Establishing an Extracellular Vesicle Isolation Process - From Stirred 3D-Stem Cell Cultures to Efficient Isolation

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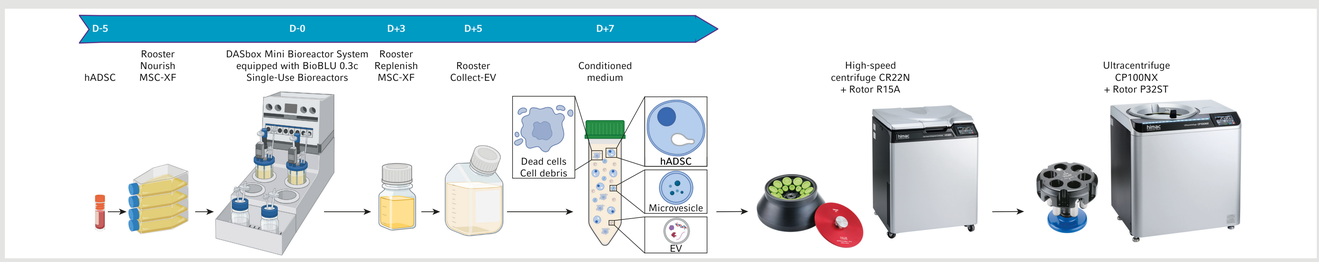
Abstract

Extracellular vesicles (EV) are secreted by different cells of the body and considered important players in cell-to-cell communication. Their biological functions stem from the ability to transfer cargo molecules, including membrane and cytosolic proteins, lipids, nucleic acids, and metabolites. EVs like exosomes, and more specifically stem cell-derived exosomes, are of great interest due to their potential role as cell-free diagnostic and therapeutic agents in various diseases models, including skin, nervous system, heart, liver, and kidney. Stirred-tank bioreactors are a powerful tool to ensure robust, reproducible, and scalable cell culture processes to meet the increasing demand of stem cells for cell and gene therapy applications. Here, we described the fast and easy isolation workflow for EVs from human adipose-derived stem cells (hADSC) by combinining the parameter control of a bioreactor with the performance of high-speed and ultracentrifuges. The cells were first cultured in the DASbox® Mini Bioreactor System and the secreted EVs were then separated by a combination of the high-speed centrifuge CR22N and the ultracentrifuge CP100NX. With this approach, we were able to achieve high quantities of pure, intact extracellular vesicles.

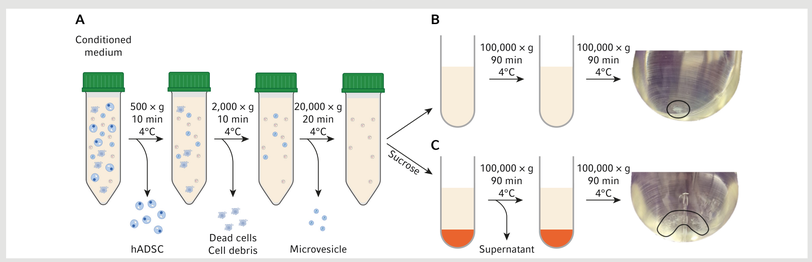
Material and Methods

Extracellular vesicle production and isolation workflow



Expansion of human adipose-derived stem cells (hADSC) for EV production was performed in the DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Bioreactors. After 7 days of incubation, EV isolation was carried out by a combination of different centrifugation steps using the high-speed centrifuge CR22N with R15A fixed-angle rotor as well as ultracentrifuge CP100NX with the swinging bucket rotor P32ST. Created with BioRender.com

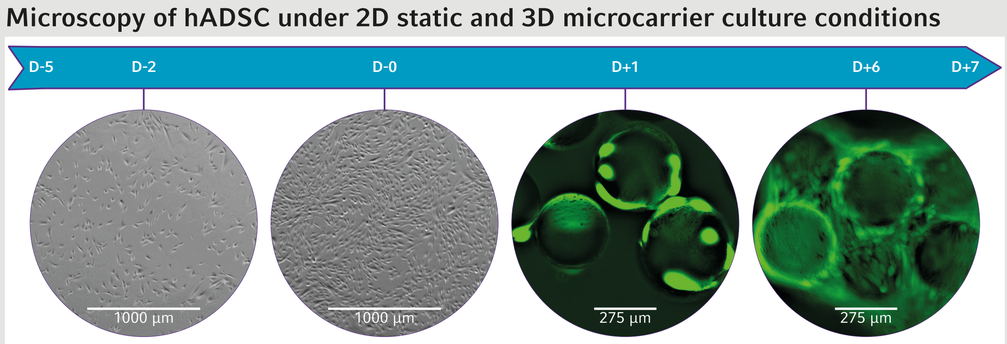
Extracellular vesicle isolation process in detail



