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**Title**

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<https://escholarship.org/uc/item/5rf0k4wv>

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**Publication Date**

2023

**Data Availability**

The data associated with this publication are not available for this reason: NA



# Three-dimensional culture system improves the yield of placental mesenchymal stem cell-derived extracellular vesicles

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## Introduction

- Placental mesenchymal stem cell derived-extracellular vesicles (PMSC-EVs) trigger cellular regeneration with less toxicity and immunogenicity compared to cell-based therapy.
- Conventional monolayer cell culture has low yield of PMSC-EVs which limits current applications.
- The CELLLine bioreactor, allows for a high-density 3D cell culture within a semipermeable membrane. It has been utilized as a large-scale tissue culture method.
- Objective-** Explore the application of the CELLLine bioreactor as a novel approach to improve the production and yield of PMSC-EVs for regenerative medicine applications.

## Design

- PMSC-EVs were isolated from the EV rich medium in the cellular compartment using ultracentrifugation isolation method weekly.
- Nanoparticle tracking analysis (NTA) was used to quantify concentration, size distribution, and relative charge
- Cryogenic electron microscopy (cryoEM) was used to confirm morphology
- Western-blot was used to confirm EV surface proteins CD9 and CD63 in addition to cytosolic proteins TSG101 and Alix.

## Acknowledgements

Support for this project comes from UC Davis Center for Surgical Bioengineering  
Special thank you to Aijun Wang PHD, Dake Hao PHD, Juan Lopez and the whole UC Davis Center for Surgical Bioengineering

