatase activity

Human periodontal ligament cells were plated at 10^4 cells/cm^2 in 12-well plates. After 24 h, medium with platelet-rich plasma or without (control) was added. At 3 and 6 days, control and platelet-rich plasma-treated cells were harvested for measuring alkaline phosphatase activity. After medium was removed, the cells were washed two times with Tris-buffered saline (20 mM Tris/HCl pH 7.4, 150 mM NaCl) and solubilized by adding 100 μl/well of lysing buffer (Tris-buffered saline plus 0.25% Triton X-100, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol). The lysates were centrifuged at 13,000 *g* for 5 min at 4°C and the supernatants used for the determination of alkaline phosphatase activity and protein concentration. Alkaline phosphatase activity was determined by measuring the release of *p*-nitrophenol from disodium *p*-nitrophenyl phosphate. The reaction mixture contained 0.1 M diethanolamine buffer pH 10.5, 0.5 mM $MgCl_2$, 12 mM *p*-nitrophenyl phosphate and cell extract, in a final volume of 0.5 ml. The mixture was incubated at 37°C for 30 min and the reaction was stopped by the addition of 0.5 ml of 0.5 M NaOH. The enzymatic activity was determined on the basis of the absorbance at 405 nm. The specific activity is expressed as Units/mg of protein. One unit is defined as the amount of enzyme that hydrolyses 1 nmol of *p*-nitrophenyl phosphate/min under the specified conditions.

Statistical analysis

All experiments were performed in triplicate and repeated three times to ensure reproducibility. Means and standard deviations (SD) were calculated, and data were statistically analysed by Student's *t*-test or one-way ANOVA followed by the Tukey *post hoc* test for comparisons. Difference was considered significant at a *p*-value < 0.05.

Results

Effects of platelet-rich plasma on human periodontal ligament cells, gingival fibroblasts and keratinocytes proliferation

The effect of platelet-rich plasma on human periodontal ligament cells, gingival fibroblasts and keratinocytes cell growth was determined by [^{3}H]thymidine incorporation. Platelet-rich plasma treatment induced a strong time-dependent increase of human periodontal ligament cells proliferation (Fig. 1). A significant difference between platelet-rich plasma-treated cells and control cells became apparent at 48 h, and further increased to up to fourfold of control at 72 h.

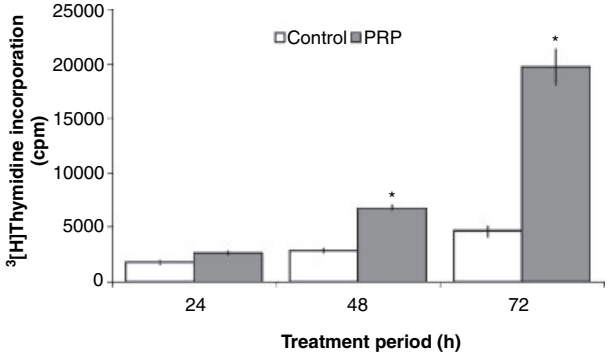

Fig. 1. Time-dependent growth stimulation of human periodontal ligament cells by platelet-rich plasma. The cells were plated at a density of 10^4/cm^2 in 12-well plates. Medium with platelet-rich plasma (PRP) or without (control) was added 24 h later. After 24, 48 and 72 h, cell growth was measured as [^{3}H]thymidine incorporation. Values are given as counts per minute (cpm). Data are shown as means ± SD of results from three independent experiments. *$p < 0.001$ vs. the respective control.

The increased growth rate of platelet-rich plasma-treated human periodontal ligament cells was associated with a remarkably more spindle-like morphology, as well as with an evident multilayered disposition of the cells (Fig. 2).

Platelet-rich plasma differently influenced human periodontal ligament cells, human gingival fibroblasts and human keratinocytes growth rate compared to their own controls. In fact, a 72-h exposure to platelet-rich plasma increased human periodontal ligament cells fourfold (Fig. 3A) and increased human gingival fibroblasts proliferation to a lesser extent (twofold) (Fig. 3B). Conversely, in the same experimental conditions, human keratinocytes growth was inhibited by 40% (Fig. 3C).