(A) 500, 2000, and 20,000 × g centrifugation steps were performed in the high-speed centrifuge CR22N to remove cells, cell debris, and microvesicles. Hereafter, EVs were isolated by two 100,000 × g centrifugation steps in the ultracentrifuge CP100NX, either **(B)** with or **(C)** without a sucrose cushion. Created with BioRender.com

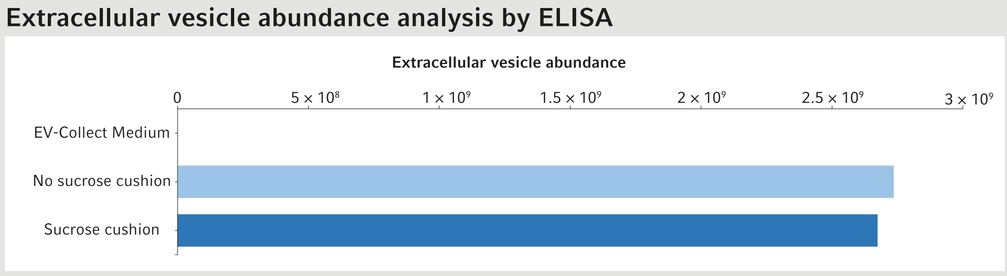
Results

Microscopy of hADSC under 2D static and 3D microcarrier culture conditions



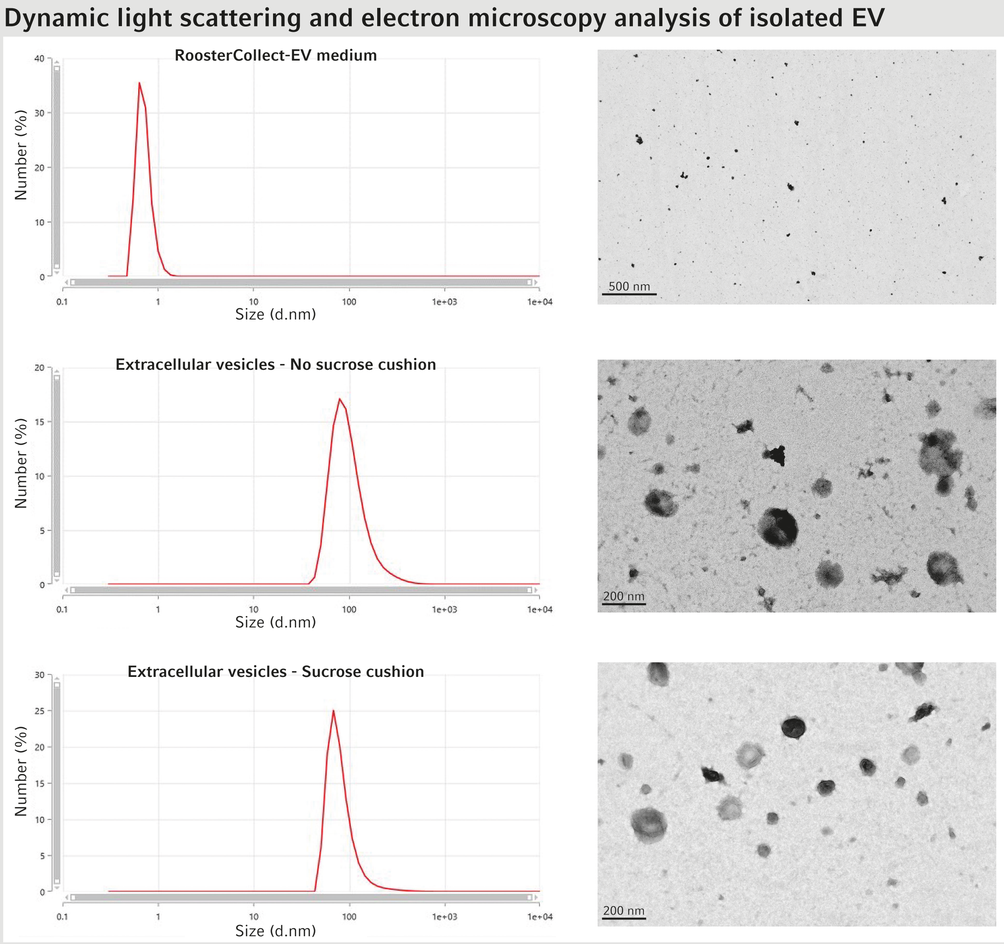
> Initial 5-day 2D cell expansion period in T75 flasks (D-2/D0)
> Subsequent hADSC trypsinization (5.3 × 10^5 cells/mL, viability: 96.7%), seeding on Synthemax II low-density microcarriers (Corning®) (cell-to-bead ratio of 3.7 cells/bead), and transfer to BioBLU 0.3c Single-Use Bioreactors
> During the 3D bioreactor incubation period under control of the DASbox Mini Bioreactor System, cells showed efficient microcarrier attachment (D+1/D+6) and proliferation (D+6)