## Design (cont.)

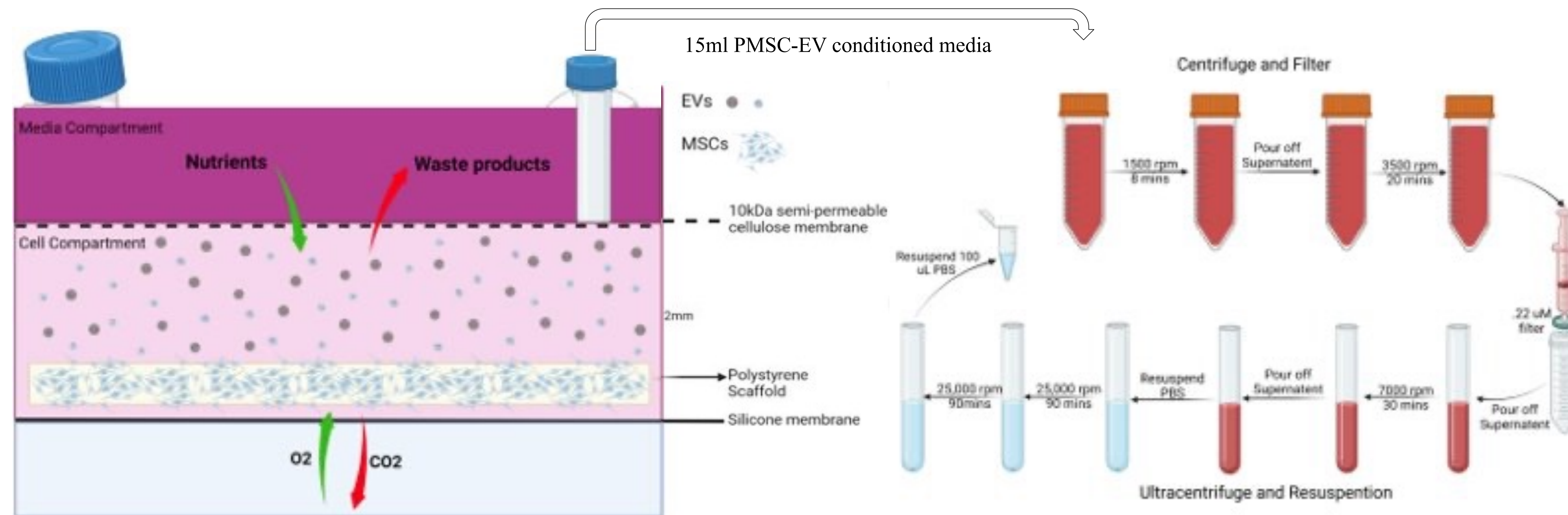
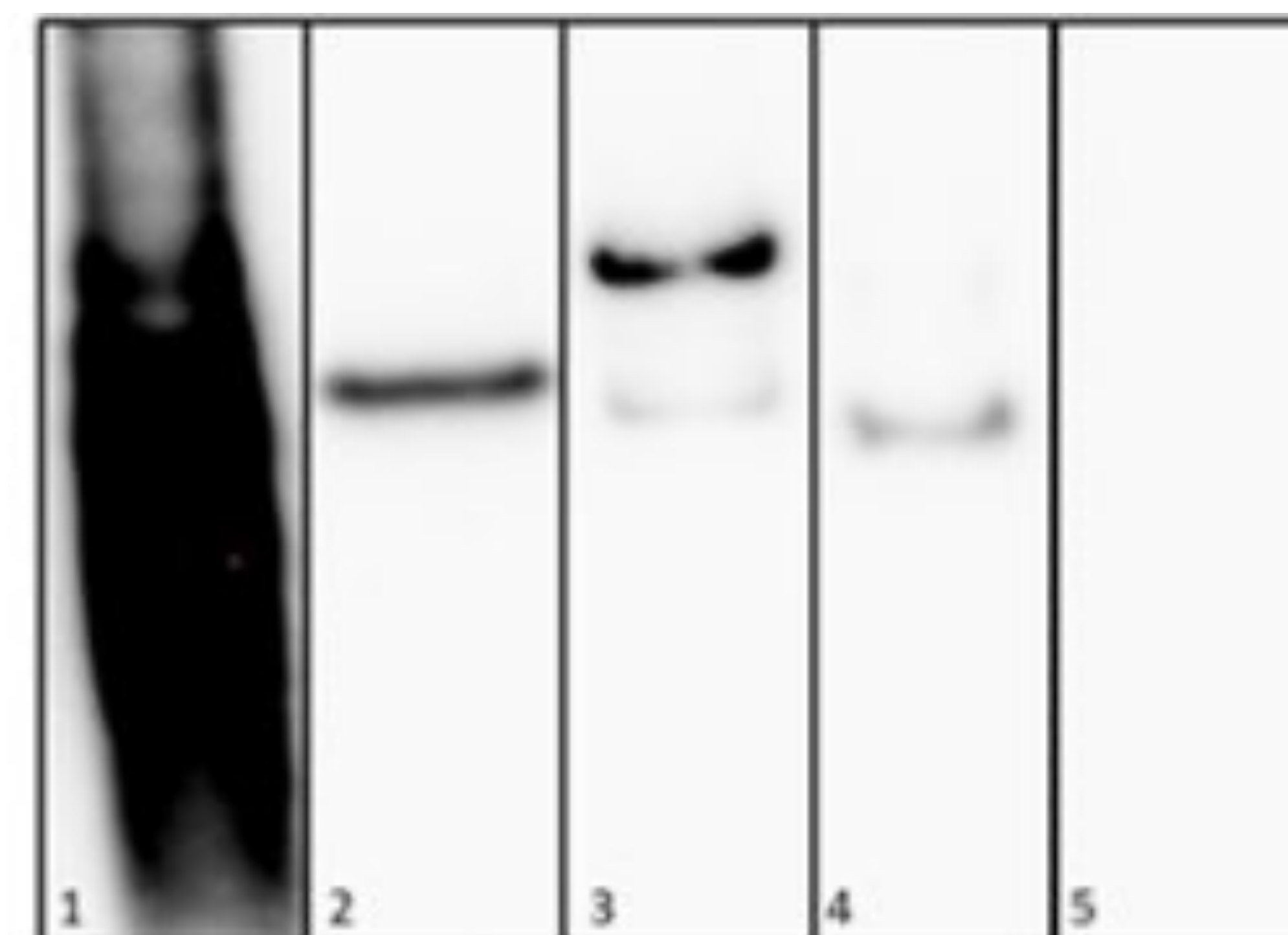


Figure 1. Components of the 3D culture and diagram of EV isolation using ultracentrifugation

