Novel Method To Isolate Mesenchymal Stromal Cells From Bone Marrow

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Introduction

Bone marrow contains mesenchymal stromal cells (MSCs), also called as mesenchymal stem cells, which can give rise to multiple mesenchymal lineages. MSCs have been applied for cell therapy in various diseases. In the most protocols, the marrow cells aspirated from bone marrow must undergo ex vivo separation and expansion to obtain sufficient dose of MSCs for the investigational treatment. However, extended tissue culture is theoretically fraught with hazards, including contamination and most worrisome, malignant transformation. Moreover, gene expression changes with prolonged culture could alter the therapeutic potential of the cells.Thus, increasing the recovery of MSCs from the freshly harvested bone marrow allowing for less culture expansion would be a major advance in MSC therapy. In this study, two independent investigators isolated MSCs from human bone bone marrow (n=8) using a novel MSC separation device(KANEKA CORPORATION, Japan) containing a nonwoven fabric filter composed of rayon and polyethylene in comparison with the conventional MSC isolation method by density gradient centrifugation. The resulting cell product was evaluated for the recovery of nucleated cells, colony forming unit (CFU) assay, MSC growth rate in culture, flow cytometric analysis of antigen expression and ex vivo differentiation into trilineages (osteoblasts, adipocytes and chondrocytes).

Materials & Methods

Bone marrow cells

Human bone marrow cells from 8 healthy donors (22y.o. - 60y.o.) were provided by Stem Cell and Xenograft Core Facility at the Perelman School of Medicine of the University of Pennsylvania.

MSC isolation protocols

Density Centrifugation was performed with lymphocyte separation medium (LSM) as described in the manufacture's protocol.
MSC separation device from KANEKA Corporation was used to isolate MSC according to the enclosed protocol.

CFU-F assay

CFU-F assay was evaluated after 13 days culture.

MSC growth

MSC growth was evaluated at each passage time point up to passage 2.

Flow cytometrical analysis

MSCs were analyzed by FACS after passage 2.

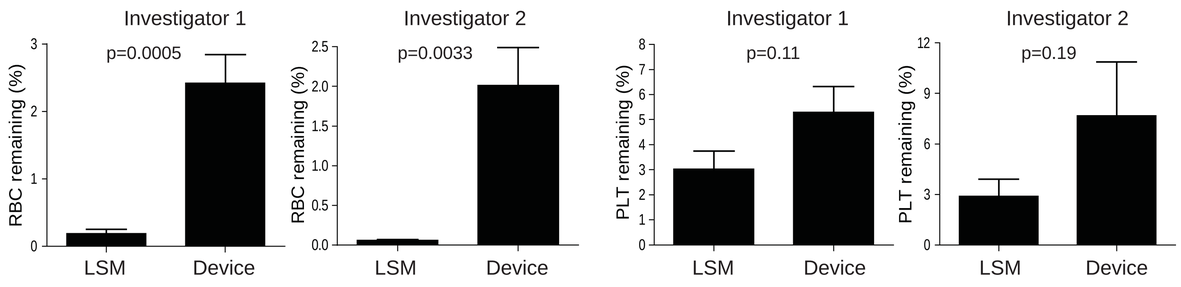
Differentiation

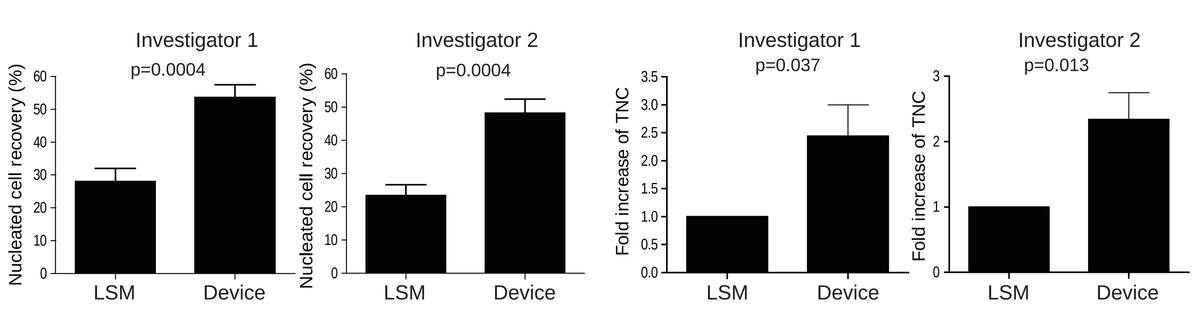
MSCs were induced into osteogenic, adipogenic and chongrogenic differentiation after passage 2.

