The signature exosome map for bone marrow stromal cells: development of an optimal sample preparation approach

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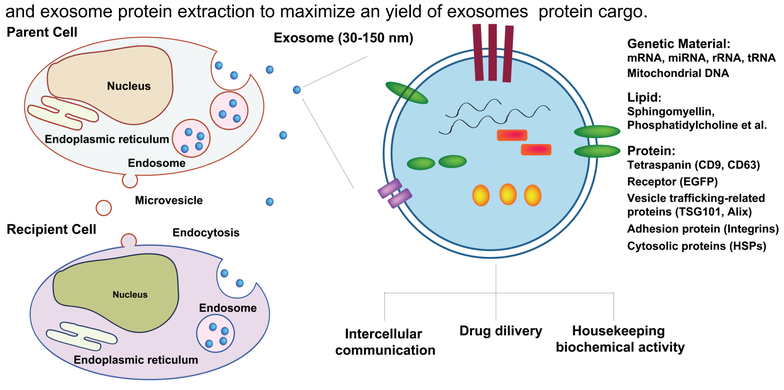
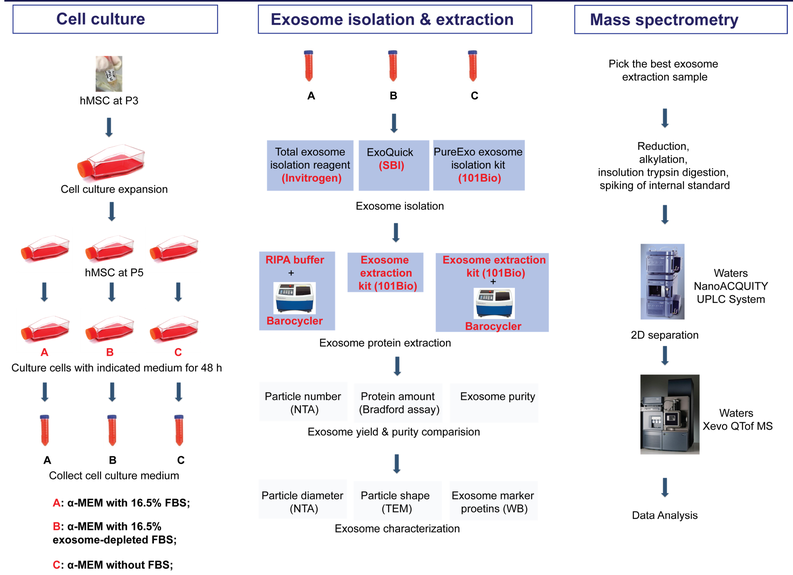
Introduction and Objective

The emergence of Human Multipotent Stromal Cells (hMSCs) as potential therapeutics in diverse range of diseases is due to three major properties: potential for cell replacement and multipotent differentiation, immune and inflammatory modulation, and tissue repair. More recent studies on MSC's biodistribution and engraftment led to the proposal that MSC's therapeutic effect is linked to the secreted extracellular vesicles (EVs). Exosomes are now considered as the specifically secreted EVs that enable intercellular communication. There is exponentially increasing interest to study exosomes' functions and to use them in minimally invasive diagnostics. However, to date, there are very few in-depth proteomic studies of hMSC exosomes contents. Our laboratory has previously performed a comprehensive proteomic analysis of hMSC and compiled and comparatively assessed the largest to date proteomic dataset of culture-expanded MSC from various human donors with a total of 7753 protein groups (FDR≤3.4)[1].

Our aim is to explore and document the influence of *in vitro* cell passaging on dynamic changes of the exosome proteome. A crucial part of any proteomic study is designing an optimal sample preparation approach; in the case of exosomes there is no established approach. Here we describe the development and comparison of various methods of cell culturing, exosome isolation, and exosome protein extraction to maximize an yield of exosomes protein cargo.



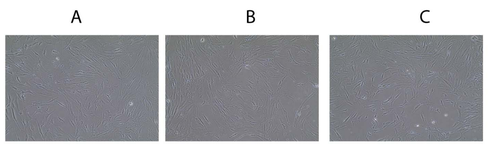
Experiment Workflow & Methods



Effect of cell culture medium on hMSC Growth

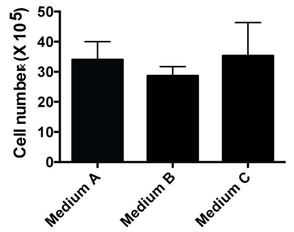
Since FBS contains bovine exosomes, we compare the cell culture condition for medium with exosome-depleted FBS or without FBS. We first investigated how the cell culture medium affect the hMSCs' growth. We reseeded cells that have been cultured with indicated medium for two days and grow them in normal cell growth medium. We monitored the cell proliferation by cell morphology (**a**), cell counting (**b**) and colony forming ability (**c, d**). No visible difference was observed among cells cultured with mediums A, B and C.

a

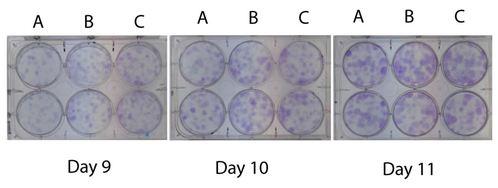


A, α-MEM with 16.5% FBS;
B, α-MEM with 16.5% exosome-depleted FBS;
C, α-MEM without FBS

