# Isolation and Characterization of Exosomes From Large-Scale Cell Cultures

Poster #: Arial Bold

Heather Branscome, MS[1,2], Siddhartha Paul, PhD[3*], Sheela Jacob, PhD[3], Dezhong Yin, PhD[3], Fatah Kashanchi, PhD[2]

[1]ATCC, Manassas, VA; [2] Laboratory of Molecular Virology, School of Systems Biology, George Mason University, Manassas, VA; [3] ATCC Cell Systems, Gaithersburg MD; contact email: spaul@atcc.org

## Abstract

The scientific community is currently experiencing an outpouring of research surrounding extracellular vesicles (EVs) and, more specifically, exosomes. This is due not only to their critical role in intercellular communication, but also to their potential to be used as diagnostic tools and/or therapeutic agents in a wide range of pathological conditions[1]. The rising interest in exosomes, coupled with the immense volume of research, underlies significant needs for both the isolation of high quality exosomes from large-scale batches and the development of industry standards for the characterization and quality control testing of exosomes. While traditional methods, which include the use of ultra-centrifugation and density gradients, are suitable for small-scale studies, the development of scalable and robust processes for the isolation of functional exosomes is essential to meet the growing needs of the scientific community[2]. Here, we report the use of tangential flow filtration (TFF) for the isolation and concentration of functional exosomes from large batches of conditioned culture medium to include lung carcinoma cells, human mesenchymal stem cells (MSCs), and human induced pluripotent stem cells (iPSCs).

## Methods

### Large Scale Isolation of Exosomes

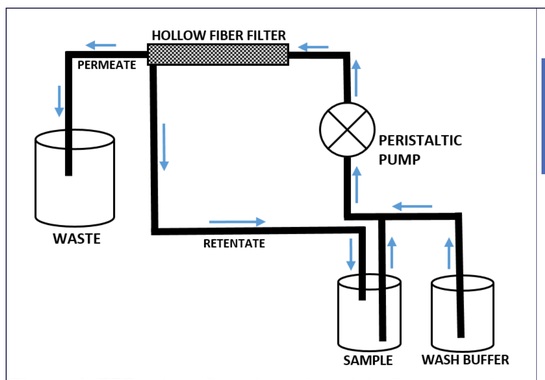


**Figure 1. TFF setup.** Sample and wash buffer are pushed through a hollow fiber filter. Larger molecules are excluded as waste while smaller molecules are retained and directed back to the sample reservoir where they are recirculated.

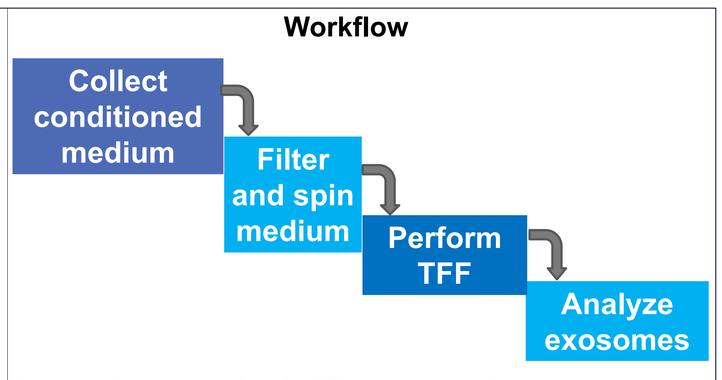


**Figure 2. General workflow for TFF exosome isolation and concentration.** Conditioned medium is collected, spun, and filtered. Clarified medium is concentrated and serially diafiltrated to isolate small EVs, such as exosomes. The retentate fraction is saved for downstream analysis.

### *In-Vitro* Wound Healing Assay

Normal primary Human Bronchial Epithelial Cells (HBECs; ATCC® PCS-300-010™) were grown to confluence in cell culture inserts. Upon insert removal an artificial gap was created and cells were treated with either iPSC- or MSC-derived exosomes. Treatment conditions were based on approximate ratios of HBECs to exosomes. To quantify cell migration, the average gap width was measured for each treatment condition over the course of 48 hours using an inverted microscope equipped with a NIST-certified stage micrometer. Analysis was performed with GraphPad Prism.

