Lipoprotein particles can be detected by high-resolution flow cytometry and potentially interfere with EV characterization

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Results: A 10-fold increase in laser power increased the SI on SSC 2.9-fold and on FSC 20-fold, whereas the rCV improved (reduced 0.67-fold and 0.97-fold, respectively). The improved confocal detection increased the SI on SSC 6.4-fold and on FSC 550-fold, while the rCV slightly worsened (increased 1.1-fold and 1.02-fold, respectively). Combining both increased laser power and confocal detection resulted in a 20-fold increase in SI for SSC and 2 · 10^4-fold for FSC, and improved the rCV (reduced 0.39-fold and 0.24-fold, respectively).
Summary/Conclusion: Adaption of the optical configuration of the FACSCanto by increasing the laser power and confocal detection improved the scatter sensitivity 20-fold for SSC and 2 · 10^4-fold for FSC. Next, we will evaluate the influence of increased measurement time and reduction of the number of particles in the sheath on the scatter sensitivity.
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Lipoprotein particles can be detected by high-resolution flow cytometry and potentially interfere with EV characterisation

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Introduction: Lipoproteins co-isolate with EVs and are potential confounders in EV characterisation. CD36 is a membrane-bound scavenger receptor located on cells and EVs capable of interacting with VLDL and LDL, which could interfere with antibody-based phenotyping. Freezing and thawing samples was shown to increase phosphatidylserine-positive (PS+) EVs while other common phenotype markers were unchanged. This could provide a method for disrupting lipoproteins and EVs. Thus, we aimed to investigate the impact of lipoproteins on EV characterisation and freezing/thawing samples on their dissociation from EVs on a high-resolution flow cytometer (hFCM).
Methods: Plasma from 6 healthy individuals was subjected to either 0, 2, 4 or 6 freeze-thaw (FT) cycles and stained with a cocktail of lactadherin-FITC, anti-CD41-BV510, anti-CD36-PE and anti-ApoB-APC or lactadherin-FITC and matched isotype controls. Samples were analysed on an Apogee A60 Micro-PLUS hFCM. Gating was performed as follows: size gates established on silica reference beads; phenotype gates set on 99th percentile of isotype control channel fluorescence.
Results: hFCM was able to detect both free apolipoprotein B (ApoB) particles and ApoB bound to PS +CD41+, PS+CD36+ and PS+CD41+ CD36+ EV phenotypes. From 0–2 FT cycles, ApoB bound to PS +CD41+ and PS+CD41+ CD36+ phenotypes tended to decrease ($p > 0.05$). Moreover, ApoB bound to PS +CD36+ increased 4.9-fold from 0–2 FT cycles for ($p < 0.05$). Interestingly, this progression mirrored that of PS+CD36+ (2.0–2.5-fold, $p < 0.05$), bulk CD36 + (1.8–2.4-fold, $p < 0.05$) and ApoB+ (4.1–5.0-fold, $p < 0.01$). Finally, in line with previous reports, PS+ tended to increase following FT (1.5-2.1-fold, $p > 0.05$). Contrary to previous reports, certain EV phenotypes decreased from 0–2 FT cycles (PS+CD41+ and PS +CD41+ CD36+, both 2.6-fold, $p < 0.05$) suggesting that EV phenotypes might perish following FT further confirmed on bi-variable plots of data.
Summary/Conclusion: This study demonstrates that ApoB can be detected on hFCM and thereby interfere with EV characterisation. What further complicates matters is that lipoproteins could carry markers traditionally associated with EVs including PS and CD36. FT cycles did not consistently dissociate EVs and lipoproteins; however, FT affected certain EV populations. Further studies are required to elucidate these findings.

PF06.06

Analysis of fluorescent labelling efficiency of extracellular vesicles derived from different kingdoms of life with lipid-binding dyes via nano-flow cytometry

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Introduction: In all domains of life – archaea, bacteria and eukarya, cells produce and release extracellular vesicles (EVs). The double-layered lipid membrane is the most prominent feature of EVs, and fluorescent labelling with lipid-binding dyes has been frequently used to visualize and detect single EVs. For example, most conventional flow cytometers rely on fluorescence threshold triggering for single EV detection upon membrane labelling with lipophilic dyes. However, the labelling efficiency of EVs with these lipid-binding dyes remains unknown. Here, we reported an approach to quantitatively analyse the labelling efficiency of lipid-binding dyes toward EVs by using a laboratory-built nano-flow cytometer (nFCM) that enables light scattering detection of individual EVs as small as 40 nm.
Methods: EVs were extracted from cultured medium of HCT15 cells (colorectal cancer cell line), *E. coli* O157: