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Effect of platelet-rich plasma on proliferation of human synovial cells

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Platelet-rich plasma (PRP) promotes early tissue healing and regeneration, and significantly induces proliferation of various cells. We investigated how PRP affects the proliferation of human synovial cells, which is not well understood. Surgical specimens of synovium were digested and the liberated cells resuspended in Dulbecco's modified Eagle's medium (DMEM). PRP was prepared using the double spin method, and activated PRP (aPRP) was prepared with calcium chloride and autologous thrombin. Cell proliferation was examined using Cell Counting Kit-8 (Dojindo Molecular Technologies, Gaithersburg, MD, USA) and the protein concentrations of IL-6 and IL-8 in culture media were determined using the Artemis TR-FRET Microplate Reader for HTRF® (Cosmo Bio, Tokyo, Japan) with HTRF® reagents (Cis-bio, Marcoule, France). We found that aPRP promoted proliferation of human synovial cells more than did PRP on day 5 of culture and that it promoted proliferation in a concentration-dependent manner. Proteins of IL-6 and IL-8 were up-regulated in culture media. 5% aPRP was the lowest relative value per cell among IL-1β, -6 and -8. Thus, we determined an optimal concentration of 5%. (J Osaka Dent Univ 2016 ; 50 : 31-35)

Key words : Platelet-rich plasma ; Human synovial cells ; Interleukins

INTRODUCTION

PRP is used in various fields of medicine, such as dentistry, orthopedics, and plastic surgery, to promote early tissue healing and regeneration, and has recently received recognition because of its increased use in injured athletes.[1] Platelets are important cells that initiate wound healing. PRP is a normal autologous component of blood that contains many platelets. There are three types of granules in platelets. The α granules are storage granules containing various growth factors, such as PDGF, VEGF and CTGF. Growth factors released from platelets become active through the coagulation process, and their release upon the destruction of α granules stimulates tissue regeneration. Previous studies have reported that PRP significantly induces the proliferation of various cells, such as human adipose-derived stem cells, human dermal fibroblasts,[2] periodontal ligament cells,[3] and alveolar bone cells.[4] A recent study reported that PRP injection into the knee joint promoted cartilage healing. However, there are few studies dealing with synovial cells, and the effect of PRP on proliferation of human synovial cells is uncertain. We investigated the influence of PRP on human synovial cells to clarify the clinical efficacy of PRP for synovial membrane tissue injury associated with temporomandibular joint disorder and related diseases in the field of oral surgery.[5]

MATERIALS AND METHODS

Cell culture

The patient was a 21-year-old male who gave informed consent. Surgical specimens of human synovium were obtained during arthroscopic knee surgery. The synovial cell culture was prepared based on the methods of previous studies.[6, 7] Briefly, specimens of synovium was minced, and digested with 0.2% collagenase in DMEM. The liberated cells were resuspended in DMEM with 10% fetal bovine serum

(FBS) and 1% penicillin-streptomycin. Cells were cultured in a monolayer at 37℃ in a 5% CO_2 atmosphere. Cells from the third to seventh passage were used in this study.

PRP preparation

PRP was prepared employing the double spin method based on a previous study[2] using a PRP kit (BS Medical, Tokyo, Japan). Briefly, blood was collected from the healthy 29-year-old male volunteer into a tube containing acid-citrate-dextrose solution formula A (1 : 7 vol/vol) anticoagulant, and centrifuged at 500 x G for 7 min using a conventional centrifuge available at the experimental facility. Plasma and buffy-coat were collected into a blood sampling tube containing no anticoagulant, followed by a second centrifugation at 1600 x G for 5 min, and 1 mL of the thrombocyte pellet precipitated on the tube bottom was used as the PRP. Activator was prepared by mixing 0.5 M of calcium hydrochloride and autologous thrombin. Activated PRP (aPRP) was made by combining the pellet with the activator at 10 : 1. The mixture was centrifuged at 13,200 rpm for 15 min, and the supernatant was collected and stored at −80℃.