### Angiogenesis Assay

ATCC Angio-**Ready™** Angiogenesis Assay System (ATCC® ACS-2001-2™) was used to assess the effect of exosomes on tubular formation. Cells were treated with exosomes derived from either MSCs or iPSCs. Treatment was based on estimated protein content of exosomes. Cells were treated every 2 days for 6 days and tubular quantification was performed on day 9 using FIJI ImageJ Software.

## Results

### Characterization of Exosomes

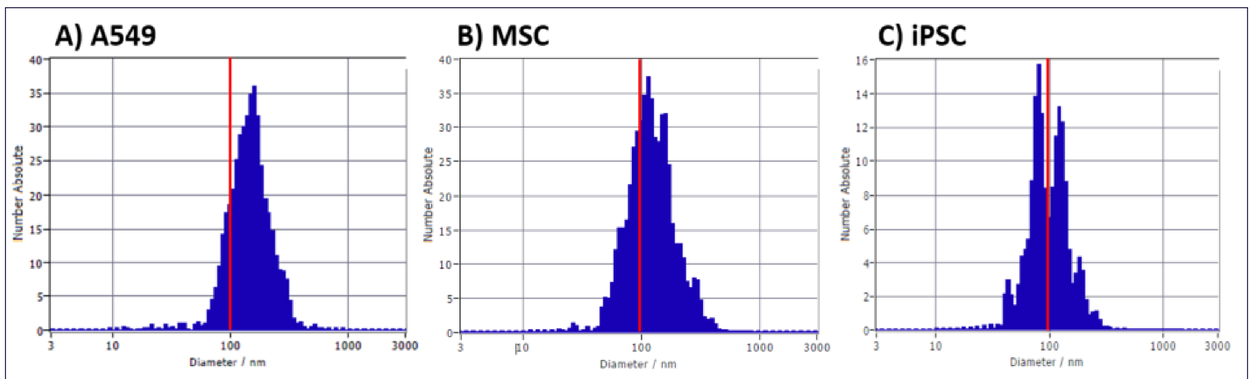


**Figure 3. Size distribution of TFF exosomes.** Analysis performed using Nanoparticle Tracking Analysis. Representative histograms of exosomes from **(A)** A549 cells, **(B)** MSCs, and **(C)** iPSCs are shown**.** The red line in each panel designates a diameter of 100 nm to show the relative size distribution of exosomes from each cell type.

| Sample | Exosomes (particles/mL) | Exosome size diameter (mean) | Exosome protein concentration |
|---|---|---|---|
| MSC Exosomes | 1.4E+10 | 113 nm | 2.8 mg/mL |
| iPSC Exosomes | 5.3E+9 | 99 nm | 2.2 mg/mL |
| A549 Exosomes | 9.6E+10 | 140 nm | 4.0 mg/mL |

**Table 1. Average exosome yield, diameter, and protein concentration of TFF-concentrated exosomes from different cell types.**

## Results

### Characterization of Exosomes and Cellular Uptake

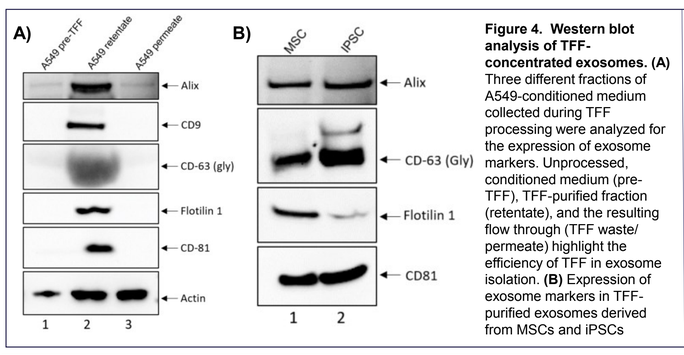


**Figure 4. Western blot analysis of TFF-concentrated exosomes. (A)** Three different fractions of A549-conditioned medium collected during TFF processing were analyzed for the expression of exosome markers. Unprocessed, conditioned medium (pre-TFF), TFF-purified fraction (retentate), and the resulting flow through (TFF waste/ permeate) highlight the efficiency of TFF in exosome isolation. **(B)** Expression of exosome markers in TFF-purified exosomes derived from MSCs and iPSCs

