Journal of Biotechnology & Biomaterials
ISSN: 2155-952X

Berezin, J Biotechnol Biomater 2017, 7:2
DOI: 10.4172/2155-952X.1000257

Review Article OMICS International

Coupling Analytical Methods for Detection of Microparticles: The Possibilities for Improvement

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Abstract

Microparticles (MPs) are considered important diagnostic biological markers in many diseases with promising predictive value. There are several methods that currently used for the detection of number and characterization of structure and features of MPs. Therefore, the MP detection methods have been remained pretty costly and time consuming. The review is depicted the perspectives to use coupling methods for MP measurement and structure assay. Indeed, there is large body evidence regarding that the combination of atomic force microscopy or coupling nanoparticle tracking analysis (NTA) with microbeads, plasmon resonance method and fluorescence quantum dots could exhibit much more accurate ability to detect both number and structure of MPs when compared with traditional flow cytometry and fluorescent microscopy. Whether several combined methods would be useful for advanced MP detection is not fully clear, while it is extremely promising.

Keywords: Microparticles; Detection; Analytical limitations; Biomarker; Probability

Introduction

Microparticles (MPs) are specified small membrane vesicles with diameter ranged from 50 to 1000 nm [1]. They are produced and actively secreted by several cells due to activation and/or apoptotic stimuli [2]. Transferring active molecules, proteins, peptides, DNAs, RNAs/micro-RNAs, hormones, circulating free-cell MPs play a pivotal role in various biological processes including immune reaction, cell-to-cell cooperation, endogamous reparation, inflammation, proliferation and growth [3,4]. MPs possess a wide spectrum of biological effects on intercellular communication by transferring different molecules, which are able to modulate other cells affecting intercellular communication, differentiation of cells, growth of tissue, reparation, vasculogenesis, inflammation, apoptosis, infection, and malignancy. Additionally, MPs are not only cargo for biological active substances. There is strong association between immune patterns of MPs originated from different cells (endothelial cells, mononuclears, dendritic cells, and platelets) and nature evolution of various diseases including cardiovascular (CV) diseases, diabetes mellitus, abdominal obesity, cancer, sepsis, eclampsia, autoimmune states, infections and thrombosis [5-10]. Moreover, number of circulating MPs has been hypothesized to be responsible for prediction of the CV risk, thromboembolic events, autoimmune crisis, bleeding, as well as risk of all-cause mortality and CV death [11-14]. In this context, measure of MPs in circulation is considered a pretty promising, not simple tool for improving personal risk stratification. On the way there are several technical limitations regarding purification of samples, determination of MP and calculation of their concentration. The review is depicted the perspectives to use coupling methods for MP measurement and structure assay.

Determination and Origin of Microparticles

MPs belong to heterogeneous family extra vesicles (EVs) that originate from plasma membranes having diameter 1000 nm and less (Table 1). In family of EVs are included the exosomes (30–100 nm in diameter), MPs (50–1000 nm in diameter), ectosomes (100–350 nm in diameter), small-size MPs (<50 nm in diameter) known as membrane particles and apoptotic bodies (1-5 μm in diameter). MPs and ectosomes have originated by direct budding from the plasma membrane, otherwise the exosomes are formed by inward budding of the endosomal membrane and then they are released on the exocytosis of multiple vesicular bodies (MVBs) known as late endosomes. However, the exosomes have been predominantly labeled in the case of immune cells and tumor cells. Unlike the exosomes, the ectosomes are ubiquitous MPs assembled at and released from the plasma membrane.

Current methods for Microparticles' Determination

Nowadays, there are several methods that currently used for the detection of number (flow cytometry technique, optical microscopy, Nanoparticle Tracking Analysis (NTA), dynamic light scattering) and characterization of structure and features (electronic and atomic force microscopy, fluorescent microscopy, Surface Plasmon Resonance (SPR) technique) of MPs [15,16].

Flow cytometry technique

Flow cytometry technique with polystyrene beads is gold standard to determine the MP sizes that has now standardized by the Scientific Standardization Committee collaborative workshop of the International Society of Thrombosis and Hemostasis [16]. However, this method of size assessment based on SSC has a low resolution of MPs that is roughly estimated to be between 60 and 200 nm, dependent on the vesicle size [17]. The liposome-based size calibration with fluorescently labeled liposomes could be improved MP size assessment with flow cytometry, because there is a single event signal at sufficiently high concentrations irrespective of the applied gating [18]. Unfortunately, determination of MP size 50 nm and less remains to be serious limitation for this method [19]. Indeed, small-size MPs have a low refractive index and are heterogeneous in their size and composition that requires advanced methods for detection [15]. Therefore, some organelles and

