INTRODUCTION:
Platelet-rich plasma (PRP) is an emerging biologic tool for regenerative medicine. It has a significant advantage over other potential therapies in that it contains abundant autologous growth factors and is easy to obtain and to manipulate. The combination of autologous PRP with stem cell therapy is promising in terms of cell expansion and transplantation. However, the interaction of PRP with stem cells and the underlying cellular mechanisms are not fully understood. Human muscle derived progenitor cells (hMDPCs), such as myoendothelial cells and pericytes have been characterized in our lab and by other researchers as multipotent cells that exhibit multilineage developmental potential and that can differentiate into skeletal myofibers, bone, cartilage, and adipocytes both in culture and in vivo. They are promising candidates for orthopedics stem cell therapy. Here, we evaluated the influence of supernatants from thrombin-activated human PRP on proliferation and osteogenic, chondrogenic and myogenic differentiations of hMDPCs, and we investigated the possible mechanisms.

MATERIALS AND METHODS:
PRP and platelet-released supernatants preparation: Six whole blood donations were used to prepare one pooled PRP derived from buffy coats (purchased from central blood bank). The platelet concentration in the pooled PRP was standardized to 2×10⁶ platelets per microliter (10 times above baseline) by centrifugation and re-suspension in platelet poor plasma (PPP) as determined by hemocytometer. The platelet-released supernatants were prepared by activating PRP with one unit of human thrombin (without calcium) per ml cell suspension for 45 minutes at room temperature.

Cultivation of hMDPCs: Three different hMDPCs were used for this study. Human pre-plated MDPCs that were isolated by pre-plate technique were bought from Cook, Inc. Myoendothelial cells (CD45−, CD146+, CD144− CD34−) were obtained by muscle biopsy from three donors, and surface markers were sorted by FACS. All hMDPCs were isolated and expanded following a standard protocol and were used between passages 5-8 for the experiments.

Cell proliferation: DNA assay was used to investigate the effects of PRP on proliferation. In vitro differentiation assays: Osteogenic differentiation was evaluated by Micro-CT scanning of mineralized cell pellets and expression level of RUNX-2; Chondrogenic differentiation was quantified by alcian blue staining and GAG quantification assay on cell pellets; Myogenic differentiation was indicated by fusion rate of fast MHC (+) myofibers. Statistical analysis: The results were expressed as the mean ± SEM. The results of the present study demonstrated that PRP holds potent mitogenic activity for hMDPCs. However, the osteogenic, chondrogenic, and myogenic differentiation abilities of hMDPCs decreased in response to PRP. These data indicate that some cytokines within PRP may keep hMDPCs in an undifferentiated stage. Further characterization of the changes of cell markers and cell differentiation ability after long-time culture need to be studied; and the mechanisms (which cytokine within PRP may play a major role?) need to be investigated as well.

REFERENCES: