Comparison of Effects of Allogenic Platelet Rich Plasma on the Proliferation and Differentiation of Mesenchymal Stem Cells from Different Tissue Sources

INTRODUCTION:
The success of tissue engineering can be improved with the addition of adjuncts that increase the proliferation and differentiation of progenitor or stem cells. Platelet-rich plasma (PRP) has recently emerged as a potential biological tool which can be supplied in autologously or allogenically to improve the healing and regeneration of tissue. However, the effects of PRP on stem cell proliferation and differentiation are quite different from different reports which imply stem cells from different sources may have various responses to PRP. In this study, we designed a donor-matched comparison of PRP on their effect of the proliferation, multi-lineage differentiation, and the ability of extracellular matrix formation of bone marrow stem cells (BMSC), ACL ligament-derived stem cells (LSC), and synovial-derived stem cells (SDS).

METHODS:
Isolation and identification of stem cells: Human BMSC, LSC, SDS were harvested from patients receiving total knee arthroplasty for advanced osteoarthritis as previously described. The immunophenotypes of stem cells, and in vitro chondrogenesis, osteogenesis and adipogenesis assays were performed as previously described.

PRP preparation: Venous blood from volunteer donors was collected with sodium citrate as anticoagulant and centrifuged at 460g for 10 min to obtain PRP. Calcium gluconate (100 mg/ml) was added to the PRP to activate platelet release action. PRP was filtered with 0.2 um pore filter and stored at –80 degrees for later use.

Effects of PRP on cell proliferation: The effect of PRP on the proliferation of BMSCs, LSCs, and SDSs were evaluated. Briefly, 40,000 cells were placed in each well of a 6-well plate. After cultured with IMDM supplemented with 10% FCS (fetal calf serum; Invitrogen Groningen, The Netherlands) or 10% allogenic PRP, cell number was determined on days 7 and 14 by trypan blue exclusion in a Neubauer counting chamber. Three independent experiments were performed at each time point.

Gene expression with PRP treatment: 40,000 BMSCs, LSCs and SDSs were placed in each well of six-well plates and cultured with mediums as in cell proliferation study. The culture medium was changed twice per week. Cells were harvested at days 7 and 14. Total RNA from the cells in each group was extracted. Complementary DNA (cDNA) was obtained by RT of 1μg total RNA using Advantage RT-for-PCR (Clontech, Palo Alto, CA, US) per manufacturer’s instructions. Quantitative PCR was conducted on a Roche LightCycler 480 (Roche Diagnostics, Laval, Quebec, Canada) real time PCR system.

Stain of collagen and total ECM proteins: Collagen and proteins were quantified by colorimetric analyses as described previously. Briefly, cells were incubated with 1 mL of saturated picric acid solution that contained Sirius Red and Fast Green for 30 min. The fluids were then withdrawn, and the plates were washed repeatedly with distilled water and then photographed under microscope.

RESULTS:

Cell morphology and growth kinetics: BMSC, LSC and SDS could be maintained in both culture conditions. The cells were plastic adherent and spindle-shaped (Fig. 1). In the experiment, the proliferation rate of cells was higher in 10% PRP than 10% FCS (Fig. 2a).

Effects of PRP treatment for 2 weeks on stem cells differentiation: Upon PRP treatment, only BMSCs have increased mRNA expression of type I collagen, osteocalcin and osteopontin (Fig. 3a, 3c, 3f). Expression of type III collagen increased in LSCs and SDS with PRP treatment (Fig. 3b). The expressions of alpha-smooth muscle actin (α-SM) are increased in all stem cells, wheather tenasin-c are increased in BMSCs and LSCs (Fig. 3e, 3d). The expressions of aggrecan and cartilage oligomeric matrix protein (COMP) are decreased with the treatment of PRP in all stem cells (Fig. 3g, 3h).

Collagen and total ECM protein production: In phase contrast microscopic examination, deeper staining was noted in the group with 10% PRP treatment compared with 10% FCS (Fig. 4).

DISCUSSION:
In our study, stem cells from bone marrow, ACL and synovial tissue possess faster proliferation rate with the treatment of 10% PRP. The genes toward osteogenic differentiation are upregulated only in BMSC with 10% PRP treatment. Expressions of gene favor ligament differentiation such as type III collagen and α-SM are increased in LSCs, BMSCs, and SDSs. However, the expression of tenasin-c only increased in BMSCs and LSCs. Cartilage differentiation are inhibited by PRP because the expression of aggrecan and COMP are low after PRP treatment in all stem cells. The extracellular collagen and noncollagen production are increased after PRP treatment in all stem cells. In summary, PRP increase the proliferation of stem cells from different tissue but possess various effect on differentiation depend on the sources of stem cells. Further evaluation of the mechanism and effect of PRP on stem cells is necessary for further application of autologous or allogenic PRP in tissue engineering.

REFERENCES:

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