Supporting information 5: Volume fraction calculations of "Removal of platelets from blood plasma to improve the quality of extracellular vesicle research"

The aim of Figures 1E and 1F in the manuscript is to show that a low concentration of particles that are relatively large compared to extracellular vesicles (EVs), such as platelets that remain after the preparation of human blood plasma, may have the same volume fraction as EVs. The aim of Figures 1E and 1F is not to make any claims on the range of the concentration of EVs and platelets in plasma. To estimate the total concentration and volume fraction of EVs and platelets, we used estimates of the size distribution of EVs and platelets in plasma based on our own data and literature values.

The size distribution of EVs is based on flow cytometry (A60-Micro, Apogee Flow Systems, UK) measurements of plasma obtained from 23 healthy volunteers from the published study "Antiplatelet therapy eFFECT on Extracellular Vesicles (AFFECT EV) [1]. The reported concentrations describe the number of particles (1) that exceeded the side scatter threshold, corresponding to a side scattering cross section of 10 nm^2, and (2) express more than 590 molecules of equivalent soluble fluorescein (FITC) of Lactadherin-FITC per mL of plasma. Lactadherin binds to phosphatidylserine and was the most specific and sensitive generic marker for EVs in plasma within the detection range of our flow cytometer [2,3]. The size distribution was obtained by Rosetta Calibration v1.11 (Exometry, The Netherlands) by relating the side scattered light signals to the diameter of EVs, assuming that EVs have a shell refractive index of 1.48, a shell thickness of 4 nm, and a core refractive index of 1.38. Size distributions were obtained by binning the data into 10-nm bins and calculating the mean concentration per bin for the 23 healthy volunteers. All details about sample preparation, assay controls, instrument calibration, data acquisition, and EV characterization are published [1], including a completed MIFlowCyt-EV template.

Figure S5.1A shows the size distribution of EVs in plasma measured by flow cytometry. To anticipate for the lower detection limit of our flow cytometer, we fitted the size distribution with a log-normal distribution. The resulting size distribution is in agreement with size distributions of EVs in plasma measured with cryo-electron microscopy [4]. Whether the resulting total concentration of $3 \cdot 10^9$ EVs mL^{-1} is reasonable remains subject to future research, especially because current literature values differ orders of magnitude.

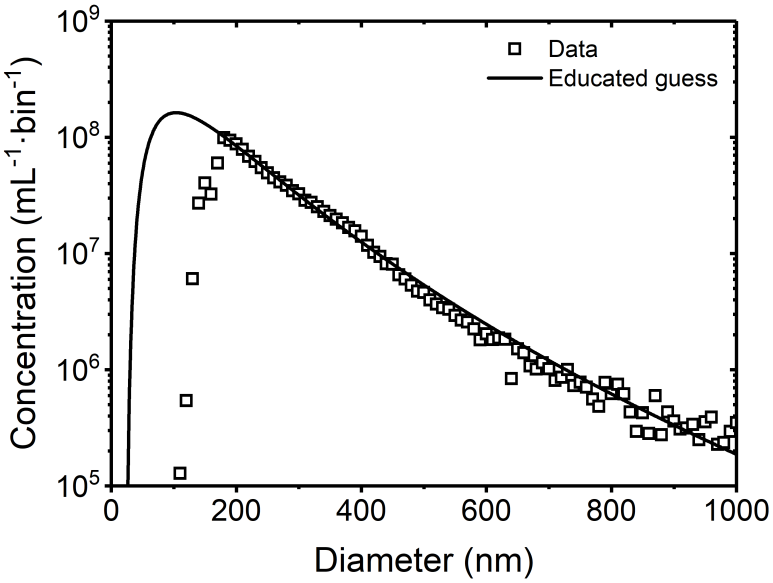

Figure S5.1: Educated guess of the size distribution of extracellular vesicles in human blood plasma of 23 healthy volunteers. The bin width is 10 nm. Data are obtained by calibrated flow cytometry, as explained in the text. The educated guess is a log-normal distribution that is fitted to the data ($y_0 = 0$, $x_c = 130.7$ nm, $w = 0.669$, $A = 2.85 \cdot 10^9$ mL^{-1}).

The total concentration of EVs in Figure 1E in the manuscript is thus $3 \cdot 10^9$ EVs mL^{-1}. The error bars span exactly 1 order of magnitude to emphasize that the displayed concentration is an estimate. The lower and upper error bar of the total concentration of platelets represents the median of the measured platelet concentrations in study C and A, respectively. The height of the bar represents the apparent mean, thus the mean of the log-transformed values, of the median platelet concentrations obtained in study A and C.

To calculate the volume fraction of all EVs in plasma in Figure 1F in the manuscript, the volume of an EV is calculated and multiplied with the concentration for each bin of the size distribution shown in Figure S1.1A. Next, the volume fractions of all bins are summed, expressed in units of mL^{-1}, and divided by 1 mL to get a dimensionless number. To calculate the volume fraction of remaining platelets in plasma, the platelet concentration is multiplied with the volume of a platelet, which is assumed to be 11 fL. The total volume fraction of platelets is expressed in units of mL^{-1} and divided by 1 mL to get a dimensionless number. Error bars are propagated from Figure 1E in the manuscript.

References

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