Serum-free and GMP compliant culture of MSCs using the CliniMACS Prodigy® for MSC-derived extracellular vesicle production

Poster ID: 314

Kathrin Godthardt, Claudia Schreiner, Ute Heider, Andreas Bosio, Stefan Wild and Sebastian Knöbel
Miltenyi Biotec B.V. & Co. KG, Bergisch Gladbach, Germany

Scan the QR code to download the poster (PDF):

Introduction

Human mesenchymal stem cells (MSCs) hold great promise for therapeutic applications. Within the last decade, MSC-derived extracellular vesicles (EVs), which include exosomes and microvesicles (MVs), are being examined for their role in MSC-based cellular therapy, as well as for their own therapeutic potential. EVs are involved in cell-to-cell communication, cell signaling, and altering cell or tissue metabolism at short or long distances in the body. EVs can also influence tissue responses to injury, infection, and other diseases. EVs comprise cytokines and growth factors, signaling lipids, mRNAs, and regulatory miRNAs, as well as present specific surface markers. As for the originating cells, a reproducible production process is important to ensure the quality of MSC-EVs.
To increase the level of process standardization and product safety, we developed an integrated workflow for MSC expansion and harvest of MSC-derived EVs on the CliniMACS Prodigy® cell processing platform.

Method

1 Isolation and expansion of bone marrow derived MSCs followed by MSC-EV production using the CliniMACS Prodigy

The CliniMACS Prodigy® provides a range of ports for connecting bags containing buffer, media, reagents, and cellular material. Various tubing sets allow for a multitude of applications. For this cultivation process, we chose the tubing set CliniMACS Prodigy TS 730, which provides up to eight connections for bags. This setup also offers the option to pre-warm solutions during transfer from an external 4 °C storage compartment to the cultivation and centrifugation unit (CCU) as well as external tissue culture vessels (ECVs) which are connected to the tubing set and are placed in an incubator next to the CliniMACS Prodigy (fig. 1A). Bags can be connected in a sterile manner prior to the installation procedure or later via sterile welding. Cellular starting material (bone marrow aspirate) is provided in the application bag (bag 1) of the tubing set. Density gradient centrifugation (DGC) of bone marrow aspirate is performed automatically by the system. After tissue preparation all following steps are performed semiautomatically by the CliniMACS Prodigy Adherent Cell Culture System, including the initial expansion step using ECVs as well as all liquid handling steps, i.e., inoculation, washing of cells and medium exchange.
To produce EVs, MSCs were washed with CliniMACS® PBS/EDTA Buffer when reached 80% confluency and EV free, adapted MSC-Brew GMP medium, including MSC-Brew GMP Basal Medium + MSC-Brew Supplement I was added. MSCs were further cultivated at 37°C and 5% CO_2 for up to five days (fig. 1B). EV rich supernatant was harvested. For comparison, the respective process was performed manually based on a small scale T75 flask.

Figure 1 A

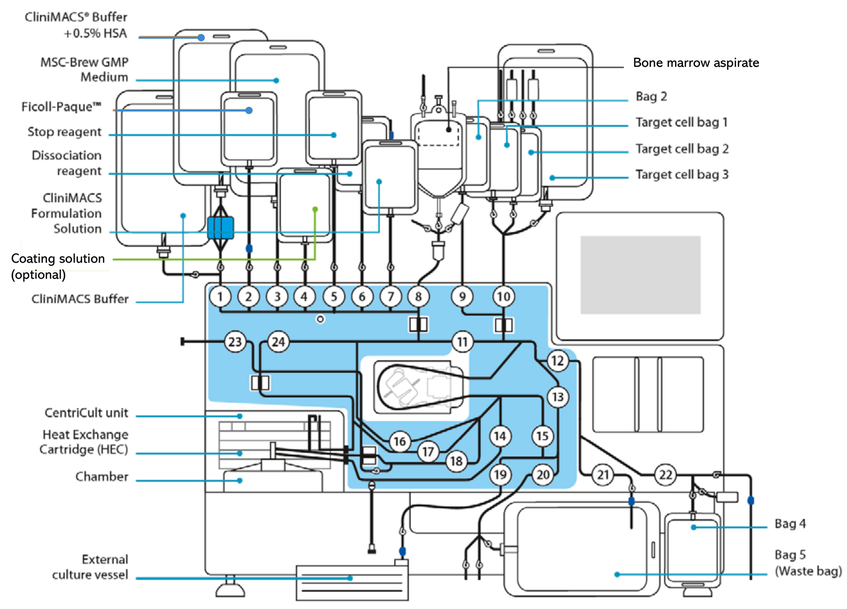
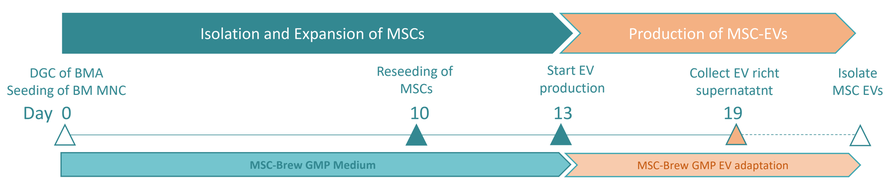


