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Effect of platelet-rich plasma (PRP) concentration on the viability and proliferation of alveolar bone cells: an *in vitro* study

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Abstract. Previous studies have shown that a combination of platelet-rich plasma (PRP) and autogenous bone graft can increase the rate of osteogenesis and enhance bone formation qualitatively. However, contradictory results were reported in a recent animal study. In order to clarify this inconsistency, this study examined the influence of the PRP concentrations on the viability and proliferation of alveolar bone cells *in vitro*. Bone cells obtained from the alveolar bone chips were exposed to various PRP concentrations. After a culture period of 7 days, cellular viability and proliferation were evaluated by counting the number of cells and a MTT assay. The results showed that the viability and proliferation of alveolar bone cells were suppressed by high PRP concentrations, but were stimulated by low PRP concentrations (1–5%). These *in vitro* results support the view that variations in the PRP concentrations might influence the bone formation within the PRP-treated bone grafts.

Key words: platelet-rich plasma; bone graft; bone formation.

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There is currently a great deal of interest in oral and maxillofacial bone grafting procedures, which involve the use of platelet-rich plasma (PRP) to enhance bone formation and, in particular, increase the rate of bone graft healing. The use of PRP is based on the premise that the platelets in PRP release growth factors that aid bone graft maturation[6,9,12,13,20]. Previous clinical and animal studies have shown that a combination of PRP and autogenous bone graft can increase the rate of osteogenesis and enhance bone formation qualitatively[3,4,13]. However, contradictory results were reported in a recent animal study by Choi et al. who investigated the effect of PRP on bone regeneration in an autogenous bone graft in a canine mandibular model[1]. They demonstrated that the addition of PRP into a bone graft retards new bone formation in autogenous bone grafts. The inconsistency of these results may have resulted from the differential use of the PRP concentrations. If this hypothesis is correct, there should be some *in vitro* evidence to support the view that variations in the PRP concentrations influence bone formation within the PRP-treated bone graft. Therefore, this study examined the influence of the

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PRP concentrations on the viability and proliferation of alveolar bone cells *in vitro*.

Materials and methods

Cultures of alveolar bone cells were prepared as previously described[11,15]. Briefly, alveolar bone chips were obtained from the 10 mongrel dogs and minced into approximately 1 mm^3 pieces. These were washed extensively with phosphate-buffered saline (PBS) to remove blood cells and fat. The fragments were placed in a 25 cm^2 culture flask and cultured in 1 ml of growth medium consisting of Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS, Gibco), antibiotics (100 U penicillin and 100 μg/ml streptomycin, Gibco), 1% non-essential amino acids (Gibco), and sodium pyruvate (100 ng/ml, Gibco). After a 14-day period, the medium was changed three times every week. When the cells grown from the explants had reached confluence, the cells surrounding the explants were detached using trypsin–EDTA (Gibco) and subcultured. In order to determine the log phase, the cell cultures were seeded into six-well plates at a concentration of 1×10^5 cells/well and cultured. The cells were removed from the wells after 1–9 days and number of cells were counted in a hemocytometer. The growth curve of the alveolar bone cells was obtained by plotting the results (Fig. 1). Only the cells in the log-phase growth were used for the *in vitro* assays.

PRP, platelet-poor plasmas (PPP), and platelet concentrates (PC) were prepared from the 10 mongrel dogs ($N = 10$), using a technique described previously[16]. Briefly, 60 ml of autologous blood, which were withdrawn from the same dog where the alveolar bone chips originated, was initially centrifuged at 2400 rpm for 10 min to separate PRP and PPP portions from the red blood cell fraction. The PRP and PPP portions were again centrifuged at 3600 rpm for 15 min to separate PRP (2 ml) from PPP. PPP was centrifuged again at 1300 rpm for 10 min. The supernatant (1 ml) was used as PPP in the study. The PRP samples were stored in Eppendorf tubes at −70 °C. After the samples were thawed, 1 ml of PRP was used for PRP assay and the remaining 1 ml of PRP was used for PC preparation. In order to produce PC, 1 ml of PRP was again centrifuged for 15 min at 3600 rpm and the supernatant was drawn off. The packed platelets were resuspended in 1 ml of DMEM in order to obtain a platelet suspension devoid of plasma. The platelet suspension was then diluted with DMEM, resulting in concentrations of 1% (11,000 platelets/μl), 5% (55,000 platelets/μl), 10% (110,000 platelets/μl), 20% (220,000 platelets/μl), 30% (330,000 platelets/μl), 50% (550,000 platelets/μl), and 100% (1,100,000 platelets/μl). PRP and PPP were also diluted with DMEM so as to produce the same concentration range (Table 1).

