A Scalable Platform for Producing Stem Cell Extracellular Vesicles with Reparative Properties

Zachary Cuba[1], Jessica Hindle[1], Sujata Choudhury[1],Dongsung Kim[1], Anastasia Williams[2], Yuriy Kim[2], Pooja Khatkar[2], Collin Nelson[3], David Routenberg[3], Fatah Kashanchi[2], Heather Branscome[1,2]

[1]ATCC, Manassas, Virginia, USA, [2]Laboratory of Molecular Virology, School of Systems Biology, George Mason University, Manassas, VA, USA, [3]Meso Scale Diagnostics, L.L.C., Rockville, MD, USA

Introduction

Extracellular vesicles (EVs), particularly those derived from mesenchymal stem cells (MSCs), offer novel solutions to many of the challenges faced by the biopharmaceutical manufacturing industry. EVs from stem cells are natural carriers of nucleic acids, proteins, and lipids and can modulate intercellular communication and contribute to tissue regeneration. However, there is an outstanding need to further develop and standardize robust methodologies for the production of well-characterized and functional MSC EVs. ATCC has recently coupled large–scale biomanufacturing capabilities with EV isolations protocols to produce functional EVs from immortalized MSCs. Isolated EVs have undergone various characterization assays to evaluate their biochemical, physical, and functional properties. Our data clearly demonstrates that MSC EVs are able to promote cell migration, reduce inflammation, and reverse cellular death across multiple cell types. Overall, this data demonstrates ATCC's ability to reproducibly manufacture MSC EVs and, furthermore, highlights the ability of these EVs to reduce cellular damage and promote reparative phenotypes.

Methods

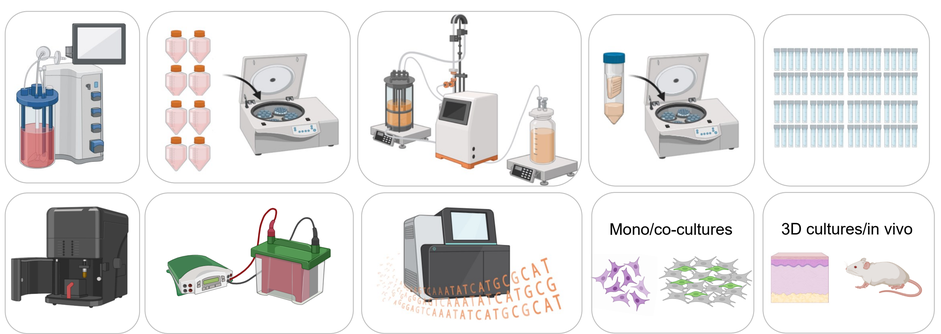

Figure 1. Large scale EV manufacturing and characterization. hTERT MSCs (ATCC® SCRC-4000™) are scaled up and EVs are isolated using a stepwise process of centrifugation, tangential flow filtration, and ultra filtration. After preservation, EVs undergo quality control testing to assess size, concentration, and sterility. Extended characterization of EVs has also been performed to better assess morphology and biochemical properties. Functionality of EVs has also been demonstrated both *in vitro* using multiple different cell types, and *in vivo.*