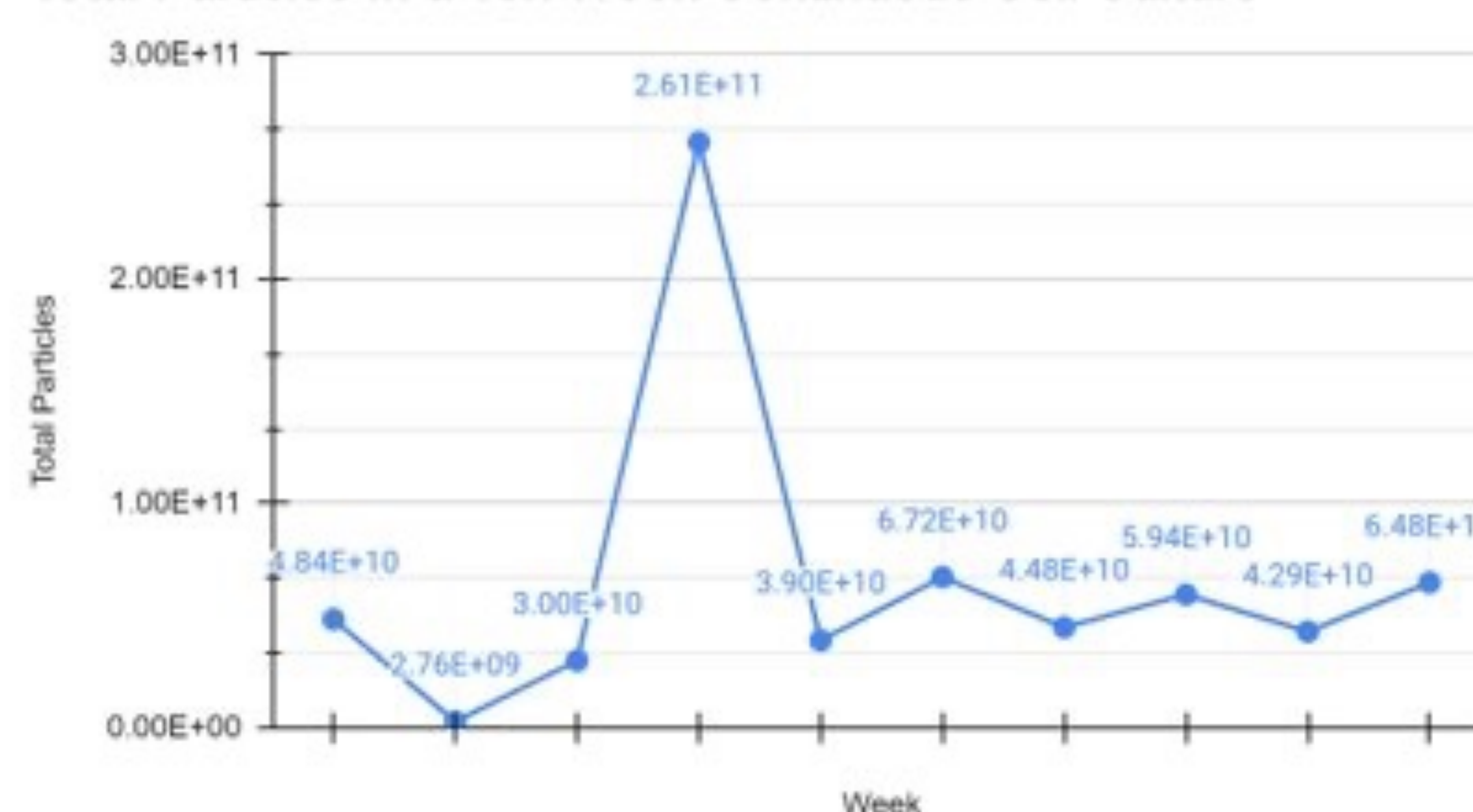
## Results

Figure 2. Western-Blot: 1-CD-63, 2-CD9, 3-Alix, 4-TSG101, 5-Calnexin (-)



- CryoEM showed EVs with morphologies mirroring those found in 2D cell culture
- NTA results showed total particle number ranging from 1.03E9-1.31E11 with an initial increase in concentration from week 1-3 and a decrease thereafter.
- The size of EVs ranged from 102.2-184 nm all presenting a negative charged phenotype.
- Western-blot revealed protein expression of EV biomarkers CD9, CD63, Alix, and TSG101.

