BioOne DIGITAL LIBRARY

PLATELET-RICH PLASMA PROVIDES NUCLEUS FOR MINERALIZATION IN CULTURES OF PARTIALLY DIFFERENTIATED PERIODONTAL LIGAMENT CELLS

Authors: KAWASE, TOMOYUKI, OKUDA, KAZUHIRO, SAITO, YOSHINORI, AMIZUKA, NORIO, SUZUKI, HIRONOBU, et al.

Source: In Vitro Cellular & Developmental Biology - Animal, 41(5) : 171-176

Published By: Society for In Vitro Biology

URL: https://doi.org/10.1290/0502013.1

The BioOne Digital Library (https://bioone.org/) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (https://bioone.org/subscribe), the BioOne Complete Archive (https://bioone.org/archive), and the BioOne eBooks program offerings ESA eBook Collection (https://bioone.org/esa-ebooks) and CSIRO Publishing BioSelect Collection (https://bioone.org/csiro-ebooks).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commmercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://complete.bioone.org/journals/In-Vitro-Cellular-&-Developmental-Biology---Animal on 23 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

In Vitro Cell. Dev. Biol.—Animal 41:171–176, May and June 2005
© 2005 Society for In Vitro Biology
1071-2690/05 $18.00+0.00

PLATELET-RICH PLASMA PROVIDES NUCLEUS FOR MINERALIZATION IN CULTURES OF PARTIALLY DIFFERENTIATED PERIODONTAL LIGAMENT CELLS

TOMOYUKI KAWASE,[1] KAZUHIRO OKUDA, YOSHINORI SAITO, NORIO AMIZUKA, HIRONOBU SUZUKI, AND HIROMASA YOSHIE

Division of Cellular Pharmacology, Department of Signal Transduction Research (T. K.), Division of Periodontology (K. O., Y. S., H. Y.) and Division of Oral Anatomy (N. A.), Department of Oral Biological Science, Division of Anatomy and Cell Biology of the Hard Tissue, Department of Tissue Regeneration and Reconstruction (H. S.), Graduate School of Medical and Dental Sciences, Niigata University, Niigata 951-8514, Japan

(Received 15 February 2005; accepted 19 May 2005)

Summary

Platelet-rich plasma (PRP) has been used to promote periodontal regeneration following the premise that constituent transforming growth factor–β1 (TGF-β1) and platelet-derived growth factor–AB will stimulate cell proliferation at the site of application. In previous studies, we demonstrated that PRP mimics TGF-β1 to modulate proliferation in a cell type–specific manner, that fibrin clot formation by PRP upregulates type I collagen, and that an unidentified factor(s) in PRP increases alkaline phosphatase (ALP) activity in human periodontal ligament (PDL) cell cultures. We have now examined the effects of PRP on in vitro mineralization. Platelet-rich plasma and PDL cells were prepared from human adult volunteers or rats. After 20 d of continuous treatment with PRP in dexamethazone (Dex)-containing osteogenic medium, PRP time dependently promoted mineralization by rat PDL cells but failed to fully induce the osteoblastic phenotype. Furthermore, when human PDL cells were induced to increase ALP activity in osteogenic medium that lacked Dex, a condition that should delay (or suppress) osteoblastic differentiation, transmission electron microscopy revealed that mineralized spicules were initially deposited onto PRP-derived platelet aggregates. Taken together with our previous data, these findings suggest that PRP provides platelet aggregates as nuclei to initiate mineralization while stimulating PDL cell proliferation, differentiation, and collagen production. The combination of these effects may effectively mediate PRP's ability to promote regeneration of periodontal tissue, including skeletal tissue, at the site of injury.

Key words: platelet-rich plasma; platelet; osteoblastic phenotype; mineralization; periodontal ligament cells; atelocollagen.

Introduction

Platelet-rich plasma (PRP) provides a concentrated natural combination of platelet-derived growth factors and materials that should exert bioactivity at the site of application. On the basis of the assumption that these biological properties can be readily applied in a clinical setting, homologous PRP has been frequently used after periodontal surgery to promote tissue regeneration. However, the scientific basis for this therapeutic protocol has not been widely discussed and the exact mechanism(s) behind PRP action is poorly understood.

In a previous in vitro study (Okuda et al., 2003), we demonstrated that high levels of both platelet-derived growth factor (PDGF) and transforming growth factor–β (TGF-β) are contained in human PRP preparations and that PRP modulates proliferation of cultured human periodontal ligament (PDL) or oral epithelial cells in a cell type–specific manner. We also found significant growth factor–independent effects of PRP on PDL cell cultures. Expression of type I collagen in these cells is stimulated by insoluble fibrin, which is converted from PRP-derived fibrinogen by cellular thrombin, without the intercession of any known growth factor (Kawase et al., 2003). Taken together, these findings clearly suggest that PRP is capable of promoting regeneration of connective tissue by stimulating both proliferation of fibroblastic cells and production of extracellular matrix.