RESULTS

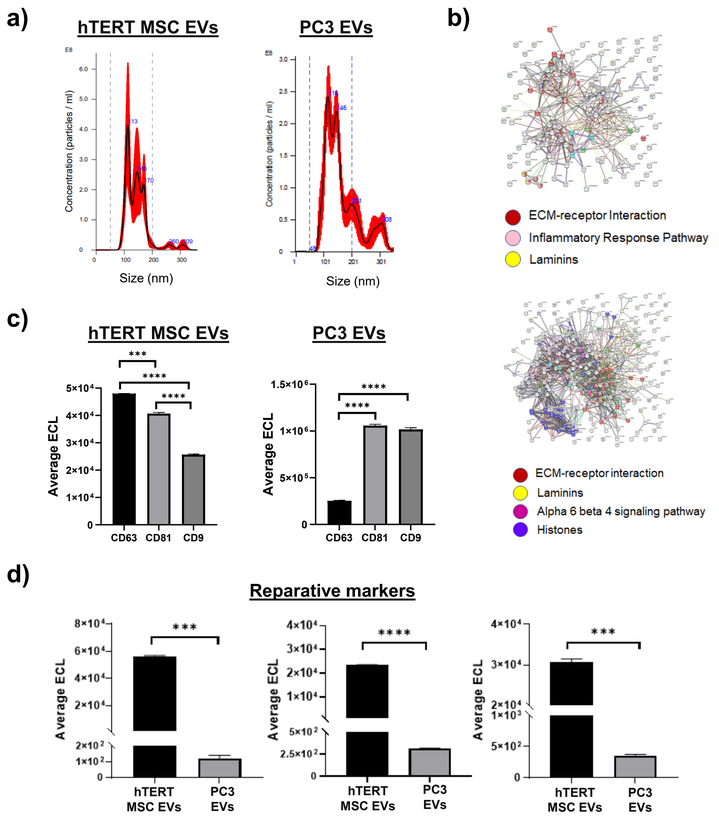

Figure 2. EV Characterization. a) Nanoparticle Tracking Analysis. PC3 (prostate cancer) EVs (ATCC® CRL-1435-EXM™ are included in all assays as a non-stem cell EV control. **b)** Proteomics results. STRING was used to calculate the interactions of peptide of hTERT MSC EVs (top) and PC3 EVs (bottom). **c)** EV were quantified with MULTI-SPOT® U-PLEX® plates displaying CD63, CD81, and CD9 antibodies. Assays were run in duplicate. **d)** Analysis of EV-surface marker proteins. Assays were run in duplicate. Average ECL values were analyzed and compared between hTERT MSC and PC3 cancer EVs. *** $p < 0.001$; **** $p < 0.0001$.

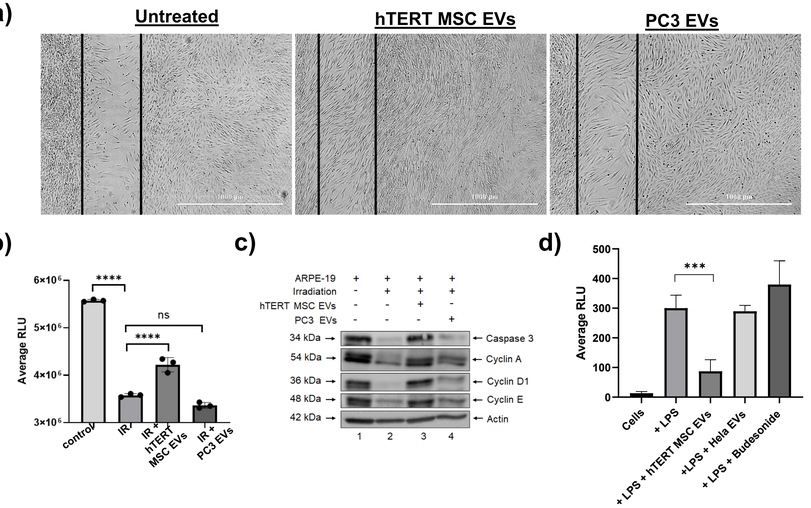

Figure 3. EV function. a) Cell migration assay using primary dermal fibroblasts (ATCC® PCS-201-012™). Photographs show gap closure 3 days post-treatment. **b)** Retinal cells (ARPE-19; ATCC® CRL-2302™) were exposed to ionizing radiation (IR) and treated with EVs. CellTiter-Glo® assay was performed to assess viability after 5 days. n=3. ****$p < 0.0001$. **c)** Western blot was performed to evaluate expression of proteins involved in apoptosis and cell cycle. **d)** THP-1 ThawReady™ cells (ATCC ® TIB-202-NF-kB-LUC2-AR™ were exposed to LPS to simulate an inflammatory response, followed by treatment with EVs or positive control drug. CellTiter-Glo® assay was performed on day 5 to assess viability. n= 3. *** $p < 0.001$.

Summary

ATCC hTERT MSC EVs meet well-established quality and characterization specifications and exert multi-functional effects in vitro, highlighting their potential for reversing cellular damage in different cell types.

For more information:

Acknowledgments: This work was partially funded by NIH Grants AI078859, AI074410, AI127351-01, AI043894, NS099029, DA050176, and R01MH13438901. Ongoing work is being funded by the ATCC Internal Reserach and Development Program.

AFS 10801 University Boulevard, Manassas, Virginia 20110-2209 **Phone:** 800.638.6597 **Email:** atccfederalsolutions@atcc.org **Web:** www.atcc.org