Effects of platelet-rich plasma on human periodontal ligament cells collagen levels

Type I collagen synthesis was markedly stimulated by platelet-rich plasma. As shown in Fig. 4, in human periodontal ligament cells cultures treated with platelet-rich plasma for 7 days, collagen levels twofold higher than control were measured.

Effects of platelet-rich plasma on human periodontal ligament cells differentiation

The basal alkaline phosphatase specific activity of human periodontal ligament cells cultures was measured and resulted in considerably higher levels (about 10-fold) than that of human gingival fibroblasts (data not shown).

When human periodontal ligament cells were exposed to platelet-rich plasma for 3 days and 6 days (Fig. 5), a significant increase of alkaline phosphatase specific activity was observed of up to fourfold over control.

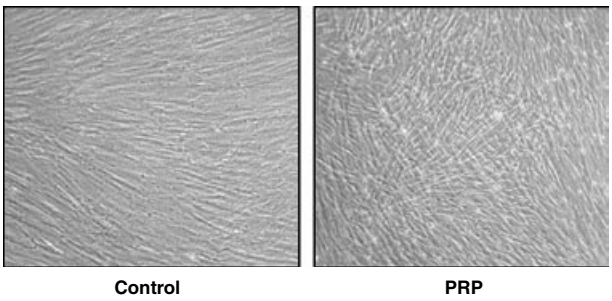

Fig. 2. Microscopic appearance of human periodontal ligament cells treated with platelet-rich plasma (PRP) or without (control) for 72 h. A multilayered disposition was evident in treated cells compared to control. The images are representative of three independent experiments (magnification ×100).

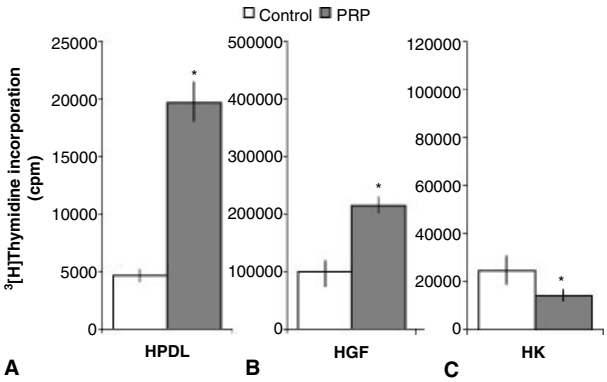

Fig. 3. Cell-type specific effect of platelet-rich plasma on human periodontal ligament cells (HPDL) (A), human gingival fibroblasts (HGF) (B) and human keratinocytes (HK) (C) proliferation. The cells were plated at low density (see Material and methods for details) in 12-well plates. Medium with platelet-rich plasma (PRP) or without (control) was added 24 h later. After 72 h, cell growth was measured as [^{3}H]thymidine incorporation. Values are given as counts per minute (cpm). Data are means ± SD of results from three independent experiments. *$p < 0.001$ vs. the respective control.

Discussion

Among the variety of periodontal regeneration techniques, the possibility to utilize a totally autogenous preparation such as the platelet-rich plasma, obtainable by simple and low cost procedures, is particularly fascinating. However, at the present time, no definite data about both the effect of platelet-rich plasma on periodontally related cells and its clinical efficacy in periodontal regeneration are available. In this *in vitro* study, we aimed to clarify some molecular aspects underlying the utilization of platelet-rich plasma for the regeneration of periodontal tissues.

Our experiments allowed the quantification of the platelet-rich plasma mitogenic activity on human periodontal ligament cells. Only one study previously reported the platelet-rich plasma-induced proliferation of periodontal ligament cells, as measured by an immunocytochemical staining method (26). In our study, human periodontal ligament cells growth was assessed measuring the [^{3}H]thymidine incorporation. After an incubation time from 24 to 72 h with platelet-rich plasma, a marked time-dependent increase of human periodontal ligament cells proliferation compared to control resulted. Conversely, a significantly different behaviour was evident for human gingival fibroblasts and epithelial cells when exposed to platelet-rich plasma, suggesting a cell-type specific effect. In fact, platelet-rich plasma-treated human gingival fibroblasts exhibited a minor increase in the growth rate compared to human periodontal ligament cells (twofold vs. fourfold of the respective controls), whereas, in the same conditions, primary keratinocytes were inhibited. From a clinical point of view, the effects induced by platelet-rich plasma