On the other hand, the anabolic action of PRP on skeletal tissue is still controversial, although our recent clinical study (Okuda et al., 2005) showed that regeneration of alveolar bone is significantly promoted in PRP-treated groups. As generally accepted, it is important to promote synchronized bone regeneration in the process of successful periodontal tissue regeneration. Therefore, to obtain scientific evidence sufficient to explain this clinical observation, we have very recently examined the effects of PRP on alkaline phosphatase (ALP), a representative marker of bone-forming activity, in human PDL cells and found that in contrast to TGF-β or PDGF, PRP time dependently increases ALP activity (Kawase et al., 2005). To further explore the possible involvement of PRP in bone regeneration, we have now examined the direct effects of PRP on in vitro mineralization in human and rat PDL cell cultures.

Materials and Methods

Preparation of PRP. As described previously (Okuda et al., 2003), PRP was prepared from the plasma obtained from three healthy adult volunteers (aged 25–47 yr) or male Lewis rats (5 wk old). In brief, whole blood in sterile

[1] To whom correspondence should be addressed at E-mail: kawase@dent.niigata-u.ac.jp

Downloaded From: https://complete.bioone.org/journals/In-Vitro-Cellular-&-Developmental-Biology---Animal on 23 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

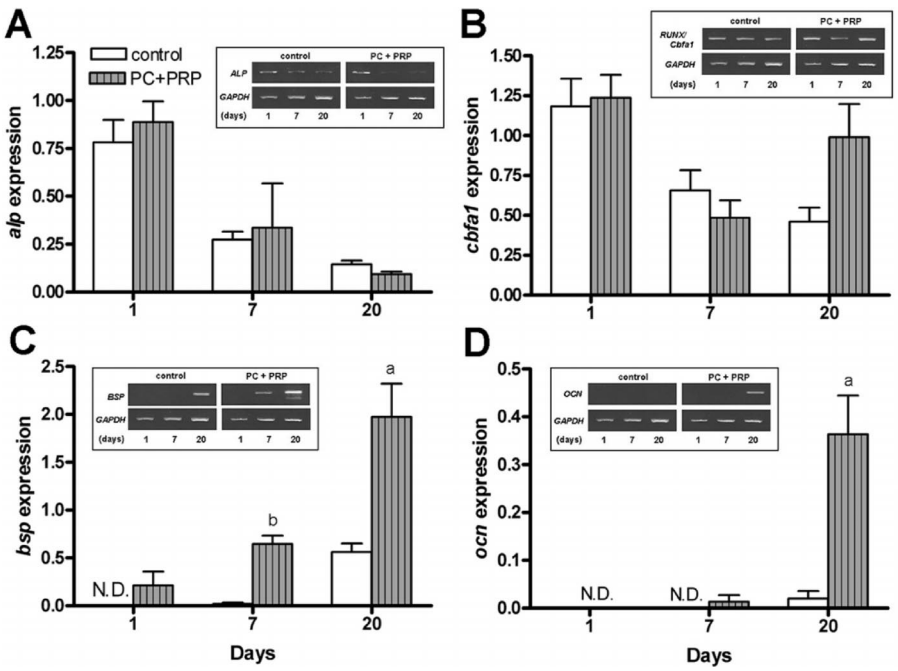

FIG. 1. Time-course effects of PRP on the osteoblatic marker mRNAs in rat PDL cell cultures. Cells were continuously treated for 1, 7, or 20 d with 0.5% PRP in 2% FBS-containing αMEM in the presence of Dex, ascorbate, and β-GP in PC-coated wells, and mRNAs were then extracted for reverse transcription–polymerase chain reaction, as described in *Materials and Methods*. Control cells were cultured in noncoated wells. Each *column* and *vertical bar* represents the mean and SD, respectively, from three independent experiments. $^{a}P < 0.02$, $^{b}P < 0.005$ versus the control cultures. The inserts represent the typical data from these experiments. Dex, Dexamethazone; FBS, fetal bovine serum; PDL, periodontal ligament; PRP, platelet-rich plasma; mRNA, messenger ribonucleic acid; αMEM, α-minimum essential medium; β-GP, β-glycerophosphate; ND, not determined.

tubes coated with an anticoagulant (acid–citrate dextrose) was centrifuged at 3600 rpm for 15 min (first step) to separate PRP and platelet-poor plasma (PPP) fractions from the red blood cell fraction. The PRP and PPP fractions were further centrifuged at 2400 rpm for 10 min (second step) to obtain PRP fraction. Because we have preliminarily confirmed that frozen PRP stimulates PDL cell proliferation as effectively as freshly prepared PRP (T. Kawase et al., unpubl. obs.), PRP was stored at −80° C until use (usually within 1 mo). The concentration of platelets in our PRP preparations ranged from 4.5×10^5 to 12.0×10^5 platelets/μl (mean = 2.7×10^5 platelets/μl).

The study design and consent were approved by the ethical committee for human subject use at Niigata University Medical and Dental Hospital in accordance with the Helsinki Declaration of 1975 and as revised in 1983. The volunteers were informed of the purpose of the study and gave informed consent.

