Characterization of microvesicles using the MACSQuant® Analyzer

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Background

Microvesicles (MVs) are small (0.1–1 µm) membrane vesicles shed from activated cells, delimited by a lipid bilayer and containing a wide range of membrane-bound or free proteins and nucleic acids (in particular mRNA and miRNA). MVs carry surface molecules that are typical for the cell that releases them. Recent studies suggest MVs as important vehicles of intercellular communication.

Materials and methods

Microvesicles have been isolated from blood samples within four hours after blood drawing.

Materials

- Centrifuge
- Ultracentrifuge
- MACSQuant Analyzer
- 0.10 µm pore size membrane-filtered PBS (e.g., using Stericup®-VP, 0.10 µm, polyethersulfone filter, Merck Millipore)
- 5(6)-carboxyfluorescein diacetate N-succinimidyl ester (CFSE)
- Fluoresbrite® Carboxylate Size Range Kit I (Polysciences, Inc.)
- CD61-PE, human

Methods

Preparation of microvesicles from blood
1. Draw blood into 7.5 mL EDTA tubes.
2. Separate plasma by centrifugation at 1,100×g for 15 minutes at room temperature.
3. Remove cell debris from plasma by serial centrifugation at 1,000, 2,000, and 3,000×g for 15 minutes at 4 °C.
4. Transfer 1.5 mL of plasma into a ultracentrifuge tube and fill up with 0.10 µm pore size membrane-filtered PBS.
5. Ultracentrifuge sample at 110,000×g for 75 minutes at 4 °C.
6. Resuspend the ultracentrifuged pellet with 500 µL triple 0.10 µm pore size membrane-filtered PBS.
7. Transmission electron microscopy (TEM) was used to check the morphology of microvesicles and aggregates.
8. 100 µL of unstained sample were used for NanoSight analysis to check for aggregates and the size and concentration of microvesicles.

Staining of sample
9. 60 µL of sample and 60 µL of triple 0.10 µm pore size membrane-filtered PBS (control sample) were stained with 0.02 µM CFSE at 37 °C for 20 minutes in the dark.
10. The CFSE stained sample and the control sample were incubated with 6 µL of CD61-PE antibody in the dark for 20 minutes at 4 °C.
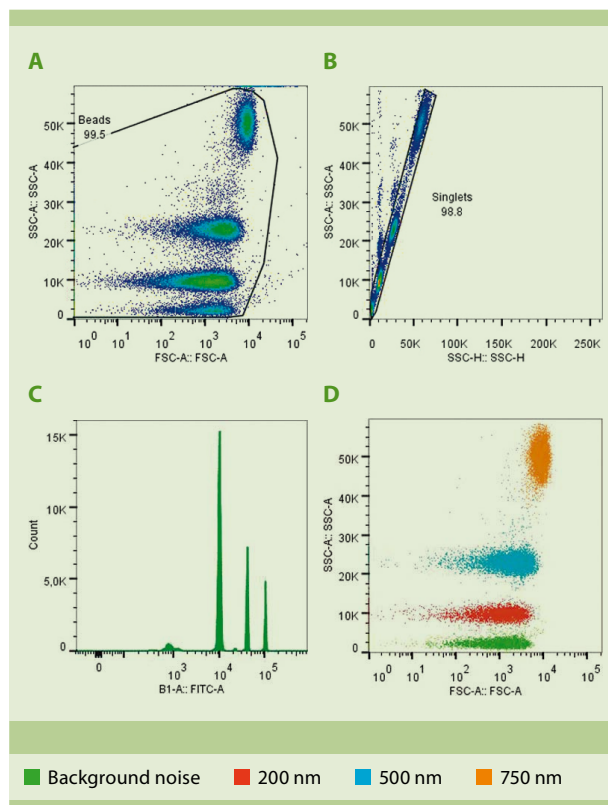
    Note: Before use the antibody was centrifuged at 17,000×g for 30 minutes at 4 °C to eliminate aggregates.