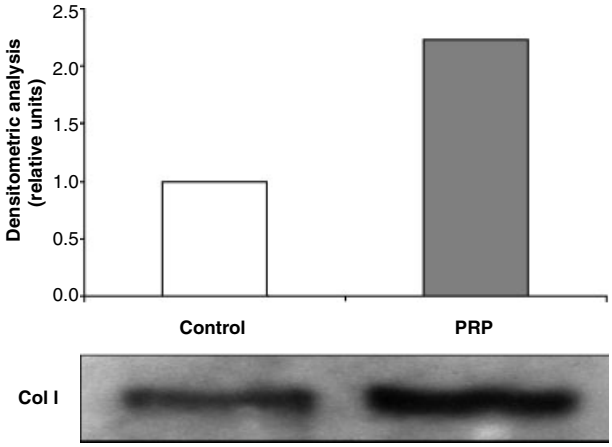

Fig. 4. Type I collagen levels increase by platelet-rich plasma in human periodontal ligament cells cultures. Equivalent numbers of cells (10^6), derived from cultures grown with platelet-rich plasma (PRP) or without (control) for 7 days, were processed for western blot analysis for type I collagen. Images were processed and the densitometric analysis performed by the ImageJ software. The results are representative of three independent experiments.

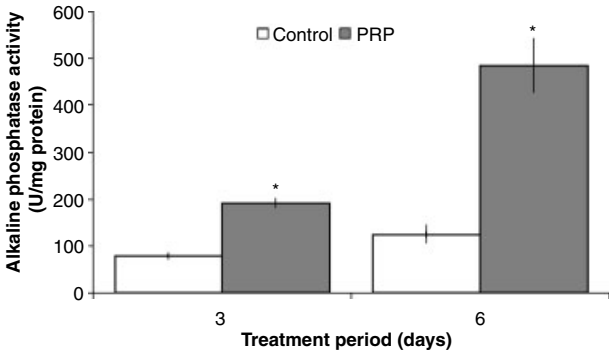

Fig. 5. Alkaline phosphatase specific activity increase by platelet-rich plasma in human periodontal ligament cells cultures. Cells were plated at a density of $10^4/cm^2$ in 12-well plates. Medium with platelet-rich plasma (PRP) or without (control) was added 24 h later. After 3 and 6 days, the alkaline phosphatase specific activity was measured by means of a spectrophotometric assay. Values are given as U/mg protein. Data are means ± SD of results from three independent experiments. *$p < 0.001$ vs. the respective control.

could synergistically operate in promoting periodontal regeneration. As known, the main reason of failure of the periodontal regenerative procedures is the apical migration of the junctional epithelium and gingival connective along the root surface inside the defect. Thus, platelet-rich plasma could enhance the regenerative process either by stimulating human periodontal ligament cells (and only to a minor extent human gingival fibroblasts) proliferation or by inhibiting that of epithelial cells. On the other hand, the mitogenic effect also observed on human gingival fibroblasts, although not completely desirable for the regeneration of deep periodontal tissues, cannot be undervalued, justifying the use of platelet-rich plasma in soft tissue grafting procedures and likewise explain the favourable wound healing observed when platelet-rich plasma is clinically applied (29, 30).

Type I collagen is the main protein synthesized by mesenchymal cells; it represents the major constituent of periodontal ligament fibres and is essential for their normal architecture. Collagen levels significantly increased when human periodontal ligament cells were exposed to platelet-rich plasma. This finding is in line with the results obtained by Kawase *et al.* (31) by means of immunocytochemical staining techniques. The increased extracellular matrix synthesis, in combination with the above-mentioned growth stimulation, could be important in promoting the regeneration of periodontal ligament fibres.