Cell isolation and cell cultures. As described previously (Kawase et al., 2003), PDL cells were harvested from extracted teeth that were removed for orthodontic reasons in four young, healthy volunteers (aged 12–16 yr) or male Lewis rats (5 wk old). The PDL tissues were minced, placed in 35-mm culture dishes, and incubated in Dulbecco modified minimum essential medium (DMEM) (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS) and kanamycin (50 mg/L) (Meiji Seika, Tokyo, Japan) at 37° C in humidified 5% CO_2 and 95% air. Cells outgrowing from the tissue pieces were further cultured in DMEM containing 10% FBS and passaged five times before use. Unless otherwise specified, cells were seeded at a density of 3×10^4 to 5×10^4 cells/well (24-well plate), and the medium was replenished every 3 d.

The remaining cells were stocked under liquid nitrogen until use. Cells from different volunteers were specifically coded, and only passages 6 through 10 were used in the experiments reported in this study. Importantly, no significant differences were observed in any data obtained from different batches of PDL cells.

Coating with atelocollagen. According to the manufacture's protocol, multiwell plates (Falcon, Franklin Lakes, NJ) were coated with a solution of type I atelocollagen (PC) (KOKENCELLGEN, I-PC; Koken Co. Ltd., Tokyo, Japan) extracted from bovine dermis for 30 min, rinsed three times with distilled water, dried in a clean bench, and stabilized under an ultraviolet ramp for 30 min. This preparation was performed 1–2 d before use, and coated plates were stored at 4° C until use, as described previously (Kawase et al., 2005).

In vitro mineralization assay. To induce relatively rapid mineralization, rat PDL cells were treated with PRP in the presence of coated PC for 20 d in "osteogenic medium" (α-minimum essential medium [αMEM] [GIBCO] with 10 m*M* β-glycerophosphate [β-GP] [Calbiochem, San Diego, CA], 50 μg/ml ascorbic acid [Wako Pure Chemicals, Osaka, Japan], and 2% FBS), with or without the addition of 1 μ*M* dexamethazone (Dex) (Calbiochem). At the end of treatment, cells were rinsed with phosphate-buffered saline (PBS), fixed with 2% ice-cold formaldehyde, and stained with 40 m*M* alizarin red–S (AR-S) (Sigma Chemical Co., St. Louis, MO). After nonspecific staining was reduced by washing with PBS (5×, 15 min), mineral deposits were photographed. For spectrophotometric determination, AR-S was solublized with 10% (w/v) cetylpyridinium chloride in 10 m*M* sodium phosphate buffer (pH 7.0) for 1 h at 22° C. The absorbance was measured at 562 nm.

Human PDL cells were treated with PRP in αMEM supplemented with 10 m*M* β-GP (Calbiochem), 50 μg/ml ascorbic acid (Wako Pure Chemicals), and 2% FBS. The medium lacking Dex significantly delayed mineralization (data not shown). At the end of treatments, the cultures were fixed and stained with AR-S, and the parallel cultures were subjected to the observation using an electron microscopy (*see below*).

Assessment of osteoblastic phenotype by reverse transcription–polymerase chain reaction. Total ribonucleic acid from cells in each group was extracted according to the acid guanidinium isothiocyanate-phenol-chloroform method. Then, the first-strand complementary deoxyribonucleic (cDNA) was synthesized using random primers (nine-mers) (Takara, Osaka, Japan) and Superscript II (GIBCO) as a reverse transcriptase. For polymerase chain reaction (PCR), aliquots of synthesized cDNA were added to PCR mixtures containing 3′ and 5′ primers (0.2 μ*M* each), diethylnitrophyenyl thiophosphate mixture (0.2 m*M* each) (GIBCO), and *Taq* polymerase (0.05 U/μl) (GIBCO). Cycling conditions were 94° C for 30 s, 58° C for 45 s, 72° C for 40 s for 24 cycles for bone sialoprotein (BSP); 94° C for 30 s, 62° C for 45 s, 72° C for 30 s for 33 cycles for Runx2/Cbfa1; 94° C for 30 s, 54° C for 30 s, and 72° C for 60 s for 21 cycles for ALP; 94° C for 30 s, 62° C for 45 s, 72° C for 40 s for 21 cycles for osteocalcin (OCN); and 94° C for 30 s, 55° C for 45 s, 72° C for 30 s for 12 cycles for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively.

Primers for PCR were as follows: 5′-CTGCTTTAATCTTGCTCTG-3′ and 5′-CCATCTCCATTTTCTTCC-3′ for BSP; 5′-CAACCACAGAACCACAAGT GCGGTGCAAAC-3′ and 5′-CCGGGCTCACGTCGCTCATCTTGCCGGGCT-3′ for RUNX2/Cbfa1; 5′-TGGAATATGAACTGGATGAGA-3′ and 5′-GTTG TTGTGAGCATAATCCAC-3′ for ALP; 5′-TCTGAGTCTGACAAAGCCTT-3′ and 5′-CTAAACGGTGGTGCCATAGAT-3′ for OCN, and 5′-CAACTCCCTC AAGATTGTCAGC-3′ and 5′-GGGACTTGCTGTTGAAGTCACA-3′ for GAPDH. To verify the identity of the PCR products, we sequenced PCR products and confirmed that sequences of PCR products matched the predicted sequences.

