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Mini Review

Biosensor Designs for Platelet-derived Microparticles Analysis

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ABSTRACT

Platelet-derived microparticles (PMPs) are often used as marker of platelet activation and their count in blood has been found to be significantly associated with many diseases like myocardial infarction, stroke, venous thrombo-embolism etc. PMPs have been proposed as potential biomarkers for these conditions. Biosensors are newer analytical tools, being developed for convenient and cost effective analysis. For PMPs analysis, biosensors offer many advantages over conventional analysis techniques. This mini review compiles designs and techniques of reported biosensors based on antibody capturing for analysis of PMPs.

Keywords: Platelet-derived microparticles, biosensors, biomarkers, electrochemical analysis, myocardial infarction.

Introduction

Platelets release extracellular vesicles (EVs) in blood. Vesicles originating from cell membrane budding are known as microparticles while smaller vesicles originating from intracellular multivesicular bodies are called exosomes. Platelet-derived microparticles (PMPs) are often used as marker of platelet activation (1, 2). PMPs are most abundant EVs in human blood (3, 4). PMPs are of heterogeneous in size ranging 0.1 to 1 micron (100- 1000 nm), while exosomes size ranges from ~40-100 nm (3). Ninety percent of platelet EV are below 500 nm in size, majority being in range of 100-250 nm (5). PMPs play significant role in cell to cell communication, homeostasis, angiogenesis and other functions. They are rich in phosphatidylserine, tissue factor and many other receptors and have procoagulant surface and ability to interact with leukocytes and endothelial cells (6-8).

PMPs count in blood has been found to be significantly associated with many acute and chronic diseases like myocardial infarction, stroke, venous thrombo-embolism, preeclampsia, fungal (candida albicans) sepsis, systemic lupus erythematosus (SLE), rheumatoid arthritis, etc. (9, 3, 10-13, 4, 14). Increased number of circulating microparticles can trigger hypercoagulable state, which may lead to thrombo-embolic complications (11).

PMPs play role in haemostasis and are considered highly procoagulant (4, 15). Increased blood PMPs concentration in patients of myocardial infarction and acute coronary syndrome, compared to healthy individuals, has already been reported (16, 10, 17, 15). Thus, PMPs level in blood has been proposed as potential biomarker (18, 10, 17). Multiple techniques for PMPs level estimation and characterization have been reported, including flow cytometry, electron microscopy, nanosight

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tracking analysis, ELISA, etc.

Biosensors are analytical devices that combine biological components with physico-chemical detectors. Biosensors offer many advantages over conventional analysis techniques like low cost, quick results, miniaturization and easy application (19). Immunosensor is a variety of biosensor where principle of antigen-antibody reaction is utilized for specificity of detection and antibodies are fabricated on sensor platform for analyte capture (20). Novel biosensors are being reported regularly and are gaining popularity in medical diagnostic field (20-22). For convenient analysis of PMPs, biosensors are being developed as newer tools.

Designs of biosensors for PMPs analysis

Our lab was first to design specific antibody based PMPs capture nano-bio-sensor platform. We reported a graphene oxide based biosensor for quantitative estimation of PMPs. Glassy carbon disc electrodes were fabricated with graphene oxide at first step, then PMP specific PAC1 (first Procaspase Activating Compound) antibody was immobilized onto it to make the sensor specific for PMPs. Detection was based on electrochemistry by frequency response analysis (FRA), which detects impedance applied by immobilized PMPs on sensor surface. The results were compared and confirmed by flow cytometry analysis of same PMPs samples. Our biosensor successfully and quantitatively estimated PMPs levels in plasma samples of healthy donors as well as acute myocardial infarction patients. The detection limit of our biosensor was 100 PMPs per μL sample, and linear response was seen upto 7000 PMPs per μL for quantitative detection. This biosensor design proved to be most suitable for quick, cost effective, sensitive and user friendly point of care testing and opened new paradigm for development of biosensor based PMPs detection techniques (10).

In 2017, building upon our PMPs biosensor technique, Singh *et al*. reported another design of biosensor based on nanosilica-PAC1 antibody, P-selectin antibody and conjugated Horse Radish Peroxidase (HRP) on ITO (Indium Tin oxide) electrode (23). Thionine doped silica nanoparticles were synthesized and applied for this biosensor design, where Nanosilica was used as 3-dimentionals platform for biomolecules encapsulation and thionine was used as mediator of electron transfer. PAC1 antibody was used for capturing PMPs on sensor surface, while HRP tagged P-selectin antibody generated detection signal after reacting with H2O2 (hydrogen peroxide). Signal generated by H2O2 oxidation through HRP tagged P-selectin was used for estimation of PMPs quantity. H2O2 oxidation was detected by cyclic voltametry, where higher concentration of PMPs in sample generates higher peaks in cyclic voltamograms, which in turn successfully detected PMPs numbers in samples. Reported detection limit was 490 PMPs per μL sample, and linear response was seen upto 4080 PMPs per μL (23).

A hybrid technique for simultaneous real time quantification and characterization of PMPs by combining surface plasmon resonance (SPR) and atomic force microscopy (AFM) was also developed and reported as an 'on-chip' NanoBioAnalytical platform in 2017 (24). Authors immobilized CD41 antibody on gold sensor chip to capture PMPs, and then used SPR and AFM to characterize captured PMPs. This technique eliminated size limitation, need for labelling PMPs and complex sample preparation. This technique allowed metrological analysis of captured PMPs and revealed that more than 95% of PMPs were smaller than 300 nm. This method reported analysis of PMPs of size range 30 nm to 1 μm and concentration range 109-1010 per μL (24). Results suggested that this NanoBioAnalytical platform, combining SPR and AFM, is a suitable method for a sensitive, reproducible, label-free characterization and quantification of various microparticles over a wide concentration range (24).

Biosensor based techniques offer many advantages by reducing analysis time, eliminating staining steps, ease of application and requirement of very small sample volume for detection. These biosensors are still under development phase, targeted for user friendly wide spread applications.

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