Finally, in our conditions, platelet-rich plasma addressed the differentiation of human periodontal ligament cells toward the osteoblastic phenotype. In fact, as shown in Fig. 5, the exposure to platelet-rich plasma significantly stimulated the alkaline phosphatase activity of human periodontal ligament cells with respect to control. As known, alkaline phosphatase represents an early marker of osteoblastic phenotype expressed at high levels by these cells (6–11). Thus, platelet-rich plasma-mediated stimulation of alkaline phosphatase could enhance periodontal regeneration, offering to human periodontal ligament cells the instruments required for the mineralization of hard tissues composing the deep periodontium.

The reported *in vitro* effects on periodontally related cells are similar to those described for some platelet-released growth factors. In particular, platelet-derived growth factor and transforming growth factor-β act as strong mitogenic and anabolic factors and are reported to markedly stimulate the proliferation of fibroblasts and periodontal ligament cells, as well as the extracellular matrix formation (32–35); moreover, transforming growth factor-β is known to have an inhibitory effect on epithelial cell proliferation (32, 36). Platelet-rich plasma contains high levels of these growth factors, together with other active molecules such as fibrin and fibrinopeptides released during clot formation (24–26). Thus, platelet-rich plasma could affect behaviour of periodontally related cells by a platelet-derived growth factor- and/or transforming growth factor-β-mediated action, although further mechanisms involving other mediators cannot be excluded.

In conclusion, the cell-type specific growth modulation, together with the

increase in human periodontal ligament cells collagenous matrix and alkaline phosphatase activity, represent important biological effects of platelet-rich plasma on periodontally related cells and appear interesting in view of its utilization in the periodontal tissue regeneration.

Acknowledgements

This study was partially supported by a PhD Studentship in Biomedical Technologies Applied to the Odontostomatological Sciences and by a Research Grant from the Second University of Naples (Italy).