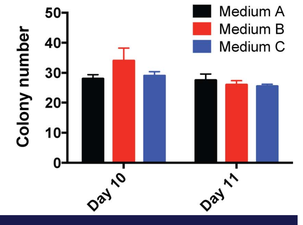
b



c



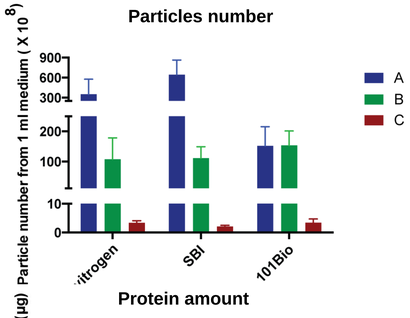
d

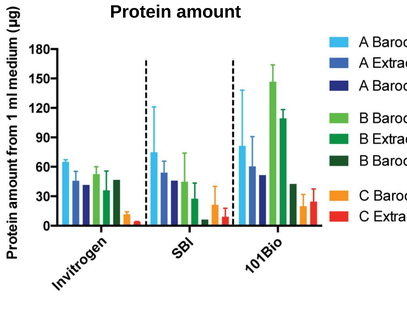


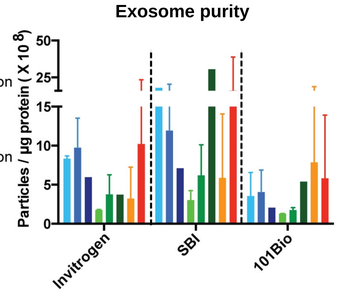
The Comparison of Exosomes' Yield and Purity

We compared the exosomes' yield and purity from cells cultured in medium with exosome-depleted FBS, without FBS , or with normal FBS as control. According to the data of particle number and protein amount, hMSCs cultured in medium **C** (α-MEM without FBS) produce least exosomes. hMSCs cultured in medium **C** (α-MEM without FBS) produce least exosomes. The exosome from hMSCs cultured in medium **B** (α-MEM with 16.5% exosome-depleted FBS) is less than that from medium **A** (α-MEM with 16.5% FBS), but much higher than medium **C**. There is not too much difference in protein amount between medium B and C.

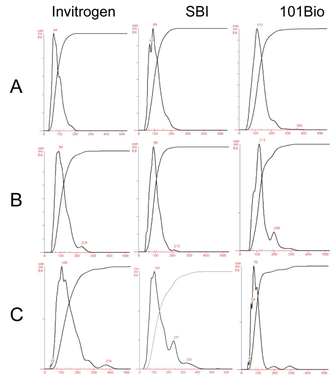
We also compared three commercial exosome isolation kits, which are Total Exosome Isolation (Invitrogen), ExoQuick (SBI) and PureExo (101Bio). There is no marked difference between Invitrogen and SBI kits regarding the exosome number and protein amount in medium B and C, respectively. And similar amount of proteins were obtained from the three exosome extraction methods



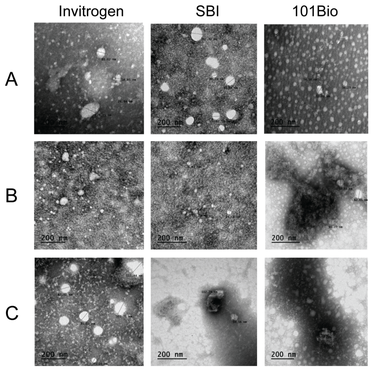




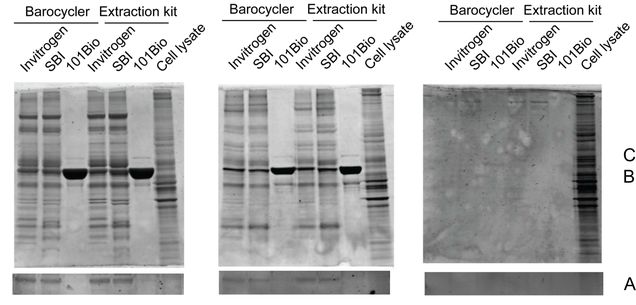
Characterization of Exosomes



Size distribution of particles isolated with indicated methods from cells cultured with indicated medium.



Transmission electron microscopy (TEM) of particles isolated with indicated methods from cells cultured with indicated medium.



1D SDS-gel analysis of exosome protein extraction showed that 1) the 101Bio kit isolated exosomes had lower protein abundance; 2) hMSC in medium C produce very little exosomes. The results have been confirmed by western blotting against known exosome marker protein Alix.

Conclusions

1. hMSCs produce very little exosomes in the culture medium without FBS compared to that in culture medium with FBS.
2. Exosomes isolated by Total Exosome Isolation kit (Invitrogen) and ExoQuick (SBI) were with comparable purity and protein yields; while protein extraction of PureExo (101Bio) -isolated exosomes had lower protein abundance according to 1D SDS-gel and western blotting.
3. Both barocycler and extraction kit (101Bio) increased protein yield .

Reference & Acknowledgement

Mindaye ST, Surdo JL, Bauer SR, Alterman MA. (2015) ***Stem Cell Res;***15(3):655-64

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