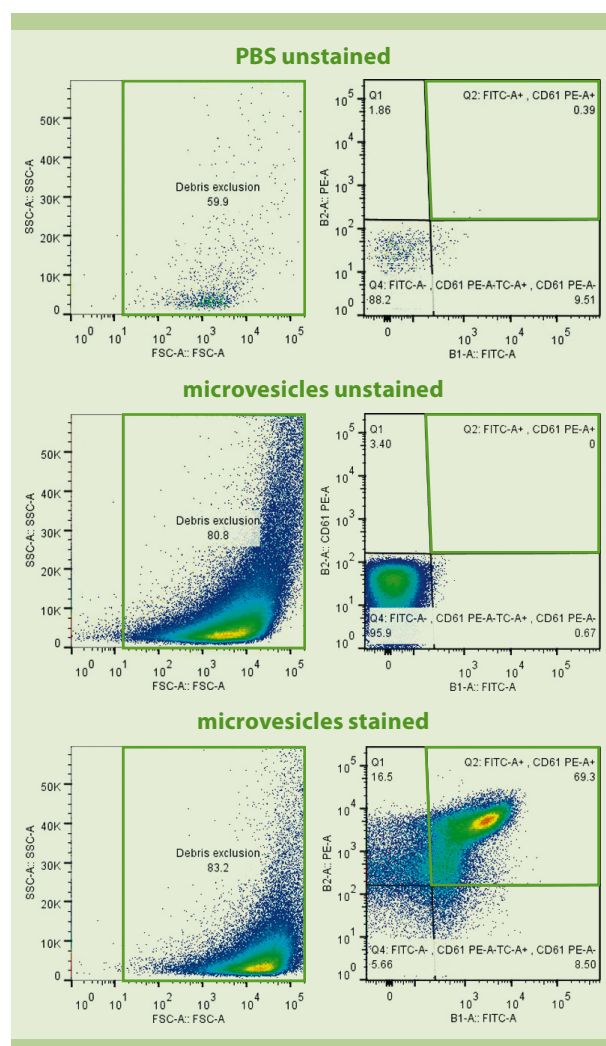**Data acquisition and analyzes using the MACSQuant Analyzer**

Note: Sheath fluid was filtered using 0.1 µm pore size filter to further improve the signal-to-noise ratio.

11. The emission spectra of FITC and PE were compensated to correct the spectral overlap.
12. Unstained triple 0.10 µm pore size membrane-filtered PBS was acquired to evaluate the buffer background noise.
13. The stained PBS control sample was acquired to detect the autofluorescence of the antibody.
14. 30 µL of unstained sample were acquired to detect the sample auto-fluorescence.
15. The Fluoresbrite® Carboxylate Size Range Kit I (0.2, 0.5, 0.75, and 1 µm) was used to set the calibration gate in the FSC/FL1 and FSC/SSC dot plots on MACQSQuant Analyzer.

16. 30 µL of double stained sample were acquired on the MACSQuant Analyzer.

17. Quantitative multiparameter analysis of flow cytometry data was carried out using FlowJo Software (Tree Star, Inc.) to determine the percentage and count of double stained microvesicles (CFSE and PE positive events).

Figure 1: Scatter and fluorescence calibration: Fluoresbrite Carboxylate Size Range Kit was used to obtain optimal resolution of scatter and FITC signals. A) Debris exclusion, B) Doublet exclusion, C) Histogram showing resolution of the different bead populations according to FITC fluorescence, D) Resolution of the different bead populations according to FSC and SSC signals.

Figure 2: Gating strategy and gating controls: Panel A: scatter (1) and fluorescence (2) dot plots for "background noise" control (PBS only). Panel B: scatter (1) and fluorescence (2) dot plots for gating control (MVs only). Panel C: scatter (1) and fluorescence (2) dot plots for experimental sample (MVs + CFSE + CD61 PE antibody).

Conclusion

Characterization and quantification (MVs/µL) of different sub-populations of microvesicles from blood plasma samples can be accomplished using the MACSQuant Analyzer.

Visit **macsquant.com** for more information on the MACSQuant Instruments.

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