The PCR products were fractionated on a 1% agarose gel (Sigma), trans-

Downloaded From: https://complete.bioone.org/journals/In-Vitro-Cellular-&-Developmental-Biology---Animal on 23 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

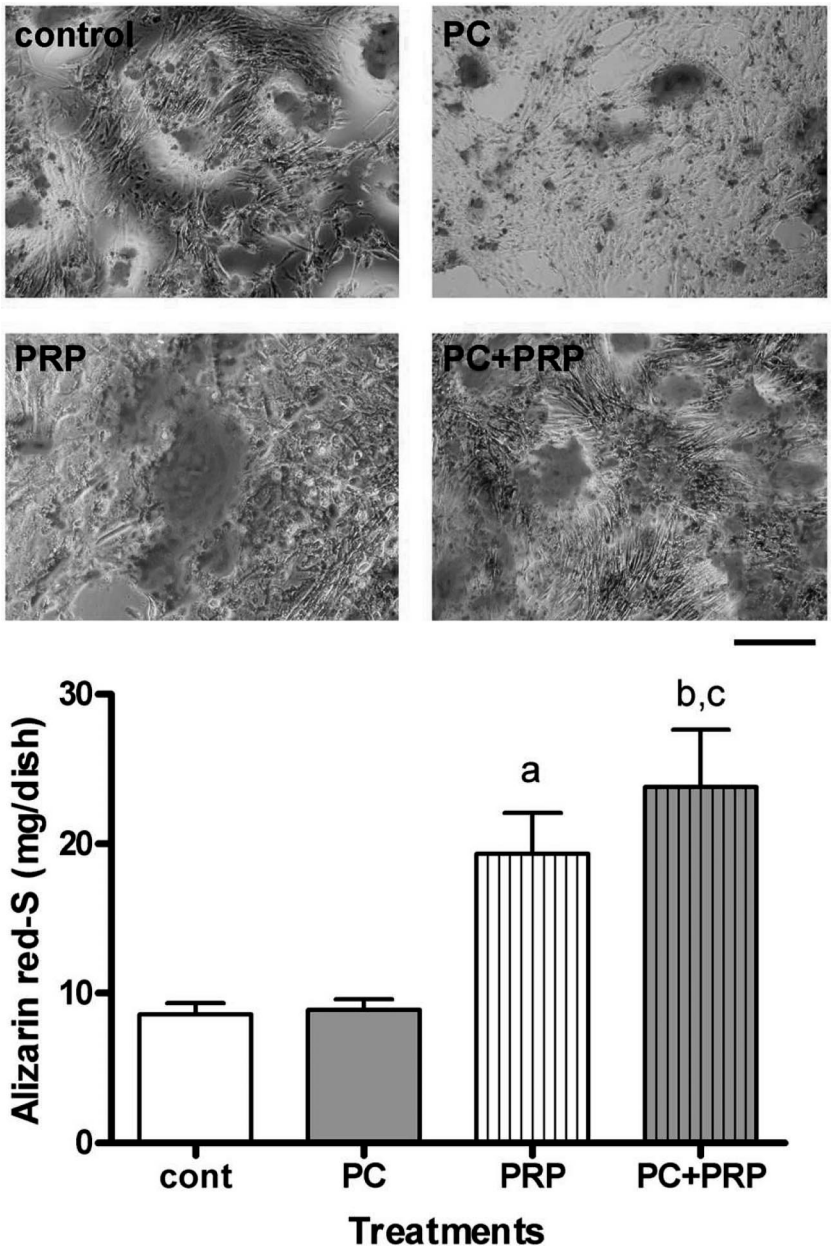

FIG. 2. Effects of PRP on in vitro mineralization in rat PDL cell cultures. Cells were treated with 0.5% PRP in noncoated or PC-coated wells for 20 d, fixed with neutralized formalin, and stained with AR-S, as described in Materials and Methods. Control cells were also cultured in noncoated or PC-coated wells. The AR-S–positive deposits were photographed (*A*) or extracted for spectrophotometrical assay (*B*). (*A*) All experiments were repeated three times with similar results, and the results shown here are representative for all these data. *Bar* represents 200 μm. (*B*) Each *column* and *vertical bar* represents the mean and SD, respectively, from three independent experiments. $^{a}P < 0.05$, $^{b}P < 0.01$ versus the control cultures. $^{c}P < 0.01$ versus the cultures with coated-PC alone. AR-S, alizarin red–S; PRP, platelet-rich plasma.

ferred to positively charged nylon membranes (Schleicher & Schnell; Einbeck, Germany), and cross-linked by ultraviolet light. Membranes were hybridized with digoxigenin (DIG) (Roche Diagnostics, Basel, Switzerland)-labeled deoxyribonucleic acid probes and detected with CDP-Star substrate (NEW ENGLAND BioLabs Inc., Beverly, MA) according to the manufacturer's standard protocols.

