Human MSCs Release Multiple EV Populations Containing Mitochondria

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Abstract:

A growing body of evidence supports intracellular mitochondrial (MT) transfer as an important intercellular signaling mechanism. Further, increasing evidence suggests that Mesenchymal Stromal Cells (MSCs) can rescue injured and dysfunctional cells by donating whole mitochondria, and this phenomenon may explain the beneficial effects of therapeutically implanted MSCs. One possible mechanism for MT transfer involves packaging mitochondria into extracellular vesicles (EVs). This would open the possibility of cell-free MT-targeted regenerative therapies. Confirming that this is possible would be an important step toward therapeutic development. As demonstrated here, human MSCs produce EVs containing MT. We have used the CNF facilities to further characterize these 'mitovesicles' and found that there are multiple populations of different sizes, indicating different modes of biogenesis and/or distinct bio-signaling functions.

Summary of Research:

The phenomenon of intercellular mitochondrial transfer, by which mesenchymal stromal cells donate whole mitochondria (MT) to other cell types undergoing MT dysfunction, is a promising avenue for therapeutic intervention in degenerative disease [1]. Mitochondrial donation has been demonstrated in multiple cell types, including neurons and myocytes. It has been shown to improve MT function and prevent apoptosis *in vitro*, as well as improve tissue repair *in vivo* [2–4].

Our lab studies MSC MT donation in the context of orthopedic disease, using *in vitro* chondrocyte cultures and explanted cartilage tissue as models. Using confocal imaging, we have identified several possible modes of MSC-chondrocyte MT transfer, including direct cell-cell contact (e.g., nanotubule-medial filipodial transfer, gap junction-mediated transfer) and what appears to be non-contact transfer, whereby MSCs release mitochondria into the extracellular space, which are then taken up by chondrocytes. We hypothesize that these are MT are packaged inside of extracellular vesicles (EVs) as so-called 'mitovesicles'.

This strategy of loading MT into EVs has precedent in literature. Phinney, et al., showed that MSCs can use mitovesicles, to outsource mitophagy of depolarized MT to macrophages, boosting bioenergetics for both the donating MSC and the recipient macrophage [5]. Furthermore, Morrison, et al., used cellular staining and flow cytometry to demonstrate that distressed lung epithelial cells can take up MT through EV-mediated transfer, and this ameliorates lung injury *in vivo* [6].

Our goal was to characterize the EVs produced by human MSCs. EVs are an inherently heterogenous population, making specific categorization difficult. However, it is widely recognized that they fall into three size categories: small (15-100 nm, exosomes), medium (150-1000, microvesicles), and large (1 μm+, apoptotic bodies). We isolated EVs from human bone marrow derived MSCs and used dynamic light scattering (DLS) to analyze their size distribution (Figure 1). As expected, we found the three categories supported by previous work [7] (Figure 1).

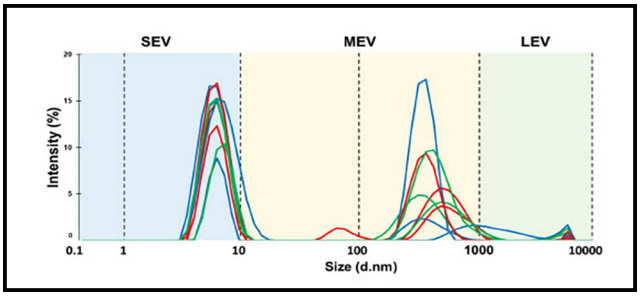

Figure 1: Dynamic Light Scattering of human MSC-derived extracellular vesicles (EVs) reveals three sub-populations of EVs based on size: small, (SEV; ~5-10nm) medium, (MEV; ~100-1000nm) and large (LEV; 5,000-10,000nm). N =3.

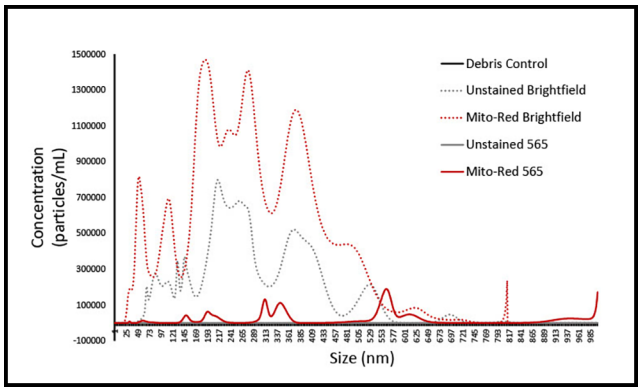

Figure 2: NTA supports trend of larger EVs containing MT. EVs were stained with Mitotracker Red and run with either a non-fluorescent (brightfield) filter, or with fluorescence exciting at 565 nm. These groups were compared to non-stained EVs and a double filtered PBS control. Unstained particles were undetectable using the 565 filter. Mitotracker positive EVs seemed to show a trend towards a larger size, with nearly all of the smallest population disappearing altogether. N = 1.

Next, we used the Malvern NS300 NanoSight to identify which, if any of these categories contain MT. We stained hMSC EVs with Mitotracker Red, then performed nanoparticle tracking analysis using the NanoSight's 565 nm fluorescent filter. We were able to validate that this method allows us to track exclusively EVs that contain mitochondrial content (Figure 2). Further, we found that mitovesicles make up around 20% of the total EV's released and appear to trend slightly larger than the general population (Figure 2). The significance of these findings is not yet clear, but likely reflects distinct modes of biogenesis and cargos for different sub-populations of mitovesicles.

Conclusions and Future Steps:

Our work thus far has confirmed our ability to isolate EVs from MSCs and identify mitovesicles within that population. Our next step is to identify and separate the EVs that contain functional and non-functional MT. This will allow us to begin identifying the role that these particles play in intercellular signaling and to further investigate MSC-EV mediated MT transfer.

This work has contributed to a poster, "MitoEVs Containing CX43 Transfer Mitochondria to Chondrocytes" that was presented at the International Gap Junction Conference 2022 and a manuscript, "Human Mesenchymal Stromal Cells Release Functional Mitochondria in Extracellular Vesicles" that is currently being proofed for publication in Frontiers in Bioengineering and Biotechnology.

References:

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