References

1. Cochran DL, Wozney JM. Biological mediators for periodontal regeneration. *Periodontol 2000* 1999;**19:**40–58.
2. Egelberg J. Regeneration and repair of periodontal tissues. *J Periodont Res* 1987;**22:**233–242.
3. McCulloch CA, Bordin S. Role of fibroblast subpopulations in periodontal physiology and pathology. *J Periodont Res* 1991;**26:**144–154.
4. Pitaru S, McCulloch CAG, Narayanan AS. Cellular origins and differentiation control mechanisms during periodontal development and wound healing. *J Periodont Res* 1994;**29:**81–94.
5. Isaka J, Ohazama A, Kobayashi M *et al*. Participation of periodontal ligament cells with regeneration of alveolar bone. *J Periodontol* 2001;**72:**314–323.
6. Murakami Y, Kojima T, Nagasawa T, Kobayashi H, Ishikawa I. Novel isolation of alkaline phosphatase-positive subpopulation from periodontal ligament fibroblasts. *J Periodontol* 2003;**74:**780–786.
7. Arceo N, Sauk JJ, Moehring J, Poster RA, Somerman MJ. Human periodontal cells initiate mineral-like nodules in vitro. *J Periodontol* 1991;**62:**499–503.
8. Groeneveld MC, Everts V, Beertsen W. Formation of afibrillar acellular cementum-like layers induced by alkaline phosphatase activity from periodontal ligament explants maintained *in vitro*. *J Dent Res* 1994;**73:**1588–1592.
9. Goseki M, Oida S, Takeda K *et al*. Identification of bone-type alkaline phosphatase mRNA from human periodontal ligament cells. *J Dent Res* 1995;**74:**319–322.
10. Ivanovski S, Li H, Haase HR, Bartold PM. Expression of bone associated macromolecules by gingival and periodontal ligament fibroblasts. *J Periodont Res* 2001;**36:**131–141.
11. Pitaru S, Pritzki A, Bar-Kana I, Grosskopf A, Savion N, Narayanan AS. Bone morphogenetic protein 2 induces the expression of cementum attachment protein in human periodontal ligament clones. *Connect Tissue Res* 2002;**43:**257–264.
12. Terranova VP, Martin GR. Molecular factors determining gingival tissue interaction with tooth structure. *J Periodont Res* 1982;**17:**530–533.
13. Karring T, Lindhe J, Cortellini P. Regenerative periodontal therapy. In: Lindhe J., Karring T, Lang NP, eds. *Clinical Periodontology and Implant Dentistry*, 3rd edn. Copenhagen: Munksgaard, 1997:598–646.
14. Bartold PM, McCulloch CA, Narayanan AS, Pitaru S. Tissue engineering: a new paradigm for periodontal regeneration based on Mol Cell Biol. *Periodontol 2000* 2000;**24:**253–269.
15. Grzesik WJ, Narayanan AS. Cementum and periodontal wound healing and regeneration. *Crit Rev Oral Biol Med* 2002;**13:**474–484.
16. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *J Oral Maxillofac Surg* 1997;**55:**1294–1299.
17. 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:**638–646.
18. De Obarrio JJ, Arauz-Dutari JI, Chamberlain TM, Croston A. The use of autologous growth factors in periodontal surgical therapy: platelet gel biotechnology – case reports. *Int J Periodontics Restorative Dent* 2000;**20:**486–497.
19. 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.
20. 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 Periodont Res* 2002;**37:**300–306.
21. 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:**746–745.
22. Gentry PA. The mammalian blood platelet: its role in haemostasis, inflammation and tissue repair. *J Comp Pathol* 1992;**107:**243–270.
23. Parise LV, Smith SS, Coller BS. Platelet morphology, biochemistry, and function. In: Beutler E *et al*., eds. *Williams hematology*, 6th edn. New York: McGraw-Hill, 2001:1357–1407.
24. Liu Y, Kalen A, Risto O, Wahlstrom O. Fibroblast proliferation due to exposure to a platelet concentrate in vitro is pH dependent. *Wound Repair Regen* 2002;**10:**336–340.
25. Gruber R, Varga F, Fischer MB, Watzek G. Platelets stimulate proliferation of bone cells: involvement of platelet-derived growth factor, microparticles and membranes. *Clin Oral Implants Res* 2002;**13:**529–535.
26. Okuda K, Kawase T, Momose M *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:**849–857.
27. Gruber R, Karreth F, Kandler B *et al*. Platelet-released supernatants increase migration and proliferation, and decrease osteogenic differentiation of bone marrow-derived mesenchymal progenitor cells under in vitro conditions. *Platelets* 2004;**15:**29–35.
28. Soffer E, Ouhayoun JP, Dosquet C, Meunier A, Anagnostou F. Effects of platelet lysates on select bone cell functions. *Clin Oral Implants Res* 2004;**15:**581–588.
29. Petrungaro PS. Using platelet-rich plasma to accelerate soft tissue maturation in esthetic periodontal surgery. *Compend Contin Educ Dent* 2001;**22:**729–746.
30. Tischler M. Platelet rich plasma. The use of autologous growth factors to enhance bone and soft tissue grafts. *N Y State Dent J* 2002;**68:**22–24.
31. 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:**858–864.
32. Sporn MB, Roberts AB. Transforming growth factor-beta: recent progress and new challenges. *J Cell Biol* 1992;**119:**1017–1021.
33. Dennison DK, Vallone DR, Pinero GJ, Rittman B, Caffesse RG. Differential effect of TGF-beta 1 and PDGF on proliferation of periodontal ligament cells and gingival fibroblasts. *J Periodontol* 1994;**65:**641–648.
34. Ivarsson M, McWhirter A, Borg TK, Rubin K. Type I collagen synthesis in cultured human fibroblasts: regulation by cell spreading, platelet-derived growth

factor and interactions with collagen fibers. *Matrix Biol* 1998;**16:**409–425.

35. Marcopoulou CE, Vavouraki HN, Dereka XE, Vrotsos IA. Proliferative effect of growth factors TGF-beta1, PDGF-BB and rhBMP-2 on human gingival fibroblasts and periodontal ligament cells. *J Int Acad Periodontol* 2003;**5:** 63–70.
36. Shipley GD, Pittelkow MR, Wille JJ Jr, Scott RE, Moses HL. Reversible inhibition of normal human prokeratinocyte proliferation by type beta transforming growth factor-growth inhibitor in serum-free medium. *Cancer Res* 1986;**46:**2068–2071.