Electron microscopy procedure. For electron microscopy, human PDL cells were treated with 0.5% PRP on PC-coated Thermanox® plastic coverslips (Nunc, Rochester, NY) in 24-well plates for 12, 18, or 25 d in αMEM supplemented with 10 m*M* β-GP, 50 μg/ml ascorbic acid, and 2% FBS. Control cells were cultured on noncoated coverslips. At the end of treatments, cells were immersed in a mixture of 4% paraformaldehyde and 0.0125% glutaraldehyde in 0.067 *M* phosphate buffer, postfixed with 1% osmium tetraoxide reduced with 1.5% potassium ferrocyanide in a 0.1 *M* cacodylate buffer for 3 h at 4° C, and subsequently dehydrated in ascending acetones before embedding in epoxy resin (Epon 812, Taab, Berkshire, UK). Undecalcified, ultrathin sections obtained using ethylene glycol were examined with a transmission electron microscope (TEM) (H-7000, Hitachi Co. Ltd., Tokyo, Japan).

Statistical analysis. Data are expressed as the mean ± SD. Statistical significance of differences between groups was analyzed by Student's *t*-test (Fig. 1) or one-way analysis of variance (Fig. 2). Comparisons between individual groups were made using Tukey's multiple comparison test. $P < 0.05$ was considered significant.

RESULTS

In our last study (Kawase et al., 2005), we found that 0.5% PRP produced almost maximal effects on ALP activity (in the absence of Dex) and that PRP at concentrations higher than 1% substantially perturbed the subsequent extraction procedure by forming a more solid fibrin clot. In a preliminary study, we also observed that PC-coated plates were somewhat more optimal for cell proliferation and mineralization than naive plates (for mineralization, Fig. 2). Therefore, we have focused on studying the effects of PRP at 0.5% in

Downloaded From: https://complete.bioone.org/journals/In-Vitro-Cellular-&-Developmental-Biology---Animal on 23 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

