Large-Scale Production of Stem Cell Extracellular Vesicles for Cellular Repair

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INTRODUCTION

Viruses such as HIV-1 and SARS-CoV-2 can persist in the body after initial infection and contribute towards sustained inflammation, autoimmune, and immunodeficiency syndromes in patents, as seen in AIDS and post-acute sequelae of COVID-19 (PASC / "long COVID") (1,2,3). Even with current antiviral therapies, patients often still experience central nervous system and immunological impairments / complications as a result (4,5). Therefore, a gap of knowledge exists in the realm of supplementary methods to aid current measures in preventing and treating these complications.

Extracellular Vesicles (EVs) derived from Mesenchymal Stem Cells (MSCs) have rapidly emerged as a promising therapeutic tool for regenerative medicine. EVs are nano-sized particles which have the unique capability of being able to deliver biological cargo across the blood-brain barrier without evoking an immune response; our prior research has provided evidence that MSC EVs have the potential to exert stem-cell-like reparative functions on many cell types where other therapies cannot (6,7,8). Here, we report a large-scale manufacturing platform for generating MSC EVs and demonstrate that MSC EVs can impart immunoprotective and anti-inflammatory responses in cells outside of and within CNS models. This work provides a foundation for future *in vivo* application and for the development of MSC EV therapies for use alongside antivirals in treating the effects of viral persistence in patients.

METHODS

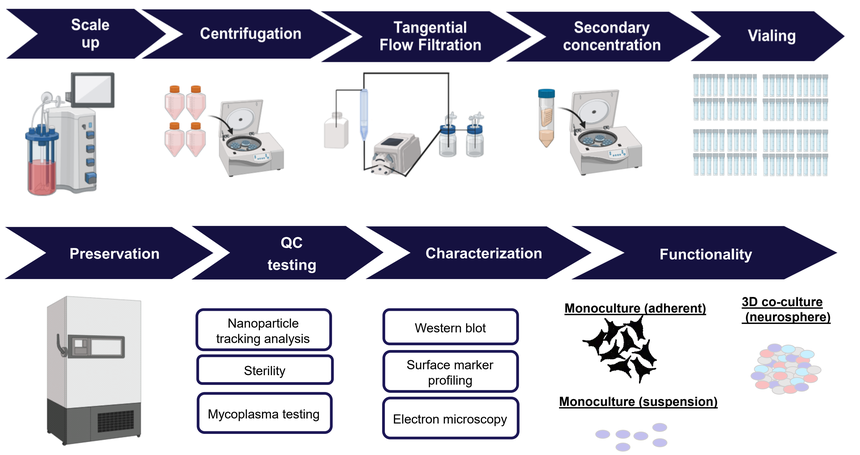

Figure 1. Large scale EV manufacturing and characterization.
hTERT-immortalized MSCs (hTERT MSCs / ATCC® SCRC-4000™) are expanded at-scale (e.g., ≥ 5L) prior to EV isolation. EVs are isolated using a stepwise process of centrifugation, tangential flow filtration, and secondary concentration to obtain the final product. After preservation, EVs undergo stringent quality control testing to assess size, concentration, and sterility. Extended characterization of EVs has also been performed to better assess morphology and biochemical properties. Functionality of EVs has also been demonstrated *in vitro* using several different cell mono-cultures and in 3D co-culture.

RESULTS

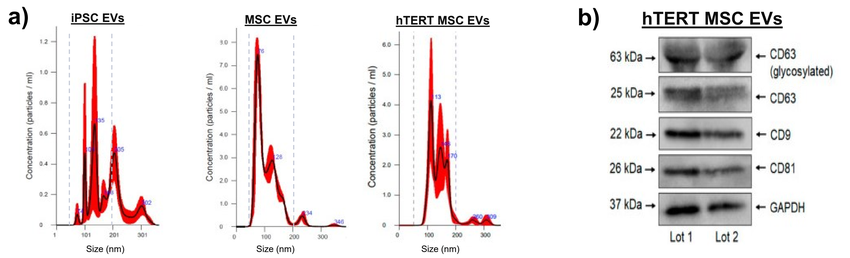

Figure 2. EV QC and Characterization.
a) Size and concentration of EVs from induced pluripotent stem cells (iPSCs), primary MSCs (ATCC® PCS-500-012™), and hTERT-immortalized MSCs (ATCC® SCRC-4000 ™) were measured by Nanoparticle Tracking Analysis. Majority of vesicles are within the expected size range of 50 to 200 nm.
b) Western blot for EV-associated tetraspanins (CD63, CD81, and CD9) was run with two independent lots of hTERT MSC EVs to. Presence of tetraspanins has also been confirmed for the other EV types (data not shown).

