Late-Adhesion Bone Marrow Stromal Cells: Evaluating their Potential
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Introduction
Bone marrow stromal cells derive from a common precursor, which differentiates into osteoblasts, chondrocytes, adipocytes, miel losses, supportive stroma, and smooth muscle cells. At low density, progenitors adhere to the culture flask, proliferate, and give rise to colonies [colony forming unit-fibroblast (CFU-F)]. Colony quantification is probably the easier and most common method used to estimate the number of progenitors. Though a three-day adherence protocol is usually used to isolate mesenchymal stem cells, some authors suggest that most undifferentiated subpopulation is in the late-adhesion compartment. In this work we aimed to compare the phenotype and in vitro osteogenic commitment of both early and late-adhesion cell populations.

Materials and Methods
Bone marrow samples were collected from eight healthy patients (aged 20-60 yrs) that underwent total hip arthroplasty. Nucleated cells were cultured in IMEM. After three days in culture, non-adherent cells were collected and replated for three more days. The “first plated” and “replated” cells were identified as early-adhesion (EA) and late-adhesion (LA) population respectively, and cultured up to 14 days. For colony forming efficiency (CFE), cells were fixed in 4% formalin, stained, and the colonies were counted and measured. Phenotypic analyses were performed by RT-PCR and FACS. Osteogenic differentiation was detected by Alizarin Red staining.

Results
CFE was five times higher and colonies were 70% larger in EA in comparison to LA (Fig.1). Both populations were positive for CD90, CD105, and CD146 and expressed osteogenic, chondrogenic, and myogenic related genes. Adipogenic related genes were observed only in LA population. Both EA and LA expressed Oct-4. Osteogenic differentiation was observed earlier in EA population.

Discussion and Conclusions
Our data showed that LA progenitors present an increased proliferation rate (larger colonies) and are less committed progenitors when compared to EA cells. Nonadherent compartment, generally discarded in mesenchymal cells isolation protocols, represent a richer source of multipotent stem cells for cell therapy.

References

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Disclosures
Authors have nothing to disclose.