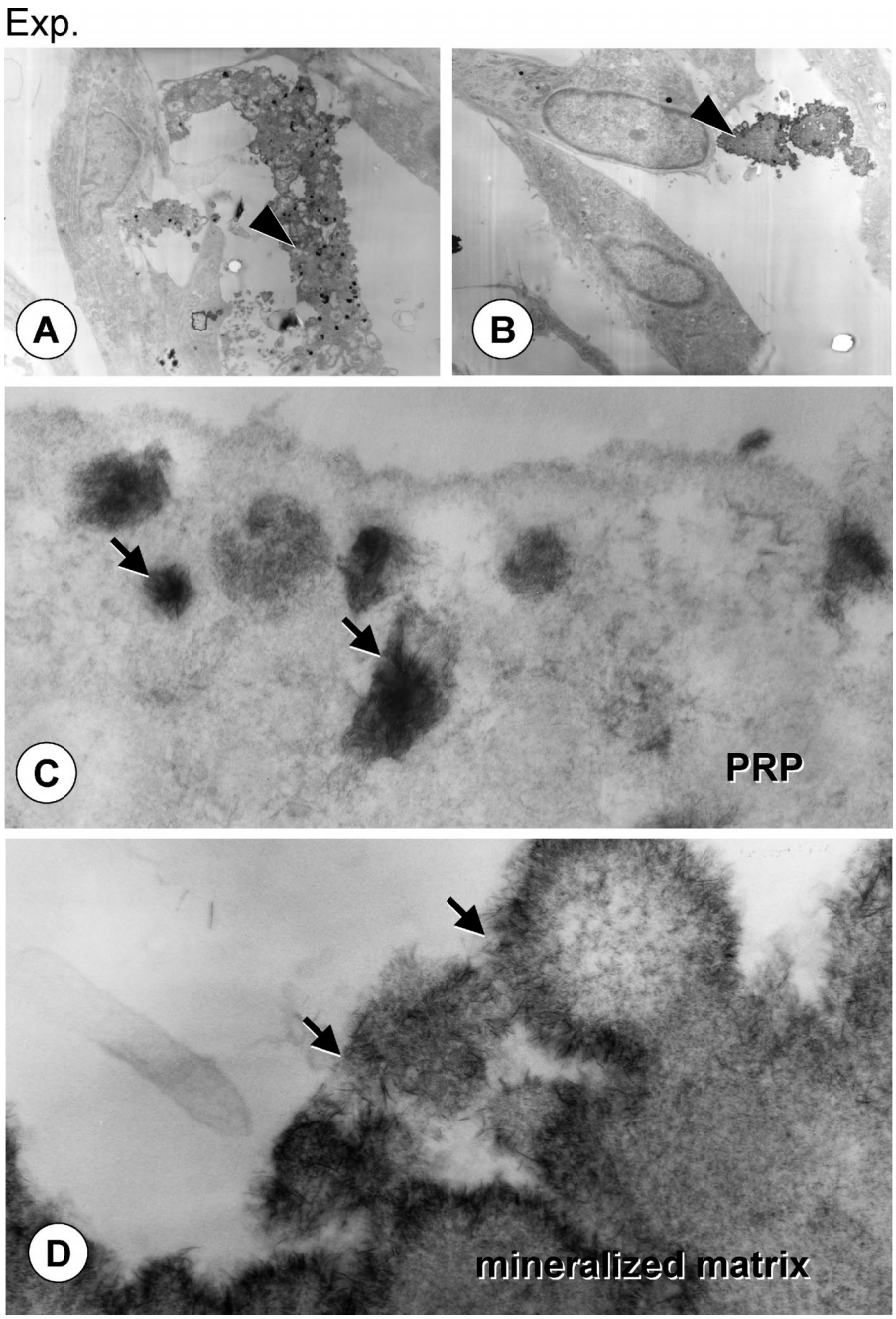

FIG. 3. TEM images of undecalcified experimental cultures on day-25. Human PDL cells were treated for 25 d with 0.5% PRP in PC-coated coverslips in medium lacking Dex. *Panels C* and *D* are higher magnified images of *panels A* and *B*, respectively. Some debris of platelets (an *arrow*) close to the human PDL cells included electron granules (*A*), and the other platelets were broadly mineralized (*B*, *arrowhead*). When observing at a higher magnification of *panel A*, mineralized nodules (*arrows*) were scattered throughout the platelet (*C*). The platelets seen in the *panel B* consisted of numerous mineralized spicules (*D*). Original magnification: *A*, ×2700; *B*, ×2500; *C*, ×50 000; *D*, ×69 600.

combination with PC-coated plates in most of the following experiments.

When rat PDL cells were cultured on native plastic culture wares in the presence of Dex, the initiation of in vitro mineralization could be usually observed by light microscopy after 15–18 d. Therefore, we have now examined PRP's time-course effects on the induction of the osteoblastic phenotype in PDL cells over 20 d (Fig. 1). In our previous study (Kawase et al., 2005), long-term treatment with PRP (0.5%) in osteogenic medium lacking Dex significantly increased the ALP activity of human PDL cells. However, in this study using Dex-supplemented osteogenic medium, mRNA expression of ALP in rat PDL cells decreased with time of treatment and was not significantly upregulated by PRP (0.5%) (Fig. 1*A*). A similar profile was observed in Cbfa1 mRNA expression; it was upregulated by almost twofold over control after 20 d of PRP treatment, but no statistical significance was observed (Fig. 1*B*). In contrast, both BSP and OCN mRNA expression increased with time and was time dependently upregulated by PRP (0.5%) (Fig. 1*C* and *D*).

Figure 2 shows the effects of PRP on in vitro mineralization in rat PDL cell cultures on day 20. Even in control cultures, AR-S–positive calcium deposits could be observed, and PC coating by itself did not appreciably stimulate this parameter. Interestingly, addition of PRP (0.5%) to noncoated or PC-coated cultures substantially increased the number and cross-sectional area of calcium

Downloaded From: https://complete.bioone.org/journals/In-Vitro-Cellular-&-Developmental-Biology---Animal on 23 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