Results

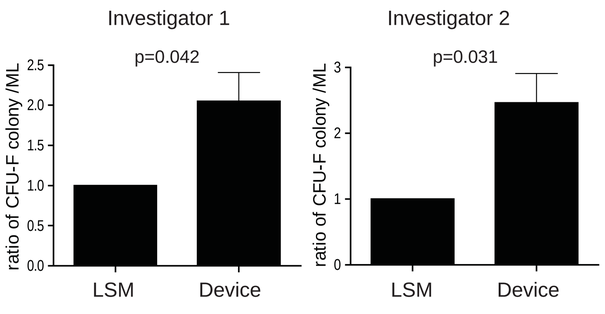
Comparison of Collection Efficacy

To evaluate the collection efficacy, a complete blood count was performed. RBC remained significantly more after the separation with the device, although almost 98% of RBC were removed. The remaining PLT was not significantly different. On the other hand, the recovery rate of nucleated cells after the separation was significantly greater with the device. The device could obtain 2.5 times more nucleated cells than LSM. (mean ± sem).



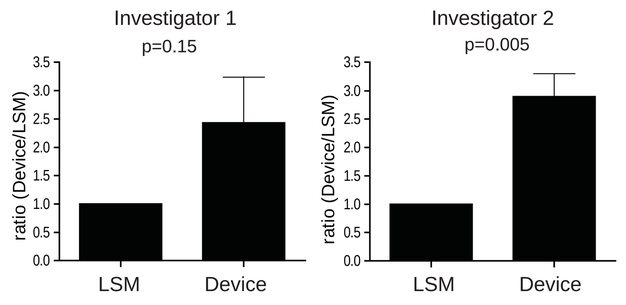


Colony Forming Unit Assay



CFU-F assay was performed. The total number of CFU-F from 1mL bone marrow was significantly greater with the device. (mean ± sem).

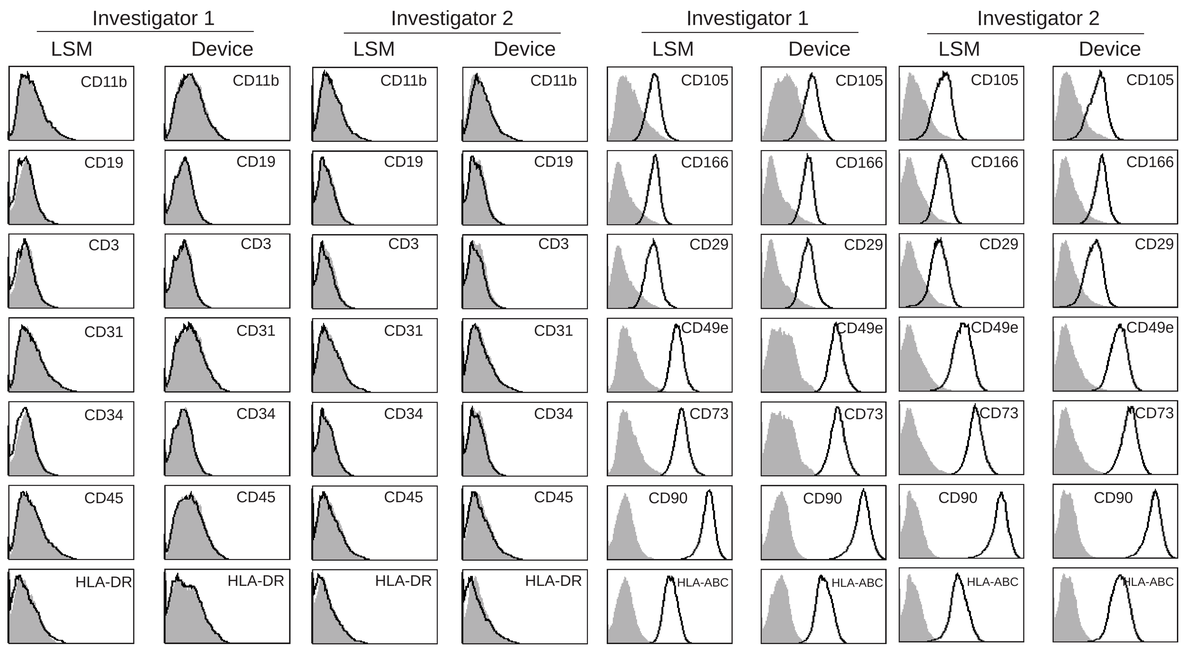
Total number of MSC after passage 2



Total number of MSC after passage 2 was examined. The total number of MSC yielded with the device was 2.9 times greater than with LSM. (mean ± sem).