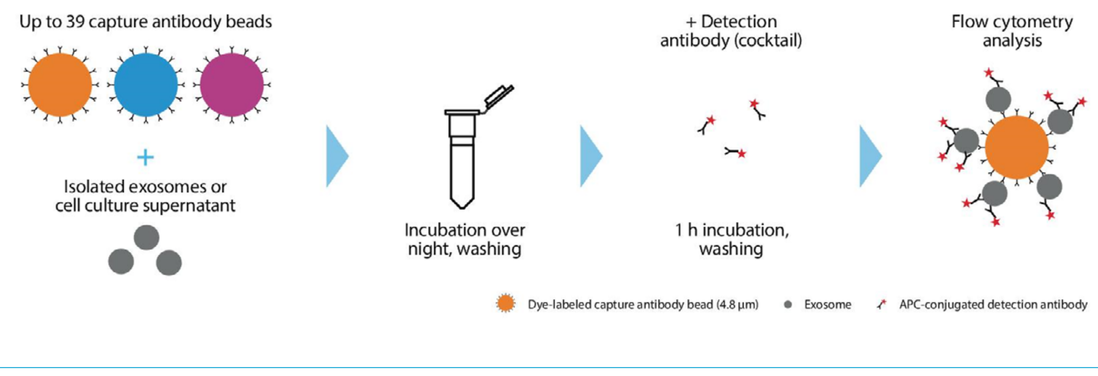
Figure 1 B



2 Characterization of MSC-EVs using multiplex bead–based assay

MSC-EVs were purified via ultracentrifugation. Purified MSC-EVs were analyzed for diameter and quantity using a myriad videodrop analysis system. To characterize surface protein composition, a prototype MACSPlex EV Kit MSC was used. 31 color-coded polystyrene beads were incubated with isolated MSC-EVs in 100 µL buffer (PBS, 0.1% HSA, 0.03% Pluronic® F127, 0.05% azide) at room temperature overnight. Beads were washed in buffer and centrifuged at 3,000×g for 5 min. The beads were resuspended in buffer and bound EVs were stained with 0.5 µg CD9-, CD63- and CD81-APC conjugates (fig. 2 A and B).

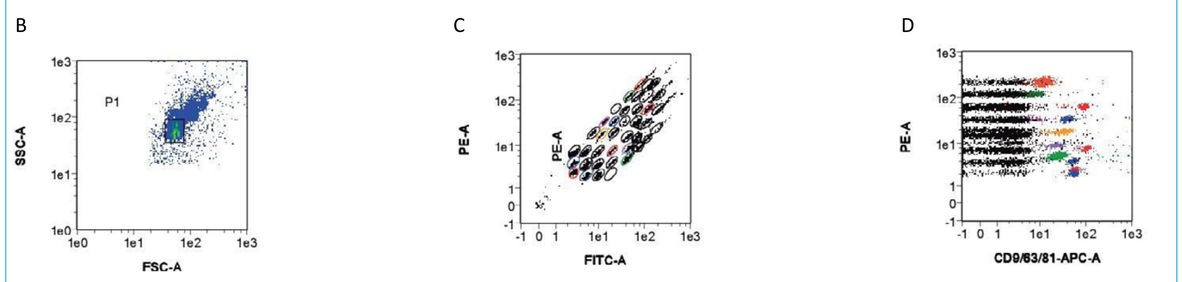
Figure 2 A



Workflow of the multiplex bead platform: Isolated exosomes were incubated overnight with 31 differently labeled beads each coupled to a different capture antibody. Bound exosomes were detected with an APC-conjugated antibody cocktail (fig 2 A).

Analysis example showing (B) gating according to bead size, (C) discrimination of differently labeled bead populations, and (D) measurement of signal intensities of the single bead populations (fig. 2B).