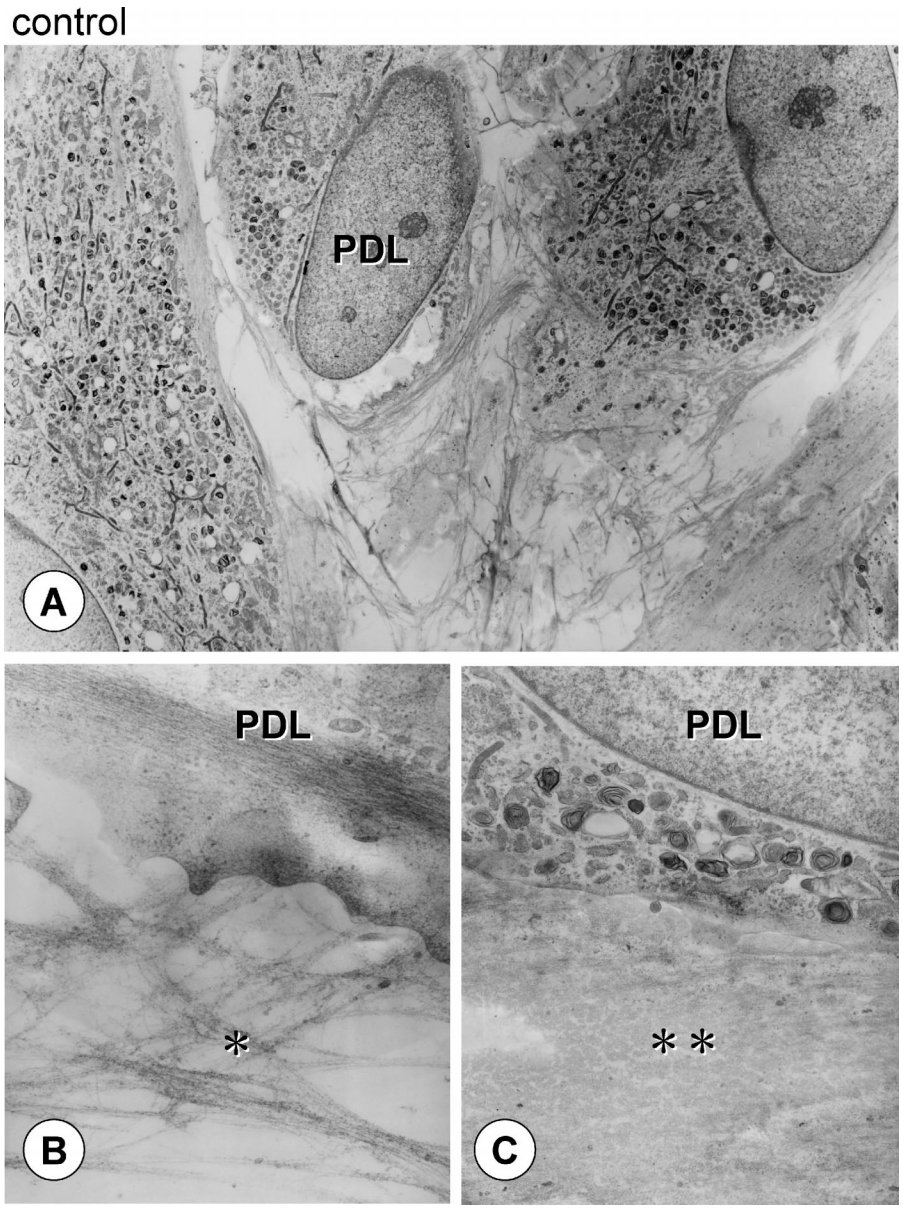

FIG. 4. TEM images of undecalcified control cultures on day-25. Control human PDL cells were cultured for 25 d on noncoated coverslips in medium lacking Dex. Cells were surrounded by fibrous materials. No electron dense deposit indicative of matrix mineralization was discernible. *Panels B* and *C* are higher magnified images of extracellular fibrous (*B*, asterisk) and amorphous (*C*, double asterisk) matrices adjacent to the human PDL cells. *Note* no mineralized nodules with electron dense profile in both fibrous and amorphous extracellular materials. Original magnification: *A*, ×2400; *B*, ×17 700; *C*, ×8900.

deposits (versus each corresponding control) (Fig. 2, *middle panels*). These data were then quantitated, and plots are presented in the *lower panel* of Fig. 2. As before, PC coating itself did not produce significant effects, although the amount of deposited calcium in PRP-treated cultures was significantly higher than that found in parallel untreated cultures. In addition, slight synergistic effects of PRP in combination with coated PC were observed, but these effects did not prove to be statistically significant.

To extend these findings to human cells and to verify the fine structure of the mineral depositions, we examined the initial phase of mineralization in human PDL cell cultures under TEM. As shown above, culture medium containing Dex is well known to promote induction of several markers of the osteoblastic phenotype and stimulate mineralization and therefore is most suitable for a differentiation study. However, we have preliminary evidence that the presence of Dex is not suitable for the purpose of demonstrating mineral nucleation in PRP-treated cultures by TEM analysis. Therefore, human PDL cells were studied in osteogenic medium that lacked Dex to determine the effects of PRP.

Under these conditions, cells generally needed more days to form AR-S–stainable calcium deposits than they did in the presence of Dex (as shown in Fig. 2), and as expected, addition of PRP stimulated cells to form calcium deposits appreciably faster. In PRP-treated cultures on day 25, we have found that platelet aggregates formed close to PDL cells (Fig. 3*A*) and contained many patchy deposits resembling the "mineralized nodules" frequently described in the literature. These mineralized nodules formed on the platelet membranes rather than within extracellular spaces that would be filled with matured collagen fibrils and matrix vesicles by adjacent osteoblasts. These areas of aggregated platelets were characterized by a gathering of mineralized spicules (Fig. 3*C*), and several PDL cells were found immediately adjacent to well-mineralized platelets

Downloaded From: https://complete.bioone.org/journals/In-Vitro-Cellular-&-Developmental-Biology---Animal on 23 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use

(Fig. 3*B* and *D*). When both cases were closely examined, matrix vesicle–like structures were not discernible between the PDL cell and platelet aggregate, suggesting that nucleation of mineralization is provided by the aggregated platelets but not mediated by matrix vesicles. In contrast, parallel control cultures on this day featured PDL cells that were surrounded by fibrous structures and amorphous organic components that did not mineralize (Fig. 4).

Discussion

In a series of studies (Okuda et al., 2003; Kawase et al., 2003, 2005), we have continuously provided positive evidence to support the therapeutic application of PRP for periodontal regeneration: (1) platelet-rich plasma contains high levels of TGF-β1 and PDGF-AB, (2) TGF-β1, rather than PDGF-AB, contained within PRP is a major factor involved in the mitogenic action of PRP, (3) fibrinogen contained in PRP forms fibrin clots in cooperation with cellular thrombin and thereby promotes initial cellular adhesion, (4) in addition to these short-term effects, long-term treatment with PRP increases ALP activity and stimulates some parts of osteoblastic differentiation in PDL cells.

To further prove that PRP is capable of directly stimulating bone formation in PDL cell cultures, we have now examined the effects of PRP on mineral deposit nucleation and formation. As expected, our present data clearly demonstrate that PRP promotes the initiation of mineral nucleation. This PRP-initiated mineralization required increased cellular ALP activity, as observed in the physiological process of mineralization that is normally observed in the mineralization front of skeletal tissue. However, in contrast to the process of mineralization in vivo, where mineral spicules are closely associated with collagen fibrils (Arceo et al., 1991), the PRP-initiated mineralization is characterized by the gathering of mineralized spicules onto platelet-derived plasma membrane. Because Marsh et al. (1995) described the possibility that cellular debris act as nucleating sites for mineralization, we suggest that PRP might initiate mineralization and thereby promote bone regeneration under particular conditions at the site of in vivo application.

