

Collagen-Induced Platelet Activation on Microvesicles in Platelet-Rich Plasma Studied with Scanning Flow Cytometry

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Background: Blood microvesicles (MVs) are small phospholipid vesicles, released from cells during stress conditions, including apoptosis and activation. MVs perform a variety of physiological functions and are considered as potential pathological markers of some diseases. MVs are believed to be involved in coagulation processes as their presence contributes to the formation of platelet aggregates. Further detailed studies of the dynamics of MV characteristics in response to the factors associated with platelet activation and aggregation may reveal MV potential as diagnostic marker of coagulation disorders and diseases associated with platelet dysfunction. In this work we studied changes in MV morphology in response to *in vitro* platelet activation by ADP and collagen.

Methods: We studied MVs and platelets in platelet-rich plasma (PRP) before and after addition of ADP and collagen. PRP was obtained by precipitation to minimize the effect of sample preparation on the state of platelets and MVs. Analysis of plasma samples was performed using a Scanning Flow Cytometer (SFC), the unique instrument developed in our laboratory, which measures light-scattering profiles (LSPs) of individual particles in flow. We utilized our previously developed method of label-free identification of single platelets, spherical MVs, and their dimers in PRP based on measured LSPs, and characterization of particle morphology, including platelet volume and MV size and refractive index. This approach provides observation of MVs under near-native conditions and also simultaneous monitoring of platelet and MV morphological changes.

Results: As expected, ADP- and collagen-induced platelet activation had a significant impact on MV characteristics. Changes caused by platelet activation were observed in the increased relative number of single MVs and their aggregates. We also observed formation of MV fractions with sizes and refractive indices different from that in control not-stimulated sample. Moreover, this effect depended on inductor, i.e. ADP and collagen action led to the emergence of different MV subpopulations.

Conclusion: Comprehensive SFC-based study of MVs in platelet-rich plasma showed changes in ability of MVs to form aggregates and the emergence of new MV subpopulations in response to platelet activation induced by ADP and collagen. MV characteristics, including size, refractive and relative count of single MVs and their dimers, which cannot be obtained by any other method, may serve as new indirect indicators of platelet functioning.

Quantification of Cancer-Specific Exosomes by High Sensitivity Flow Cytometry

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Exosomes (30-150 nm) are lipid-bilayer-enclosed extracellular vesicles (EVs) derived from all cells and circulating in the blood. Because exosomes carry molecular contents of the cells from which they originate, isolation and identification of cancer-specific exosomes in body fluids have shown great potential for cancer

diagnosis. The large intrinsic heterogeneity of exosomes in particle size and molecular contents calls for advanced analytical tools to probe exosomes at the single-particle level. Flow cytometry is a well-established technique for the multiparameter analysis of single cells or cell-sizes particles. However, conventional flow cytometry is incompetent to analyze single exosomes due to the limited sensitivity and the large background signals generated from the impurity particles in the sheath and sample fluid.

By reducing the detection volume for background reduction and extending the particle transit time through the laser beam for enhanced photon generation, we have developed high-sensitivity flow cytometry (HSFCM) that allows light scattering detection of low refractive index particles as small as 24 nm in diameter [1]. Particularly, the significantly reduced sheath flow rate renders the interference of impurity particles considerably diminished. Here we report the HSFCM analysis of exosomes isolated from cell culture supernatant of cancer cells, and a good discrimination of single exosomes against the background was achieved. In order to accurately measure the particle size of single exosomes, monodisperse silica nanoparticles (refractive index of 1.46) with diameter ranging from 40 to 100 nm were synthesized and employed as the size reference standards. The standard curve of the side scattering intensity versus particle diameter was calibrated with the refractive index of exosomes (1.41). With a throughput up to 10,000 particles per minute, high-resolution size distribution of exosomes can be acquired in 2-3 minutes. The detected size distribution agrees well with Cryo-TEM measurement. As CD147 has been identified as a specific surface protein marker for exosomes secreted by colorectal cancer cells, cancer-specific exosomes from the cell culture supernatant were quantified via immunofluorescent labeling and single particle enumeration. Moreover, analysis of circulating CD147-positive exosomes in the blood of colorectal cancer patients will be reported. HSFCM offers an advanced platform for the quantitative multiparameter analysis of disease-specific exosomes and may serve as a potential non-invasive diagnostic and screening tool to detect colorectal cancer to facilitate possible curative therapy.

[1] S. Zhu, L. Ma, S. Wang, C. Chen, W. Zhang, L. Yang, W. Hang, J. P. Nolan, L. Wu, X. Yan, Light-Scattering Detection below the Level of Single Fluorescent Molecules for High-Resolution Characterization of Functional Nanoparticles. ACS Nano 2014, 8, 10998-11006.

Identification of Small Molecule Inducers of FOXP3 in Human T Cells Using High-Throughput Flow Cytometry

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The increasing use of disease relevant human primary cells is driving the requirement for more sensitive high throughput technologies that derive maximum information from fewer and fewer cells. It is widely recognised that heterogeneous primary cell populations are more suited to high content single cell analysis techniques such as flow cytometry.

Regulatory T (Treg) cells play an important role in maintaining immune homeostasis, they suppress auto reactive lymphocytes, and control innate and adaptive immune responses. Treg cells express the transcription factor FOXP3 which is necessary for the development of this lineage and plays a critical role in coordinating the expression of several genes involved in immune regulation. Mutations in the FOXP3 gene lead to the inherited multi-organ autoimmune disease IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked) syndrome, and a similar disease in the Scurfy mouse.

There is considerable interest in strategies to increase FOXP3+ Treg cells in patients with chronic GVHD (graft-versus-host disease) and other disorders. Most strategies are currently focused on the adoptive transfer of Treg cells that have been purified and expanded ex-vivo. These cellular therapies have been difficult to standardize in humans, but measurable clinical benefit has been