Determination of the protein concentrations of PDGF-AB in PRP and aPRP

PDGF-AB levels in PRP and aPRP were determined by a commercially available sandwich enzyme-linked immunosorbent assay technique kit (Quantikine ; R &D Systems, Minneapolis, MN, USA) to confirm that the PRP was activated.

Cell proliferation assay

The obtained human synovial cells were seeded in a 96-well dish at 1.0 x 10^3 cells /well. After confirming that cells had adhered to the dish, 5% PRP, 5% aPRP, and 10% FBS were added and the cells were cultured for 5 days as Experiment 1 ($n = 5$). In Experiment 2, cells were cultured with 1, 5, 10 and 20% aPRP and 10% FBS (control) for 1, 3 and 5 days ($n = 5$). Cell proliferation was examined using a Cell Counting Kit-8 (Dojindo Molecular Technologies, Gaithersburg, MD, USA), and cells were counted following the manufacturer's instructions. The number of cells on the fifth day was confirmed under a microscope.

Determination of interleukin protein concentrations in the culture media

In Experiment 3, human synovial cells were seeded in a 12-well dish at 1.0 x 10^4 cells /well. After confirming that cells had adhered to the dish, 1, 5, 10 and 20% aPRP and 10% FBS were added and the cells were cultured for 5 days, we determined the protein concentrations of the interleukins (IL-1β, IL-6, IL-8) in culture media. The concentrations were determined using the Artemis TR-FRET Microplate Reader for HTRF® (Cosmo Bio) with HTRF® package insert reagents (Cisbio) following the manufacturer's instructions.

Statistical analysis

One-way ANOVA was used for comparison among the groups. Statistical significance was established at the $p < 0.05$ level. Data are presented as means the mean and standard deviation.

RESULTS

Blood test

Blood testing was performed by LSI Medience. The test results are shown in Table 1. The platelet counts

Table 1 Blood tested by LSI Medience

	WBC (μl)	RBC ($\times 10^4/\mu$l)	HGB (g/dL)	HCT (%)	PLT ($\times 10^4$/ml)
Whole blood	4750±212.1	471.5±7.7	13.4±0.2	40.5±0.2	24.5±0.2
PRP	18600±565.6	531.5±23.3	14.9±0.4	46.7±2.1	156.6±41.2
PPP					9.2±7.4

PRP : Platelet-rich plasma, PPP : Platelet-poor plasma.

for PPP and PRP were 9.2 ± 7.4 and 156.6 ± 41.2, respectively, showing that the count for PRP was about 10 times higher. The PRP platelet count had previously been reported to be about 7 times higher.[2] This was close to our count, demonstrating that we had successfully prepared the PRP.

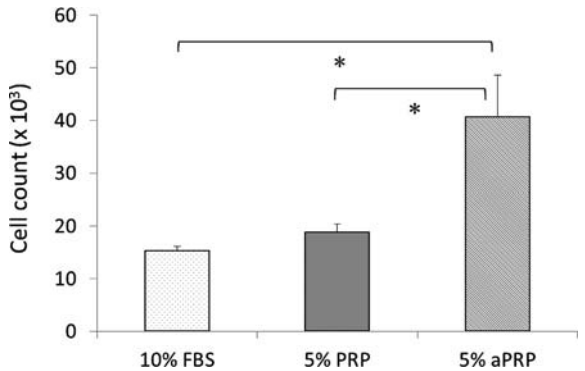

Fig. 1 Human synovial cell proliferation on day 5 after the addition of 10% FBS, 5% PRP and 5% aPRP (*p＜0.05, n＝5).

