PS09.12

High-visibility detection of exosomes by interferometric reflectance imaging

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Background: Optical characterization of exosomes in liquid media has proven extremely difficult due to their very small size and refractive index similarity to the solution. We have developed Interferometric Reflectance Imaging Sensor (IRIS) for multiplexed phenotyping and digital counting of individual exosomes (>50 nm) captured on a microarray-based solid phase chip. These earlier experiments were limited to dry sensor chips. In this work, we present our novel technology in exosome detection and characterization.
Methods: We present advances of IRIS technique to improve the visibility of low-index contrast biological nanoparticles such as exosomes in a highly multiplexed format. IRIS chips are functionalized with probe proteins and exosomes are captured from a complex solution. We have recently demonstrated the integration of pupil function engineering into IRIS technique. By tailoring the illumination and collection paths through physical aperture masks we achieved significant contrast enhancement. For in-liquid detection of exosomes, we have also developed disposable cartridges amenable to high quality optical imaging. Furthermore, we have refined the acquisition and analysis of IRIS images to enable accurate size determination of exosomes.
Results: We have shown that IRIS can enumerate, estimate particle size and phenotype exosomes from purified samples from cell culture, or directly from a small of volume clinical sample. We have conducted preliminary experiments utilizing silica nanoparticles. The results demonstrated a nearly 10-fold signal enhancement for 50 nm silica nanoparticles. Given that the nanoparticle signal in an interferometric measurement scales with particle polarizability, and hence particle volume, we expect to be able to detect low-index nanoparticles down to 30 nm with better than 1% contrast. In liquid exosome detection and characterization experiments are currently ongoing.
Summary/Conclusion: IRIS technique represents a unique capability to count and characterize individual exosomes directly captured from a complex solution in a multiplexed format. With this unprecedented capability, we foresee revolutionary implications in the clinical field with improvements in diagnosis and stratification of patients affected by different disorders.
Funding: This study was funded by EU Horizon 2020 programme under grant agreement No 766466.

PS09.13

Small-particle flow cytometry: a new frontier in detection and characterization of extracellular vesicles in liquid biopsies

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Background: Flow cytometry has been a widely used method for characterization of extracellular vesicles (EVs). However, the applicability of flow cytometry has been somewhat limited due to the inability of conventional flow cytometers (FCM) to detect smaller EVs and discriminate between single events and so-called swarms of EVs. To overcome these issues, recent advances in flow cytometry have led to the development of FCMs dedicated to the analysis of small particles (spFCM). Thus, the aim of this study is to benchmark a novel FCM platform against a conventional FCM with regard to sensitivity, resolution and reproducibility in characterizing EVs directly in plasma.
Methods: Flow cytometry is performed on FACSAria III high-speed cell sorter (BD) and Apogee A60 Micro-PLUS (Apogee Flow Systems) platforms. Sensitivity and resolution are assessed using 100 nm fluorescent silica beads and a cocktail of non-fluorescent silica beads ranging from 180 to 1300 nm respectively. Reproducibility of concentration determinations and fluorescence signals are assessed by measuring platelet-poor plasma (PPP) from a pool of healthy donors both in a single day (n = 20) and spread out over a whole week (n = 4 × 5). PPP is labelled with lactadherin-FITC, anti-CD41-APC and anti-CD36-PE. EVs are defined as phosphatidylserine-exposing (PS+) events ≤1000 nm.
Results: Initial results demonstrate that spFCM is able to measure EVs down to 100 nm. We additionally demonstrated that the bulk of EVs detected with spFCM are within the 100–300 nm range, which is in accordance with observations from previous studies. Additionally, concentration determination of EVs on spFCM was reproducible (CV = 3.68–7.32%), as was median positive channel fluorescence (MPCF) of EV phenotypes (CV = 1.44–6.63%). However, experiments are currently still ongoing and final results pending.
Summary/Conclusion: Although spFCM has been around for several years, few research groups have access to this platform due to its expensive and specialized nature. Thus, little is known about its applicability in the field of EV research, and to the authors' knowledge, this study is the first to provide a direct benchmark against a more commonly used conventional FCM.

PS09.14 = OWP2.01

Isolation and phenotype characterization of microvesicle subpopulations from mixed cells in an *in vitro* model of lung microvascular injury

PS09.15

Nanoarray for single exosome-like extracellular vesicle proteomics

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Background: The heterogeneity of extracellular vesicles (EVs) requires new tools to characterize subpopulations and elucidate the effects and mechanisms by which they shape cellular processes. Recently, significant progress has been achieved in flow cytometry and fluorescence microscopy for high-throughput analysis of high-abundance markers in single EVs but none have yet been validated for single proteins on single vesicles. Here, we identify exosome-like extracellular vesicle (ELEV) subpopulations from breast cancer cell lines enriched on nanoarrays with single-ELEV resolution and single-molecule sensitivity.
Methods: A nanoarray of anti-mouse IgGs was printed onto a glass slide using lift-off nanocontact printing, and the surface was passivated before incubation with mouse monoclonal capture antibodies. The nanoarray consists of 100 nm capture spots spaced 2 m apart that capture single ELEVs by virtue of their small size. ELEV samples, purified from cell supernatant using size exclusion columns, were incubated on the nanoarray overnight and detected using fluorescently tagged detection antibodies.
Results: Single ELEV capture was demonstrated on the nanoarray using AFM correlated with fluorescence microscopy. ELEVs could by detected with a single antibody as shown by single molecule photobleaching traces. Known exosome markers, integrins and general cancer markers were probed on exosomes derived from breast cancer cell lines, defining initial subpopulations.
Summary/Conclusion: The heterogeneity of EVs calls for methods that can measure single vesicles to allow for an accurate description of vesicle composition. With the nanoarray's ability to enrich single ELEVs of interest in a high-throughput manner, ELEV subpopulations with unique co-expression patterns can now be studied for their distinct effects.
Funding: This study was funded by Genome Canada Disruptive Innovation in Genomics and NSERC.