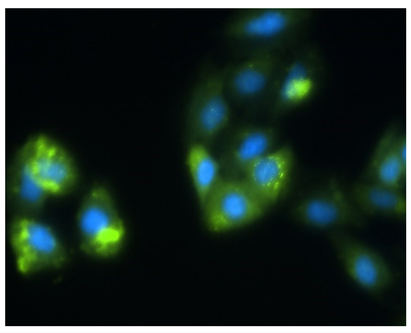

**Figure 5. Exosome uptake.** A549 exosomes were labeled with RNA-binding dye (green) and added to the recipient cell line (A549; ATCC® CCL-185™). Cells were then fixed and stained with DAPI (blue). Images (4X) were captured with a fluorescent microscope.

### Functionality Testing: Wound Healing

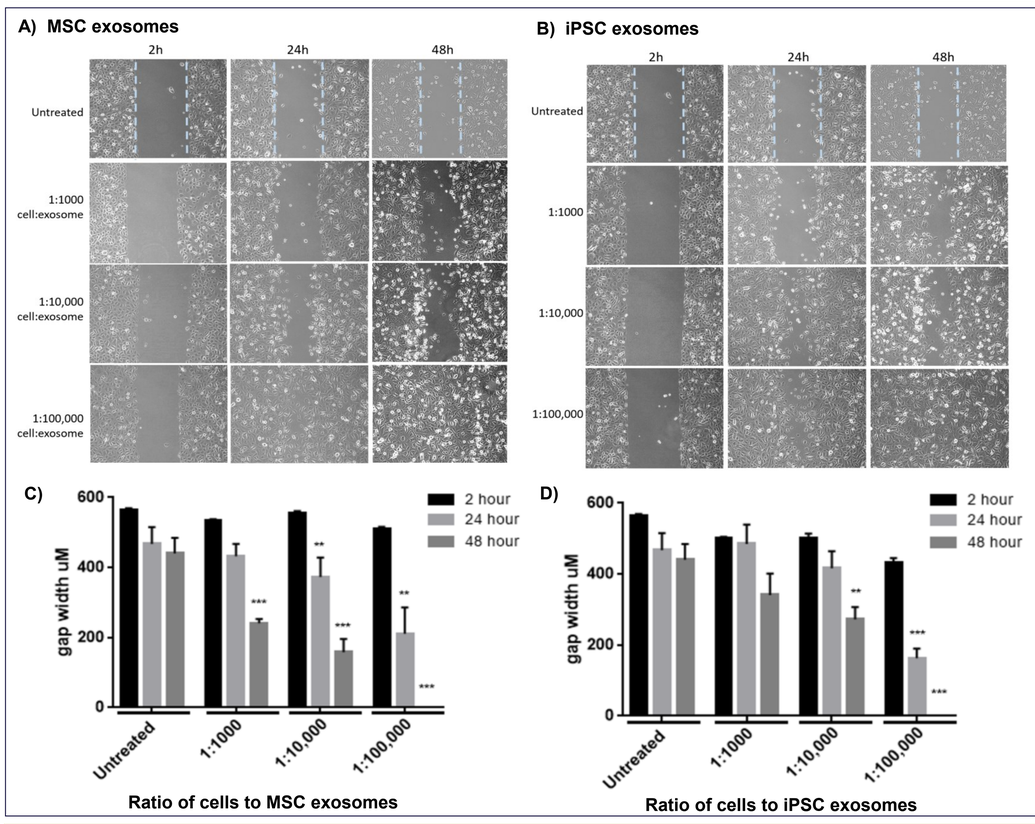


**Figure 6. *In vitro* wound healing assay.** Images (10X) of gap closures over time in normal HBECs treated with increasing concentrations of exosomes from **(A)** MSCs and **(B)** iPSCs. Untreated cells received basal medium only. **(C)** shows gap width for HBECs treated with exosomes derived from human MSCs. N=3; *** p <0.0001 relative to same treatment at 2 hours. ** p <0.0005 relative to same treatment at 2 hours. **(D)** shows the gap width for HBECs treated with exosomes derived from human iPSCs. N=3; *** p <0.0001 relative to same treatment at 2 hours. ** p <0.005 relative to same treatment at 2 hours.