Extracellular vesicle abundance analysis by ELISA



> Efficient EV yields by using the CR22N and CP100NX centrifuges
> Achieved EV abundance of more than 2.5 × 10^9

Dynamic light scattering and electron microscopy analysis of isolated EV



> Dynamic light scattering (left) and electron microscopy (right) analysis of EV fractions after ultracentrifugation

> Top: Clean medium control (RoosterCollect-EV by RoosterBio, Inc.)
Middle: Without sucrose cushion
Bottom: With sucrose cushion

> Extracellular vesicle size distribution more homogeneous after isolation with sucrose cushion

Conclusion

The DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Bioreactors offers important advantages to optimize the upstream process of extracellular vesicle production workflow. It allows the precise control of critical process parameters, such as temperature, pH, DO, and gas flow. Additionally, with its possibility to control 4 bioreactors in parallel with working volumes of 100 to 250 mL, it provides an optimal starting point for EV culture process development. Furthermore, ultracentrifugation, known as the gold standard, is a good choice for the downstream isolation process with its ability to separate the EVs from a large quantity of biomaterials at relatively low cost, and without additional potentially contaminating chemical reagents. Nevertheless, ultracentrifugation also has disadvantages which are labor intensity because of repetitive centrifugation steps of large volume of medium, dependence of separation efficiency on the rotor type (fixed-angle or swing-bucket) and its specific capacity. Another significant disadvantage is also the presence of non-EV impurities in the EV fraction. To increase their purity and integrity, EVs are most often isolated in a sucrose gradient (2.0–0.25 M) and centrifuged at 210,000 x g for 16 hours. In order to improve the protocol and reduce time, the sucrose cushion technique was used in this application note. This approach led to a reduction in ultracentrifugation time to 90 min and an EV abundance of more than 2.5 × 10^9.
Therefore, the present work illustrates the DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Bioreactors coupled with the Eppendorf high-speed centrifuge CR22N and ultracentrifuge CP100NX as a viable combination for the successful isolation of an intact and homogeneous EV population derived from hADSC. Indeed, using the high-speed centrifuge CR22N with the R15A fixed-angle rotor allowed a maximum volume of 650 mL to be centrifuged in one run, considering its ability to hold 10 x 15 TC and 10 x 50 TC conical tubes at once. This unique feature allows the clearance of cells, cell debris, and microvesicles from 650 mL medium in less than 1 hour, which saves time and work during repetitive medium centrifugation steps. The ultracentrifuge CP100NX and the swinging bucket Rotor P32ST allow spinning a total volume of 240 mL, as it can hold 6x40 mL at once. In this application note, each step required only one centrifuge run for the content of two BioBLU 0.3c Single-Use Bioreactors in the high-speed Centrifuge CR22N and two runs to concentrate the EVs with ultracentrifuge CP100NX. EV isolation time was therefore reduced to 4 hours in total, making the combination of these tools a valuable time-saver.