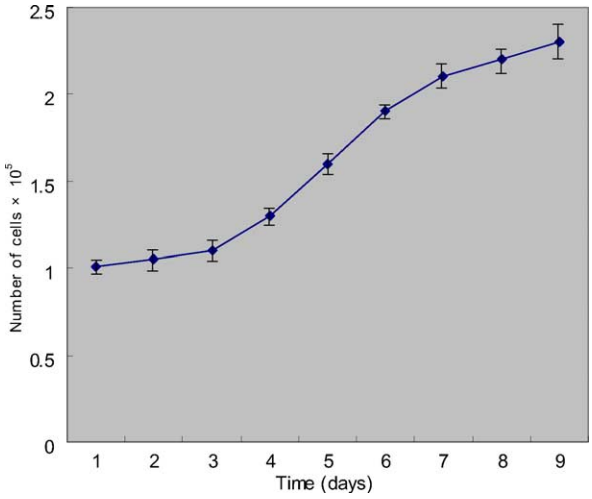

Fig. 1. Growth curve of alveolar bone cells in culture. The log phase started after day 3 of the lag phase with a greater inclination.

The cells were seeded at a density of 2×10^5 cells/35-mm culture dish or 3×10^4 cells/96-well plate and were allowed to attach overnight in 5% CO_2 at 37 °C, using the growth medium consisting of DMEM supplemented with 10% FCS, antibiotics, 1% non-essential amino acids, and sodium pyruvate. They were then incubated with various concentrations of PRP, PPP or PC. The control cells were exposed to DMEM only. After a 7-day culture period, the cell viability and proliferation were measured. The medium was not changed during the 7 days period. The justification of culturing the cells for 7 days was that the life span of a platelet and the period of the direct influence of its growth factors are approximately 5 days[13].

The MTT assay, which was used to estimate the cell viability and proliferation, was performed according to the procedure described by Ciapetti et al[2]. 3-(4,5-Dimethylthazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) was dissolved in PBS (1 mg/ml) and cells in the 96-well plate were incubated with 50 μl of the solution for 4 h at 37 °C. The MTT solution was then discarded and formazan crystals were dissolved with 100 μl of dimethyl sulfoxide. Subsequently, the plates were centrifuged, the supernatants were transferred to a new plate and the absorbance of each sample recorded at 620 nm in an ELISA reader was expressed as OD after blank subtraction. For the cell number count, the cells in the 35-mm dish were dissociated with trypsin and treated with trypan blue. The cell numbers were then counted in a hemocytometer using a phase-contrast microscope.

The significance of the difference between the test and control groups was tested with the paired Student's *t*-test. A *P*-value < 0.05 was considered significant.

Results

Cellular outgrowth from the alveolar bone chips was observed after 2 weeks of incubation. The cells grown from the fragments reached confluence after a 7–10 week culture. The PRP, PPP and PC samples had platelet counts of $124.2 \pm 24.3 \times 10^4$/μl, $1.2 \pm 0.3 \times 10^4$/μl, and $110.0 \pm 34.3 \times 10^4$/μl, respectively.

Table 1. Platelet counts in different PRP, PPP and PC concentrations

Concentration (%)	PRP (10^8/ml)	PPP (10^8/ml)	PC (10^8/ml)
1	0.12	0.001	0.11
5	0.62	0.006	0.55
10	1.24	0.012	1.10
20	2.48	0.024	2.20
30	3.72	0.036	3.30
50	6.20	0.060	5.50
100	12.40	0.120	11.00