

The Advanced Bright-field Light Optical Polarization Microscopy: Novel Coupling Method for Detection of Micro Vesicles

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Abstract

Micro vesicles (MVs) are secreted as a component of secretome of various cells including stem cells, progenitors/precursors and mature cells with different origin. MV are found to be a biological markers reflecting pathophysiological stages of disease, i.e. cardiovascular (CV) and metabolic diseases, autoimmune diseases, malignancy, infections and eclampsia. The fundamental effect of MV affects cell-to-cell cooperation, regulation of immune interrelations and transferring of several molecules. Therefore, there is a large body of evidence that confirmed the fact of number and phenotypes of MVs may be as a target for personal medical care as well as cargo for drug to the target cells. In this context, laboratory methods regarding purification of MV samples, determination of MVs, identification of origin of them and measure their concentration are fairly promising. The short communication is devoted to the consideration of advantages of novel bright-field light optical polarization microscopy, which is posed as an alternative quantified free-labeled measurement of main characteristics of MVs.

Keywords: Micro vesicles; Phenotype detection; Optical polarization microscopy; Biomarker; Probability

Introduction

Micro vesicles (MVs) are determined as small membrane particles the diameter of which ranged from 50 to 1000 nm [1]. They are secreted by various cells and play a pivotal role in cell-to-cell communications, cargo of active molecules, immune reaction, inflammation, proliferation, growth and malignancy [2]. Additionally, MVs may coordinate biological activity of some cell components incorporated in the endogenous repair systems. Therefore, number and/or immune patterns of MPs predicted a risk of manifestation of different diseases including cardiovascular (CV) and metabolic diseases, autoimmune diseases, malignancy, infections and eclampsia [3]. Moreover, the number of MVs in circulation exhibited a predictive value for all-cause mortality and premature CV death [3]. In this context, measure of MVs in circulation is discussed fairly promising to personify risk of CV disease and premature death rate.

There are several methods regarding purification of MV samples, determination of MVs, and identification of origin and measure their concentration [4]. To increase sensitivity and specificity in the enumeration of MVs we have been used the advanced bright-field light optical polarization microscopy [5]. Recently we have reported practical advantages of this method as an alternative free-labeled optical method for quantified measured of sizes and size-related characteristics of MVs [6]. We focused the research on a development of photodiode grid and PDA matrix, both of which are able to reply immediately, but not consequently as it was performed in the traditional polarized spectrophotometry.

Additionally, original soft helps to recognize replies from surfaces under interfering of various length waves and produced by two source

of polarized light, i.e. ultraviolet (λ =from 240 nm to 410 nm) and visual spectrum VIS (λ =from 590 to 950 nm). We obligatory excluded from grid photo electronic multiplier for range of length waves about 240-680 nm. The diapason of scanning was one second and less that leads to increased performance and reproducibility of results even applied for single MV in the sample. The original types of prisms and diffraction grids mediate a narrow strip of the light about 5 nm and even less and thereby they produce higher quality and clarity of the light that go around a sample. The original images are recognized MV in wide range of diameter in the mater cells that allow determining origin of the MVs on real time in higher resolution and automatic manner. The examples of the low-contrasted images received by light optical polarization microscopy and mathematically modeled images received through advanced bright-field light optical polarization microscopy are reported Figures 1.

The low-contrasted objects in red blood cells (RBCs) are visualized by application of monochromatic light with $\lambda=370+30$ nm (Figure 1). At the figure B we can see MVs with diameter less than 1 mm secreting by RBCs. Because the cell free RBC-MVs and cell debris could not be distinguished with the traditional optical polarization microscopy (C), we consequently applied ultraviolet emanation with high sensitive polarized capture through original soft to construct the image with improved contrasted features suitable for analysis of shaping, number and structure of RBC-EVs (D). Finally, this method can lead to measure a concentration of MVs in the sample without higher cost expenditure and it does not require much equipment and staff persons.

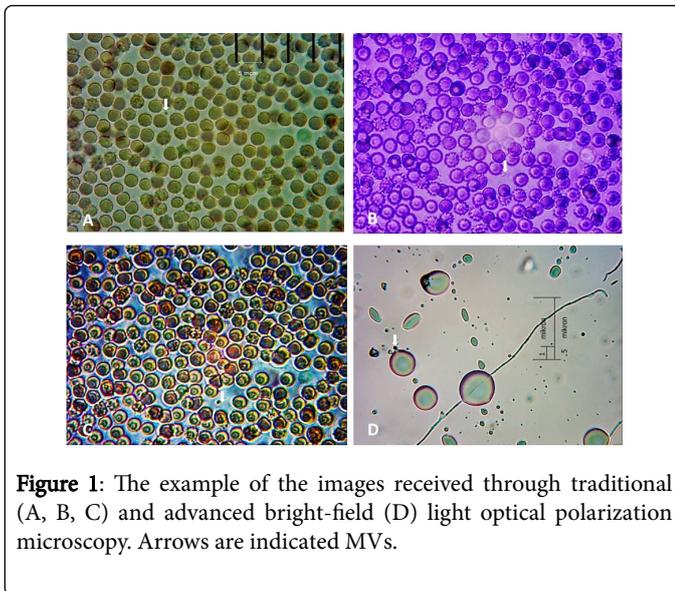


Figure 1: The example of the images received through traditional (A, B, C) and advanced bright-field (D) light optical polarization microscopy. Arrows are indicated MVs.

Conclusion

In conclusion, the advanced bright-field light optical polarization microscopy is simple method of MVs determination with low cost,

high resolution and reproducibility that requires to be investigated in future.

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