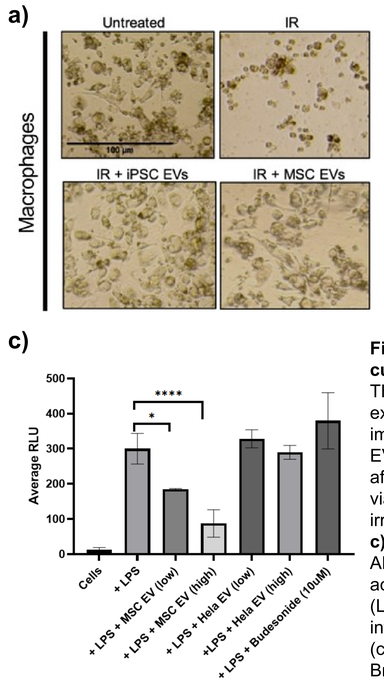

Figure 3. Reparative effects of stem cell EVs on 2D cell cultures under stress. Macrophages derived from the THP-1 monocyte cell line (ATCC® TIB-202™) were exposed to ionizing radiation (IR) and were then immediately treated with PBS (Untreated and IR), MSC EVs, or iPSC EVs. **a)** Representative morphology images after incubation for 5 days. **b)** Cells were assessed for viability. *** p <0.0001; ** p <0.005; * p <0.05 relative to irradiated cells
c) THP-1 reporter cell line (ATCC® TIB-202-NFkB-LUC2-AR™) was engineered to express luciferase upon NF-κB activation. Cells were exposed to LPS (Lipopolysaccharides) for three hours to stimulate an inflammatory response before hTERT MSC EV or HeLa (cervical cancer cell line) EV treatment. After 5 days, a Bright-Glo Luciferase assay was performed . n= 3. **** p < 0.0001; * p < 0.01.

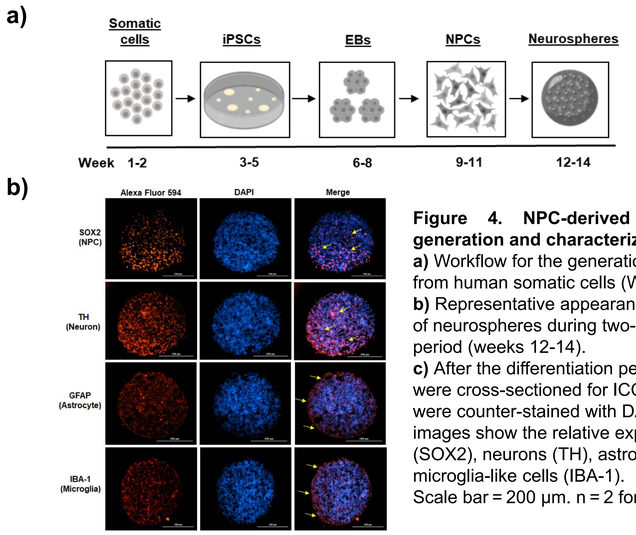

Figure 4. NPC-derived 3D neurosphere generation and characterization.
a) Workflow for the generation of neurospheres from human somatic cells (Week 1 to Week 14).
b) Representative appearance and morphology of neurospheres during two-week differentiation period (weeks 12-14).
c) After the differentiation period, neurospheres were cross-sectioned for ICC staining. Nuclei were counter-stained with DAPI. Representative images show the relative expression of NPCs (SOX2), neurons (TH), astrocytes (GFAP), and microglia-like cells (IBA-1).
Scale bar = 200 µm. n = 2 for ICC imaging.

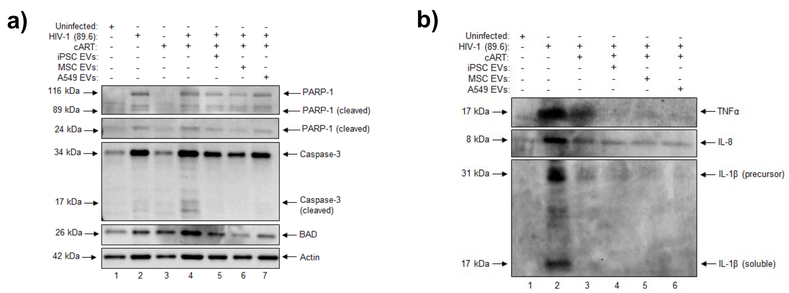

Figure 5. Effects of stem cell EVs on HIV-1 infected Neurospheres.
Differentiated neurospheres were exposed to dual-tropic HIV-1 89.6 with or without cART for 14 days. Additionally, a portion of the HIV-1 + cART treated neurospheres were additionally treated with either iPSC, MSC, or A549 (lung cancer cell) EVs at an approximate ratio of 1:250 (recipient cell to EVs).
a) Western blot from neurosphere lysates shows the relative expression of apoptotic markers PARP-1, Caspase-3, and BAD. **b)** Western blot was performed on neurosphere supernatants to evaluate the relative expression of pro-inflammatory cytokines TNFα, IL-8, and IL-1β.

CONCLUSIONS

- EVs from ATCC-sourced stem cell lines offset / reverse the effects of cellular stress/damage in 2D cell types relevant to the CNS.
- 3D neurospheres can be constructed and be shown to sustain glial cells as well as differentiated neurons.
- Stem cell EVs exert anti-apoptotic and anti-inflammatory properties in HIV-1 infected neurospheres, demonstrating their potential to be used for other disease modeling and repair assays.

ACKNOWLEDGEMENTS

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Works cited:
1. doi:10.1038/s41392-021-00749-3
2. doi:10.3390/cimb45010003
3. doi: 10.1038/s41584-023-00964-y
4. doi: 10.1016/S1473-3099(24)00436-5
5. doi: 10.17879/freeneuropathology-2024-5343
6. doi: 10.1007/s11481-019-09865-y
7. doi: 10.1038/s41598-022-05848-x
8. doi: 10.3390/cells13100861

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