determined by nanoparticle tracking analysis (NTA) and the bicinchoninic acid assay, respectively. LDL and albumin were measured using Clinical Chemistry Analyser Architect C8000 by photometric determination method and immunoturbidimetry assay, respectively . In addition, fractions were investigated by imaging flow cytometry (IFCM) and by western blot, which we also probed for the lipoprotein ApoB.

Results: According to our analysis, 35 nm qEV columns provided higher EV yields but did not separate plasma-derived EVs and ApoB as efficiently as the 70 nm qEV columns. Noteworthy, both columns failed to efficiently separate EVs and ApoB from serum samples. Furthermore, our data confirm our published findings that NTA detects far more particles than just EVs and that in its traditional form NTA should not be considered as an appropriate method for the reliable evaluation of given EV preparation protocols.

Summary/Conclusion: To conclude, 35 nm qEV column have an increased EV yield compared to 70 nm for both plasma and serum. However, both columns were not able to separate EVs and LDL for sera samples. Moreover, our data further confirm our recent publication that NTA is insufficient for EV characterization as its measures other particles in addition to EVs.

PF01.08 | Heat inactivation of fetal bovine serum increases protein contamination of extracellular vesicles

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Introduction: Extracellular vesicles (EVs) released in cell cultures are influenced by the cell culture conditions, such as the use of fetal bovine serum (FBS). FBS contains EVs and it is usually depleted of EVs by ultracentrifugation (UC) and/or heat inactivated (HI). Several studies have evaluated the effect of different UC protocols for FBS by evaluating both cells and EVs. However, less is known about the effect of HI on the cells and the released EVs. The aim of this study was therefore to evaluate the effect of HI on EV purity.

Methods: To determine the effect of heat inactivation, three different protocols were applied based on different combinations of: 1) UC at 118,000 × g for 18h and 2) HI at 56°C for 30 min. The three conditions tested were: FBS ultracentrifuged but not heat inactivated (no-HI), FBS heat inactivated before UC (HI-before EV-dep), and FBS heat inactivated after EV depletion (HI-after EV-dep). The FBS was add to the media of three melanoma cell lines (MML1, UM22Ctr and UM22BAP1) at a final concentration of 10%. After 72h, large and small EVs were isolated by differential UC. The EV purity was determined by protein quantity, electron microscopy (EM) and nanoparticle tracking analysis (NTA).

Results: The protein quantity ($\mu g/\mu l$) of large EVs was similar in the three conditions analyzed. On the contrary for small EVs, the protein amount was higher when the HI was performed after EV depletion as compared to HI before the UC and UC alone. However, significantly more particles were not detected in the HI-after EV-dep which resulted in a lower purity of small EVs in HI-after EV-dep illustrated by calculating the ratio of number of particles/μg proteins. Presence of contaminants (indicated by strong background) was observed in EM pictures of small EVs isolated in HI-after EV-dep condition differently from large EV samples.

Summary/Conclusion: The HI of FBS induces release of contaminating elements that end up in small EV pellets if not previously removed.

PF01.09 | Hydrophobic effects in membrane sensing peptide for small extracellular vesicle isolation and analysis

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