Figure 2 B

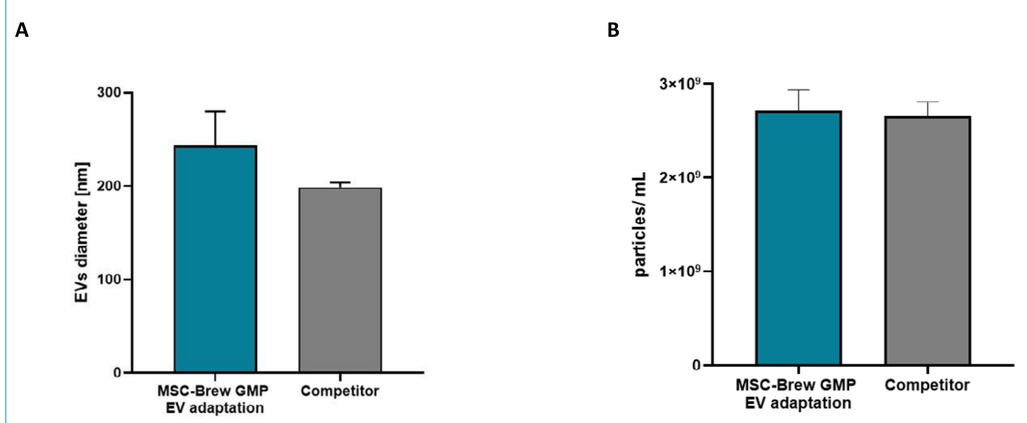


Results

1 MSC-EV diameter and yield

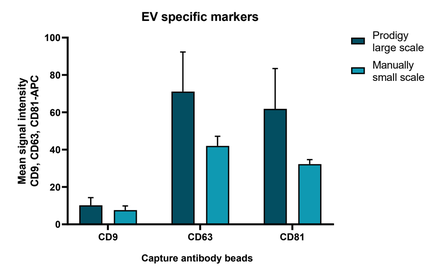
MSC-EVs showed comparable diameter (fig. 3 A) as well as yield per mL (fig. 3 B) using MSC-Brew GMP EV adaptation medium or a competitor medium for EV production.

Figure 3



2 MSC-EVs show standard ISCT markers

Figure 4 A



Characterization of MSC-EVs via multiplex bead-based assay using the prototype of MACSPlex EV Kit MSC enables fast and reliable screening of up to 31 markers within just one sample. MSC EV from all conditions show high expression of EV specific markers CD9, CD63 and CD81 (fig 4A).

As expected, the MSC specific markers CD73, CD90 and CD105 were detected while CD14, CD19, CD34, CD45 are lagged as recommended by the ISCT guidelines for MSCs. It has to be mentioned that the CD90 signal is quite low on EVs, whereas the corresponding marker expressed on MSCs is detected (fig 4B). A number of additional markers show comparable results for small scale as well as for large scale MSC EV production. The MSC-Brew GMP EV adapted medium used for all conditions is free of contaminating platelet EVs as shown by low signal for platelet specific markers CD41b, CD42a and CD62P in the same range as the negative controls (fig. 4 C).

Figure 4 B

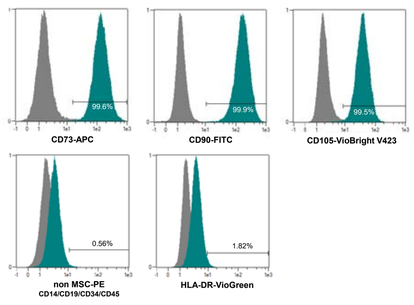
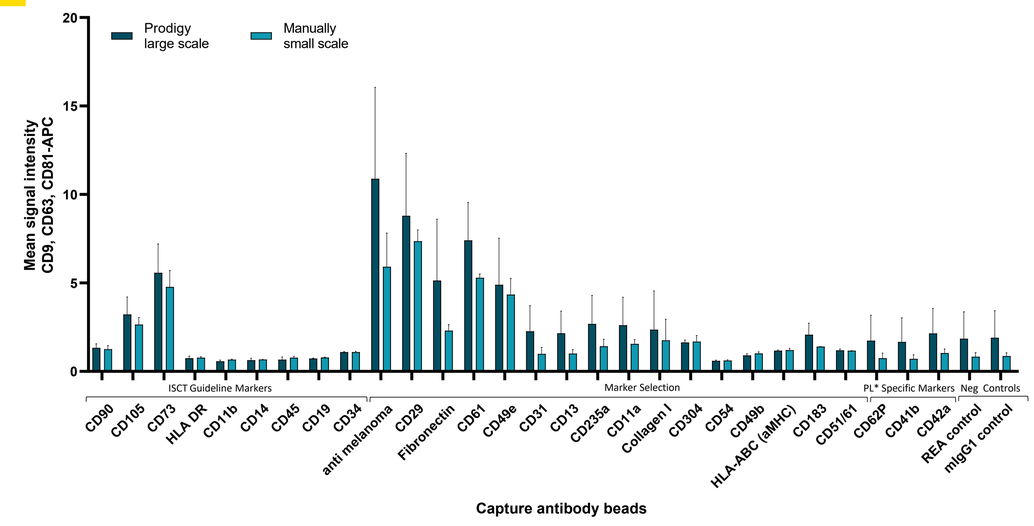


Figure 4 C



Conclusion

- MSCs were successfully isolated and expanded using the Prodigy Adherent Cell Culture System and the xeno- and serum-free MSC-Brew GMP Medium.
- MSC-EVS can successfully be produced using an adapted MSC-Brew GMP Medium consisting of MSC-Brew GMP Basal Medium plus MSC-Brew GMP Supplement I
- The prototype of MACSPlex EV Kit MSC is a fast and reliable way to analyzes up to 37 markers to characterize the surface protein composition of MSC specific extracellular vesicles using flow cytometry:
- Confirmation of positive as well as negative markers for MSCs recommended by the ISCT guidelines.
- Control for contamination of platelet exosomes.
- Further markers including MHC molecules, integrins and extracellular matrix proteins can be used to profile EVs from different production protocols or batches.