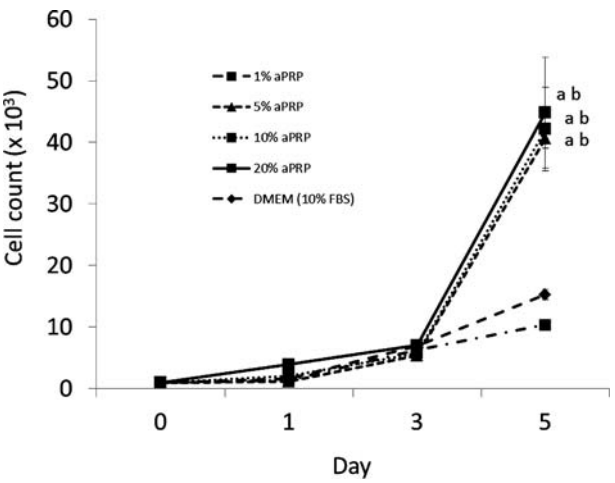

Fig. 2 Human synovial cell proliferation on days 1, 3 and 5 after the addition of various concentrations of activated platelet-rich plasma (a : p＜0.05, versus 1% aPRP, b : p＜0.05, versus 10% FBS, n＝5).

PDGF-AB levels in prepared PRP and aPRP

The PDGF-AB levels in aPRP was about 30 times higher than those in PRP (data not shown).

Effect of PRP on proliferation of human synovial cells

On day 5 of the culture in Experiment 1, the proliferation of human synovial cells was significantly greater with the 5% aPRP compared with the 5% PRP and the 10% FBS (Fig. 1). In Experiment 2, rapid growth was noted from day 3 in the 5, 10 and 20% aPRP combined groups, and significant differences were noted

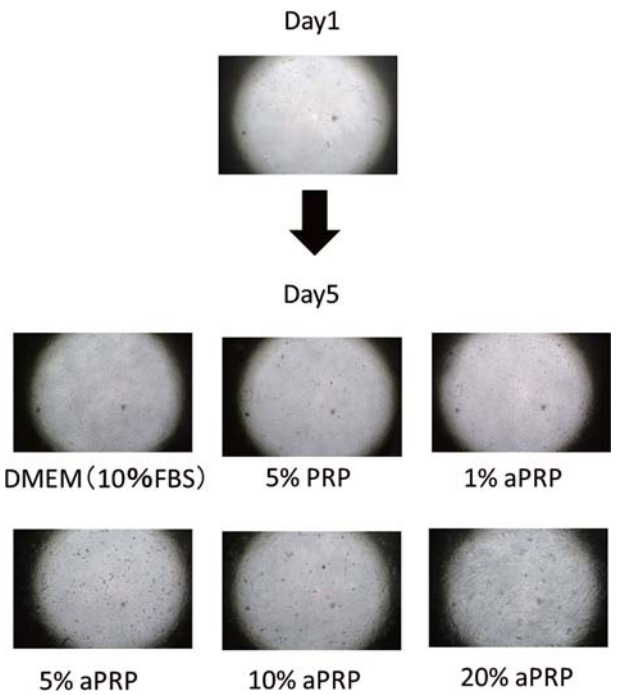

Fig. 3 Microscopic images of the proliferation of human synovial cells.

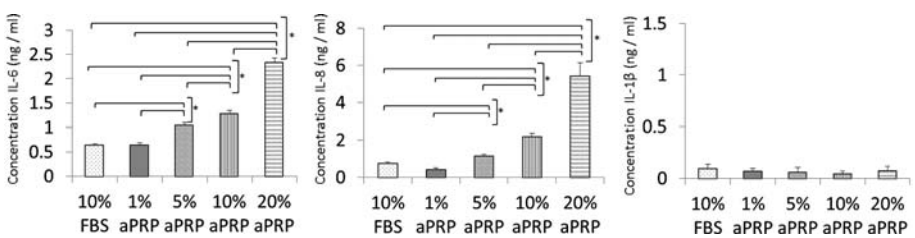

Fig. 4 Protein concentrations of IL-6, IL-8, and IL-1β in culture media on day 5 after the addition of various concentrations of activated platelet-rich plasma (*p＜0.05, n＝5).