Type I collagen fibril is found as a major matrix protein within the extracellular space of skeletal tissue. In addition to forming much of the protein scaffold, it is generally accepted that type I collagen is also important in maintaining tissue-specific functions in vitro (Rocha et al., 1985; Reznikoff et al., 1987). Particularly in osteoblastic cell cultures, type I collagen has been shown to be important in supporting expression of mature bone cell phenotypes (Andrianarivo et al., 1992; Lynch et al., 1995) and formation of mineral deposits (Marsh et al., 1995). In addition, a recent study has suggested the mechanism of action by demonstrating that the amino acid sequence, Arg-Gly-Asp (RGD), a motif enriched within the sequence of type I collagen, functions as a specific extracellular attachment site for osteogenic cells (Sano et al., 2003). Therefore, we thought that collagen-coated plates should be a better in vitro model to simulate periodontal injury sites. Compared with native plastic wares optimized for cell culture, PC coating was found to suppress cell proliferation and increase collagen production in early cultures of PRP-treated PDL cells (Kawase et al., 2005); unfortunately, no convincing synergistic effects of PRP and PC were found on the expression of osteoblastic markers or in vitro mineralization in this study. Despite the lack of support given by the present experiments, the possible combinational effects on osteoblastic differentiation or mineralization cannot be completely ruled out at present because PRP clearly influenced early, sparse cultures of PDL cells when grown on coated PC. Further study using a three-dimensional culture system is needed to develop a better understanding.

In conclusion, the overall process of PRP-initiated mineralization seems different from the typical progression of osteogenic conversion of an extracellular collagen-rich matrix, and in particular, mineral nucleation begins on the membranes of fibrin-entrapped platelets instead of on collagen fibrils. However, PRP was found to increase ALP activity and important osteogenic markers (such as BSP and OCN, both associated with control of biomineralization) over time, consistent with osteogenic developments within PDL cells. Although PDL cells do not fully differentiate into osteoblasts, PRP-initiated mineralization should be considered a consequence of a similar biological process. As a result of osteogenic induction of adjacent PDL cells coupled with providing abundant nucleation sites for biomineralization, PRP should promote general skeletal regeneration at the site of in vivo application.

Acknowledgments

This study was supported by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (14571979, 14771216) and Grant for Promotion of Niigata University Research Projects.

References

Andrianarivo, A. G.; Robinson, J. A.; Mann, K. G.; Tracy, R. P. Growth on type I collagen promotes expression the osteoblastic phenotype in human osteosarcoma MG-63 cells. J. Cell. Physiol. 153:256–265; 1992.

Arceo, N.; Sauk, J. J.; Moehring, J., et al. Human periodontal cells initiate mineral-like nodules in vitro. J. Periodontol. 62:499–503; 1991.

Kawase, T.; Okuda, K.; Saito, Y.; Yoshie, H. In vitro evidence that the biological effects of platelet-rich plasma on periodontal ligament cells is not mediated solely by constituent transforming-growth factor-β or platelet-derived growth factor. J. Periodontol. 76:760–767; 2005.

Kawase, T.; Okuda, K.; Wolff, L. F.; Yoshie, H. Platelet-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro. J. Periodontol. 74:858–864; 2003.

Lynch, M.; Stein, J. L.; Stein, G. S.; Lian, J. The influence of type I collagen on the development and maintenance of the osteoblast phenotype in primary and passaged rat calvarial osteoblasts: modification of expression of genes supporting cell growth, adhesion, and extracellular matrix mineralization. Exp. Cell Res. 216:35–45; 1995.

Marsh, M. E.; Munne, A. M.; Vogel, J. J.; Cui, Y.; Franceschi, R. T. Mineralization of bone-like extracellular matrix in the absence of functional osteoblasts. J. Bone Miner. Res. 10:1635–1643; 1995.

Okuda, K.; Kawase, T.; Momose, M., et al. Platelet-rich plasma contains high levels of platelet-derived growth factor and transforming growth factor-β and modulates the proliferation of periodontally related cells in vitro. J. Periodontol. 74:849–857; 2003.

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. 76:890–898; 2005.

Reznikoff, C. A.; Loretz, L. J.; Pesciotta, D. M.; Oberley, T. D.; Ignjatovic, M. M. Growth kinetics and differentiation in vitro of normal human uroepithelial cells on collagen gel substrates in defined medium. J. Cell. Physiol. 131:285–301; 1987.

Rocha, V.; Ringo, D. L.; Read, D. B. Casein production during differentiation of mammary epithelial cells in collagen gel culture. Exp. Cell Res. 159:201–210; 1985.

Sano, A.; Maeda, M.; Nagahara, S., et al. Atelocollagen for protein and gene delivery. Adv. Drug Deliv. Rev. 55:1651–1677; 2003.

Downloaded From: https://complete.bioone.org/journals/In-Vitro-Cellular-&-Developmental-Biology---Animal on 23 May 2025
Terms of Use: https://complete.bioone.org/terms-of-use