ISAKOS

International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine

9th Biennial ISAKOS Congress • May 12-16, 2013 • Toronto, Canada

Paper #108

Negative Effect of Platelet Rich Plasma on the Differentiation of Synovium-Derived Mesenchymal Stem Cells

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Summary:
Platelet rich plasma has negative effect on the differentiation of synovium derived stem cells.

Abstract:
Introduction:
One of the current issues studied and gaining popularity is platelet rich plasma (PRP). It is reported that PRP has proliferative effect on the various types of human cells including chondrocytes and mesenchyaml stem cells (MSCs). There also are reports that PRP enhances differentiation of MSCs especially in chondrogenesis. However, the number of studies regarding the effect of PRP on the differentiation of MSCs is very few and the methods of these studies vary considerably. MSC has gained a lot of attention as a source of cell based treatment for articular cartilage lesion, because these cells have extensive self-renewal capability, multi-lineage differentiation potential, and high capacity of in vitro expansion. Several studies have demonstrated the multi-lineage differentiation potential of human synovium-derived stem cells (SDSCs) including chondrogenesis, osteogenesis and adipogenesis. In addition, there were some reports that the differentiation potential of SDSC is better than those of other tissue derived MSCs. The purpose of this study was to evaluate whether PRP has additive or synergistic effect on the differentiation of SDSCs treated with differentiation media. In addition, we also evaluated whether PRP alone has sufficient effect to induce differentiation of SDSCs.

Methods:
Human synovial tissues were harvested from patients undergoing total knee arthroplasty for osteoarthritis and expanded in monolayer. Allogenic platelet concentrates obtained for experimental use only were used as PRP after platelet number count with addition of heparin. The media used for 4 study groups were high glucose Dulbecco's modified Eagle's medium (HG-DMEM)(Group 1), HG-DMEM with 10% PRP (Group 2), differentiation medium (Group 3), differentiation medium with 10% PRP (Group 4). Chondrogenic, osteogenic and adipogenic differentiation medium was made according to the previous reports (Lee et al., Tissue Engineering, 2009; Sakaguchi et al., Arthritis & Rheumatism, 2005). PRP concentration of 10% was determined by a pilot study on the cell proliferation using MTT assay. Evaluations were performed at 7, 14, 21 days of differentiation. Safranin-O staining of proteoglycan and immunohistochemial staining of type I, II, and X collagen for chondrogenesis, von Kossa staining for osteogenesis, and Oil Red O staining for adipogenesis were performed. Also, mRNA expression levels of typical genes regarding differentiation were analyzed using real time polymerase chain reaction. Collagen type I a1, Collagen type II a1, Aggrecan, Sox-9, Collagen type X for chondrogenesis, Collagen type I a1, Alkaline phosphatase, Osteocalcin, Runx2, Osteopontin for osteogensis, and PPAR gamma, FABP4 for adipogenesis were the genes evaluated. The study was approved by the Institutional Review Board at our institute, and informed consent was obtained from all study subjects.

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Results:
For chondrogenesis, Group 3 showed the most notable finding for proteoglycan staining and type I, II, X expression by immunohistochemistry. Type II collagen expression was observed only in Group 3. Group 1 and 2 showed positive staining only in type I collagen expression. Group 4 was stained more densely than Group 1 and 2 in all stains, but less than Group 3. For osteogenesis and adipogenesis, von Kossa and Oil Red O stains were prominent in Groups 3 and 4. Differences between the groups were not significant. Groups 1 and 2 were rarely stained. As for the gene expression level analysis, most of the typical gene expression levels for chondrogenesis, osteogenesis, and adipogenesis were increased in Group 3 and 4 compared to Group 1, but the extent of increases were greater in Group 3 than Group 4. Group 2 gene expression levels were not different from that of Group 1 and even less in some cases.

Discussion and Conclusion:
This study demonstrated that the effect of PRP was negative on the chondrogenic, osteogenic and adipogenic differentiation of SDSCs cultured in each ideally combined differentiation medium. PRP alone was not capable of inducing differentiation of SDSCs. However, PRP did enhance proliferation of SDSCs.