### Functionality Testing: Angiogenesis

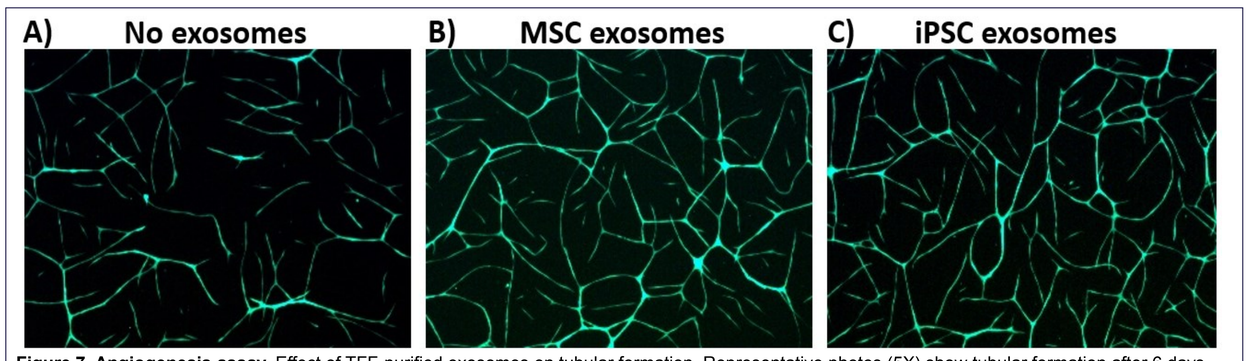


**Figure 7. Angiogenesis assay.** Effect of TFF-purified exosomes on tubular formation. Representative photos (5X) show tubular formation after 6 days in culture for **(A)** untreated cells, **(B)** MSC exosome-treated cells, and **(C)** iPSC exosome-treated cells. Untreated cells received angiogenesis medium only.

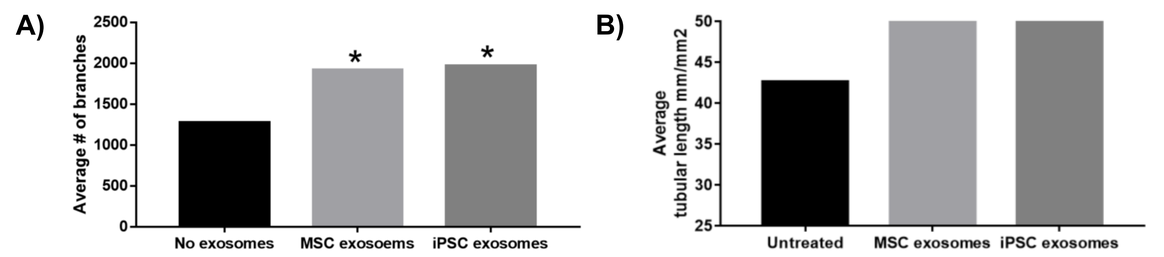


**Figure 8. Tubular quantification**. (A) Average number of branches and (B) average tubular length were quantified on day 9. N=4. *p <0.05 relative to untreated.

## Conclusions

- **Here we demonstrate a reproducible and scalable method for the isolation of exosomes from large sample volumes from different cell types.**
- **EVs isolated by TFF display the expected size distribution and express characteristic markers of exosomes.**
- **Functionality assays (wound healing and angiogenesis) have supported the potential of these exosomes to be utilized in a variety of downstream applications.**

**References:**

1. Roy S, Hochberg F, Jones P. Extracellular vesicles: the growth as diagnostics and therapeutics; a survey. *J Extracell Vesicles* 7(1):1438720, 2018.
2. Heinemann M, *et al*. Benchtop isolation and characterization of functional exosomes by sequential filtration. *J Chromatogr A* 1371:125-135, 2014.

**Reagents**

| Designation | ATCC® No. |
|---|---|
| A549 | CCL-185™ |
| Bone Marrow-derived Mesenchymal Stem Cells; Normal; Human | PCS-500-012™ |
| ATCC-DYS0100 Human Induced Pluripotent Stem Cells | ACS-1019™ |
| Primary Bronchial/tracheal Epithelial Cells; Normal, Human | PCS-300-010™ |
| Angio-***Ready***™ Angiogenesis Assay System | ACS-2001-2™; ACS-2001-10™ |

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