Total Particles In a Ten Week Continuous Cell Culture



NTA Size

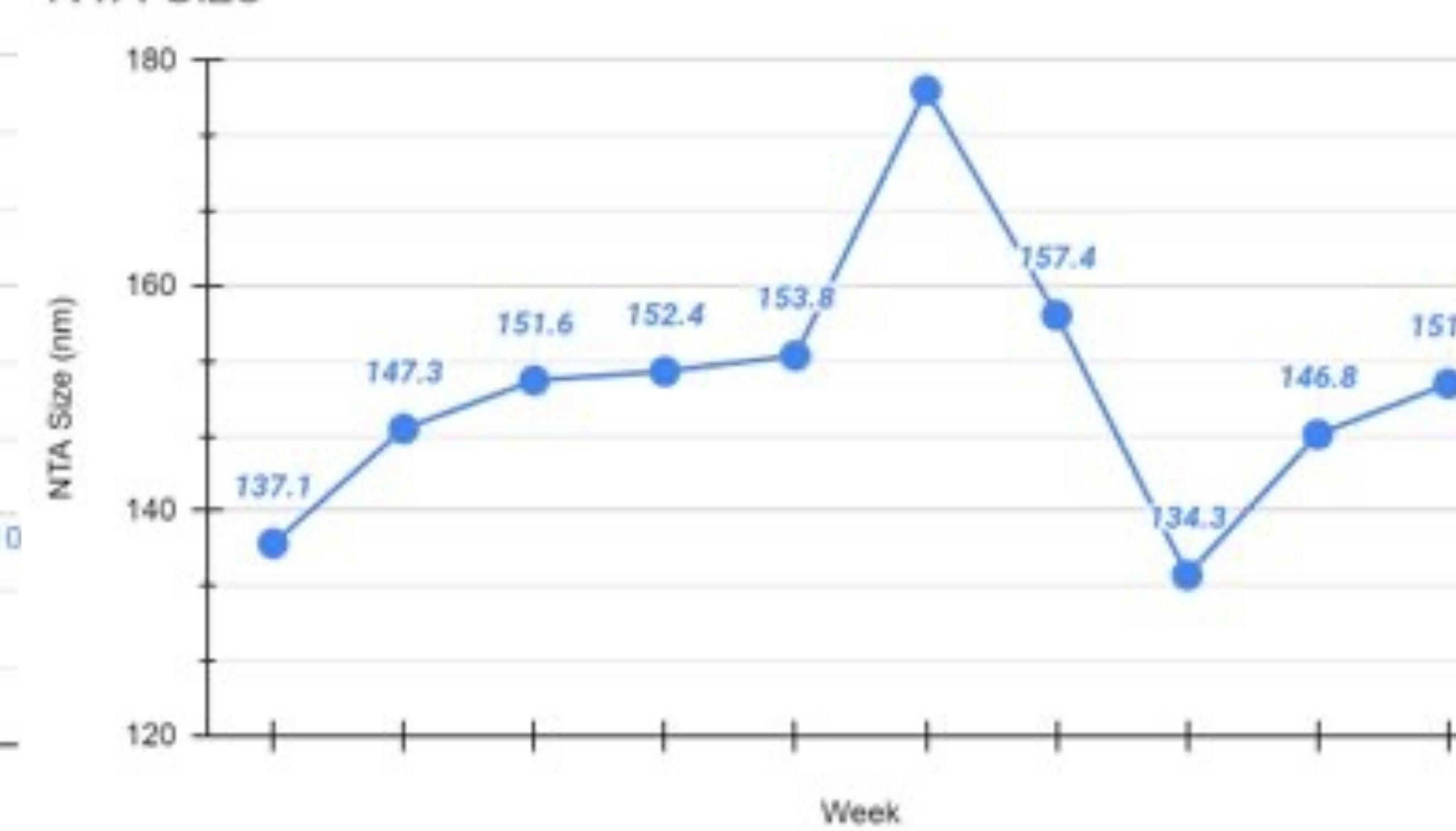


Figure 3. NTA results showing total particle count and size distribution of PMSC-EVs

## Conclusions

- The CELLLine bioreactor represents a promising new approach for large scale PMSC-EV production
- EV concentrations and size distribution shows the improvement and convenience of EV isolation from concentrated 3D culture conditioned medium.
- When cultured over an extended time, the presence of EV protein markers and morphologies of EVs remains consistent with EVs found in conventional culture methods.
- The CELLLine bioreactor design has some limitations. Since the scaffold is encased in a compartment it is difficult to get a total cell count and evaluate the general health of the cells seeded on the scaffold.

## Next Steps

- Monitor cell behavior and status on the 3D matrix by measuring the cell metabolomic activities.
- Conduct proteomics and RNA seq analyses of PMSC-EVs to further characterize PMSC-EVs protein profile and molecular cargo.
- Characterize PMSC-EV's neuroprotective function using established protocols to validate its therapeutic potency in vitro.
- To further increase the yield of EV isolation, we plan to use new isolation methods, such as tangential flow filtration and size exclusion chromatography as alternative isolation methods than ultracentrifugation