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Received April 05, 2017; **Accepted** May 15, 2017; **Published** May 22, 2017

Citation: Berezin AE (2017) Coupling Analytical Methods for Detection of Microparticles: The Possibilities for Improvement. J Biotechnol Biomater 7: 257. doi: 10.4172/2155-952X.1000257

Population of vesicles	Diameter, nm	Origin	Main contained components	Best characterized cellular sources	Markers
EV	30-1000 nm	Cell membranes	Regulatory proteins (i.e., heat-shock proteins, tetraspanin), lipids, active molecules, nucleic acids (mRNA, miRNA), cytokines, growth factors, hormones, procoagulant phosphatidylserine, likely complement	All cell types	Annexin V binding, tissue factor and cell-specific markers
MPs	100-1000 nm	Plasma membranes		Platelets, RBC and endothelial cells	
MV	50-1000 nm	Plasma membranes		Platelets, RBC and endothelial cells	
Small-size MPs	<50 nm	Plasma membranes		Endothelial cells	$CD133^{+}$, $CD63^{-}$
Exosomes	30-100 nm	Endosomal membranes		Immune cells and tumors	CD63, CD61, CD63, CD81, CD9, LAMP1 and TSG101
Ectosomes	100-350 nm	Plasma membranes		Platelets, RBC, activated neutrophils, and endothelial cells	TyA, C1q
Late endosomes	50-1000 nm	Endosomal membranes	Close-packed lumenal vesicles	Immune cells and tumors	Annexin V binding, DNA content
Apoptotic bodies	0.5-3.0 μm	Plasma membranes	Pro-apoptotic molecules, oncogenic receptors	Cell lines	
EVs: Extracellular Vesicles; MPs: Microparticles; MV: Microvesicles; RBC: Red Blood Cells					

Table 1: Classification and key features of extracellular vesicles.

macromolecules (i.e., DNAs) that release from necrotic cells may bind to MPs and lead to "big" aggregates with altered optical and density and impaired immune features. All these may negatively affect specificity and sensitivity of flow cytometry-based enumerations of MPs. Overall, the main limitations of flow cytometry methods of MPs' identification are several requirements for biofluid fractionation in particularly use of exosome enriched fractions, high risk of sample contamination and increased biological variability that negatively effects on precision of measurement [20,21].

Electron microscopy

Electron microscopy (EM) is widely used technique, which allows having strong evidence regarding structure of MPs including their morphology, size and the presence of immune markers (immune complexes). The main limiting factor of EM is a need to have an enriched MP sample, which requires to be fixed with some agents, i.e., paraformaldehyde. Rarely, suspension with MPs could be much simpler object for EM, while some MPs concentrated in suspension are not able to adhere to the grid prior to EM. There is a method of measure of MPs with EM using frozen samples. This approach appears to be promising in investigation of MP structure in detail, because there are no effects of dehydration and fixation by chemicals of samples.

Atomic force microscopy

Atomic force microscopy (AFM) is used to investigate the structure of MPs due to high resolution when compared with EM [22]. This fact is especially important for so called small-size (<50 nm) vesicles. Frequently, AFM is performed prior to other methods of quantitative MP determination [23].

Nanoparticle tracking analysis

Nanoparticle tracking analysis (NTA) is based on optical method, which allows fetching of particle tracing for independent measure of both concentration and size distribution of MPs with very low limit (<50 nm) [24]. On this occasion it is so difficult to distinguish MPs from other particles and vesicles with similar size distribution, which could express similar Brownian motion. Thus, NTA analysis is extremely sensitive to quality of preparation of biofluid with enriched MP avoiding contamination with lipoprotein particles, microbial/viral bodies, and protein complexes. Noted that even after careful purification some particle may be found in final mixture prepared for MP measurement and the estimated concentrations of MPs with a use of this technique may be not pretty accurate. Finally, all these finding require improvement of the NTA technique using some fluorescence technologies or non-optical enumeration of MPs.

Dynamic light scattering

Dynamic light scattering (DLS) is recommended for assessment of the MP size distribution, while the biofluid enriched of MPs should be relatively monodispersed to avoid some problem with enumeration of vesicles by specific software [25].

Resistive pulse sensing

The resistive pulse sensing (RPS) allows detecting the absolute size of MPs in average from 50 nm to 1000 nm in depending on pores' diameter of non-conductive membrane [26]. The resistive pulse detector counts MPs when they pass in flow through an appropriate pore in the membrane under electrical power. The method is pretty accurate utilize the MPs in suspension, which is in the camera with probe volume. The calculation of MPs requires beads of known concentration and preforms using calibration with liposomes with known diameter. The main limitation of the method is heterogeneity of suspension fetching by flow under electrical power through pores with known diameter. Using membrane with pores 500 nm, it has been postulating that MPs ranged 50-500 nm with other small-size molecules (fibrinogen and other low weight molecules, apoptotic bodies, small cells,) could be detected and enumerated as MPs. Consequently, the method requires high accuracy in purification and preparation of samples prior to measure. Therefore, calibration needs before each investigation.