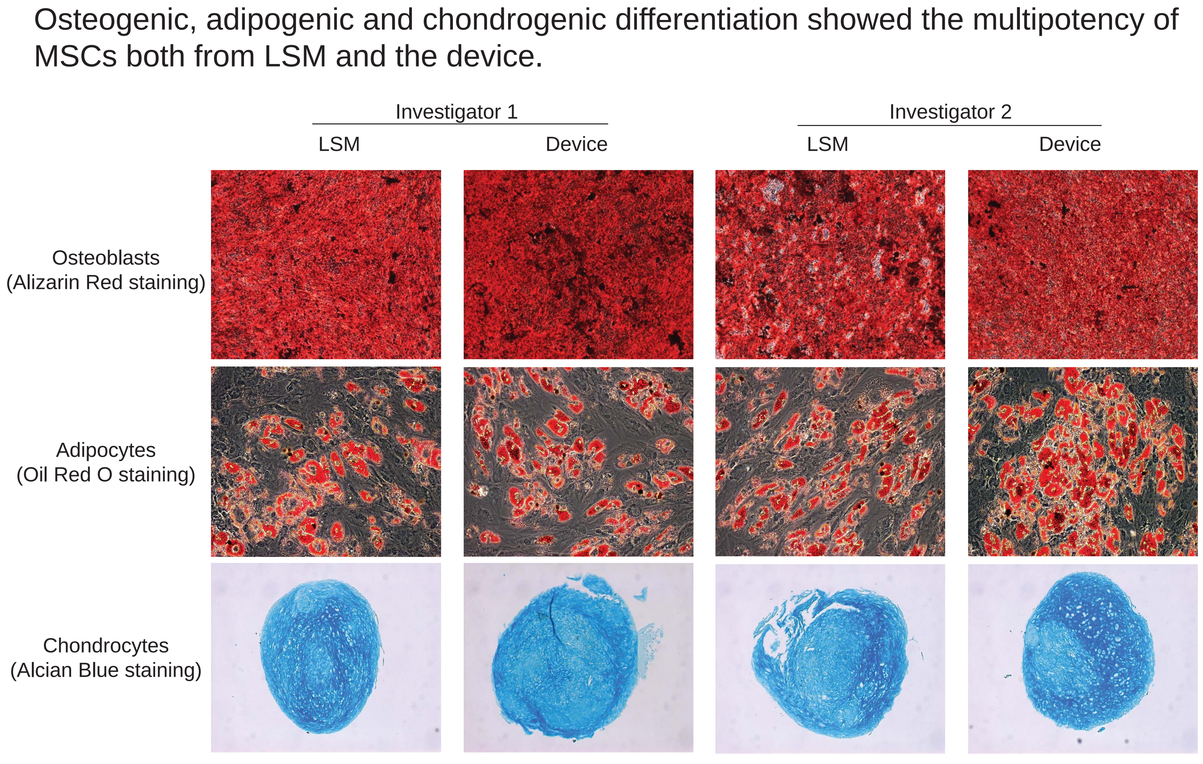
Characterization of MSCs

The ex vivo expanded MSCs were analyzed by flow cytometry. The pattern of surface markers on the MSCs from the device was similar to the one on the MSCs from LSM. Both MSCs were negative for hematopoietic markers and positive for mesenchymal markers (black solid line is experimental, filled with gray is isotype control).



Multipotency of MSCs

Osteogenic, adipogenic and chondrogenic differentiation showed the multipotency of MSCs both from LSM and the device.



Conclusion

Our data indicate that the device can provide significantly more MSCs than the conventional isolation procedure using LSM. The quality (character and multipotency) of MSCs obtained with the device is equivalent to that of MSCs obtained by the conventional method. All the results are similar between investigators indicating that personal skill is less likely to affect the results, which is often seen in the conventional procedure. The device is a closed system so that it has lower risk of contamination. Additionally, centrifugation is not rquired when isolating MSCs withthe device, resulting in less processing time. Thus, this novel MSC separation device is a fast, efficient, and reliable system to isolate MSCs and will greatly expedite preclinical and clinical investigations of MSC therapy.