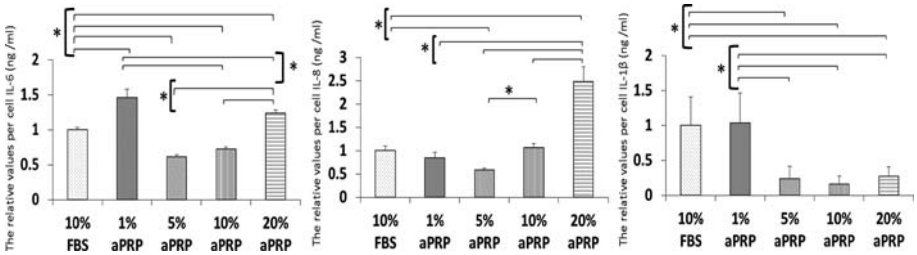

Fig. 5 The relative values per cell on protein concentrations of IL-6, IL-8, and IL-1β (*p＜0.05, n＝5).

on day 5 of culture compared to growth in the 10% FBS and 1% PRP groups (Fig. 2). Microscopic examination revealed that the number of cells increased in a concentration-dependent manner (Fig. 3). In Experiment 3, no significant difference was noted in the IL-1β level of the culture supernatant among the groups. However, the IL-6 and IL-8 protein production levels increased in a concentration-dependent manner, showing significant differences (Fig. 4), and 5% aPRP was the lowest in the relative values per cell (Fig. 5).

DISCUSSION

The synovial membrane closely adheres to the inner surface of the articular capsule in the temporomandibular joint, and the upper and lower articular spaces are individually lined. The synovial membrane produces synovial fluid, lubricates the joint, and nourishes the joint cartilage and disc. When the articular disc dislocates forward due to temporomandibular joint disorder, the synovial membrane attached to the posterior tissue of the disc directly receives a masticatory load, and this excess loading induces inflammation, causing injury and perforation of the synovial membrane, For this reason, we studied PRP which is frequently used to promote early tissue healing and regeneration. We investigated the influence of PRP on human synovial cells to clarify its clinical efficacy for treatment of temporomandibular joint disorder-associated synovial membrane tissue injury. Experiment 1 clarified that aPRP more markedly promoted synovial cell proliferation, suggesting the involvement of PDGF released from destroyed and activated platelets. PDGF has been reported to induce fibroblast proliferation.[8] We also observed significant synovial membrane growth in the aPRP group.

Experiment 2 clarified that cell proliferation was enhanced when the aPRP level was 5-20%. A previous study reported that alveolar bone cell proliferation was most markedly promoted at 5-10 %.[4] We observed aPRP concentration-dependent synovial cell proliferation. However, longer-term observation may be necessary because the duration of our experimental measurement was short (5 days), and it has been suggested that a high PRP creates cytotoxicity.[4]

A correlation between inflammatory findings of the synovial membrane on arthroscopy and temporomandibular joint pain has been reported.[9] Various proinflammatory cytokines, such as IL-1β, IL-6, and IL-8 have been measured in the synovial membrane and in fluid collected from patients with degenerative changes in the temporomandibular joint and osteoarthritis.[10, 11] The results of Experiment 3 demonstrated significant increases in the measured values of IL-6 and -8. However, those of Experiment 2 show that the proliferation rate of synovial cells was increased by raising the concentration of PRP, with the productions of IL-6 and -8 also increased in a concentration-dependent manner. Hence, 5% aPRP was the lowest relative value per cell among IL-1β, -6, and -8. Thus, we determined an optimal concentration of 5%. However, reportedly, matrix metalloproteinases (MMPs) are produced in synovial fibroblasts on day 2 after the addition of PRP.[12, 13] Thus, further studies are needed in the future. We found that aPRP promoted proliferation of human synovial cells more than did PRP, and that it promoted this proliferation in a concentration-dependent manner. This study suggests that PRP effectively promotes proliferation of human synovial cells.

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