Raman spectroscopy

Raman spectroscopy is used the monochromatic laser-based scattering of inelastic features of living cells that allows detecting their structure and chemical compositions. The main advantage of the method is avoiding labeled marker use, because the wavelength spectrum is highly specific for each molecule [27]. Moreover, a quantitative assay is possible too as a result in measure of amplitude of signal obtained by Raman microspectroscope. On the other hand, this method is relatively expensive and requires a large experience especially affected data interpretation.

Small-angle X-ray scattering

The small-angle X-ray scattering (SAXS) is the useful method that is based on scattering of the elastic features by X-ray photons at low angles [28]. In contrast to X-Ray crystallography SAXS is able to perform in closer-to-native molecular conditions, but in respectively low resolution manner. However, SAXS could present an ultrastructure model for compositions incorporated into MPs and provide more information about molecular conformation that may have an important value for determination of membrane-related proteins and organization of lipid

layers of membranes [29]. Overall, the role of SAXS in the identification of MPs is not clear and requires more investigations.

Surface plasmon resonance technique

Surface plasmon resonance (SPR) technique is well-established methods that in cooperation with to fiber optic technology may determine MPs after their absorption to beads [30]. Because gold and silver are plasmon active metals, they are used as a component a graphene-coated bead, which helps in preventing oxidation and shows better adsorption to biomolecules. This graphene-based surface is a key tool for performing SPR and features of one ensure complex processes of metal deposition, which are needed to absorption of the investigating substrate to the surface. SPR allows detecting morphology of MPs, as well as calculation of them in the solution by laser-based scattering. The main advantage of the method is pretty low cost and high reproducibility that meets rarely amongst similar methods. Moreover, currently conventional prism-based SPR platforms are simply in used, have cost-effectiveness and miniaturization [31].

Western blotting

Western blot is a useful tool for detecting some proteins, which express on the surfaces of MPs in carefully purified biofluid. Although Western blotting is not able to provide quantitative information regarding MPs in mixture, this method could be useful for determining several sub-populations of MPs distinguished each other with immune phenotypes. The cost and difficulty of this method is one of leading limitation to use one in MPs' detecting.

Overall, the current MP detection methods have been remained pretty costly and time consuming. Additionally, majority of them requires be standardizing and approving. In this context, combined methods might to quantify and qualify MP detection.

Couple Methods for MP Identification

A combination of optical or non-optical enumeration as well as functional methods may be required for a complete profiling of circulating MPs [32]. There is large body evidence regarding that the combination of SPR or RPS methods to atomic force microscopy or coupling NTA with Raman microscopy, microbeads and fluorescence quantum dots exhibited much more accurate ability to detect both number and structure of MPs when compared with traditional flow cytometry and fluorescent microscopy [33-35].

To fetch axillary information regarding the number, size distribution and accurate chemical compositions of MPs in biofluid Raman microspectroscopy with RPS could be useful. The additional attractive service of the methods' combination is avoiding fluorescence labeling with appropriate antibodies versus specific antigens that contributes in reducing analytical time. It has been postulated that consequently performing SAXS, AFM and X-ray diffraction technique could be useful tool for identification of structural, mechanical and electrical properties of MPs [36]. Interestingly, that SPR imaging could be more useful in determining MPs with low expression of antigens, but NTA analysis contributed axillary to SPR could improve information regarding structure and morphology of MPs. Therefore, a signal of SPR is weaker than in NTA that create a possibility to perform both methods consequently to increase sensitivity and specificity in enumeration MPs [37].

Moreover, all these new methods could be used as screening method for MP detection and they would not only be much more reproducibility, specificity and sensitivity, but also they should be pretty inexpensive and assessable [38,39]. Therefore, combined methods might assay some components of MPs including RNAs, lipids, proteins and active molecule profiling. Probably, similar approach would attenuate pre- and intra-analytical errors and improve entire precision of the methods. Thus, coupling some methods based on different principles might allow detecting numerous and structure of MPs. All these could be useful for providing the necessary information to clear biological role of MPs as diagnostic and predictive biomarkers.

Conclusion

Currently used analytical methods as only technique for detection of MPs exhibited serious limitation to interpretation of received results. Each of these biosensor diagnostic platforms has its own advantages and disadvantages in detecting MPs, identification of their size distribution and composite chemicals. The combination of MPs' detection methods allows sufficiently increasing their specificity, sensitivity and probability.

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