Trends in Analytical Chemistry 79 (2016) 326–334

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Signal amplification strategies for microfluidic immunoassays

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ARTICLE INFO

Keywords:
Limit of detection
Sensitivity
Biomarkers
Preconcentration
Magnetic beads
Immuno-PCR

ABSTRACT

Immunoassays have become much more sophisticated since the enzyme linked immunoassays became widely used. Microfluidics in particular, coupled with advanced optical and electrochemical readout systems have reduced the limits of detection, decreased assay time, and simplified automation. Yet the sensitivity of the microfluidic immunoassays is still limited by the ability of the detector to discriminate between signal and background. Three main approaches to produce higher signal/background are reviewed and critiqued: target preconcentration, reaction confinement in a small detection volume and signal amplification strategies. Combinations of these strategies can be used to increase sensitivity and may provide clinical diagnostics for biomarkers present in very low concentrations.

Contents

Abbreviations: AFP, α-fetoprotein; BTV, blue tongue virus; CA, cancer antigen; CD, compact disc; CEA, carcinoma embryonic antigen; DNA, deoxyribonucleic acid; E coli, Escherichia coli O157:H7; ELISA, enzyme-linked immunosorbent assay; hCG, human chorionic gonadotropin; HRP, horseradish peroxidase; ICP, ion concentration polarization; IgG, immunoglobulin G; IL, interleukin; ILGF1R, insulin-like growth factor 1 receptor; IMS, immunomagnetic separation; LOC, lab-on-chip; LOD, limit of detection; MBs, magnetic beads; MMA, murine monoclonal antibody; PCR, polymerase chain reaction; PDMS, polydimethylsiloxane; PMMA, poly(methylmethacrylate); QDs, quantum dots; SIV, swine influenza virus; SPR, surface plasmon resonance; TNF-α, tumor necrosis factor alpha; ZMW, zero mode waveguide.

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http://dx.doi.org/10.1016/j.trac.2015.10.021
0165-9936/

1. Introduction

Biomarkers such as proteins, nucleic acids, and small molecules are found in trace levels, especially in the early stages of disease progression. Reliable determination of one or more of these analytes in complex sample matrices, such as blood serum, is crucial for early diagnosis and treatment of cancer and other diseases [1]. Immunoassays including enzyme-linked immunosorbent assay (ELISA) are widely used for biomarker detection because of their speed, sensitivity, selectivity, and cost effectiveness [2,3]. However, many conventional immunoassays are still labor intensive, consume large volumes of expensive reagents and precious samples, and require long assay times, thus limiting the scope of their application.

Miniaturization using microfluidic platforms can overcome some of the limitations of conventional assay protocols based on 96-well microtiter plates and hold a great potential for extending the use of immunoassays as point-of-care diagnostic tools. Microfluidics has evolved as a powerful platform for fundamental and applied biomedical research. An assay in a microfluidic device can be completed with extremely small volumes of sample and reagents (~1 μL per assay) and in significantly reduced time (e.g. 5–30 min) [4,5]. Most of the current immunoassay methods, however, even in microfluidic platforms that provide results comparable to conventional tests, are still not sensitive enough for many applications. As a result, development of assay strategies to achieve a limit of detection (LOD) below concentrations typical of low abundance analytes is still a significant challenge. In this review, we critically evaluate the major new developments in microfluidic immunoassay methods that can contribute to increased detection sensitivity.

2. Overview of principal strategies

Increasing the LOD requires that a higher signal be measured above the background generated by 1) the noise from the measurement technique and 2) nonspecific signal from complex sample components. Three main approaches can produce higher signal/background, and we have evaluated progress in increasing signal to background for microfluidic immunoassays in each of these three areas (see Fig. 1). First, the target (antigen or antibody) can be preconcentrated before binding with their counterpart (antibody or antigen) in the assay, simultaneously removing sample components that might increase the background. Second, the immunocomplex or the signal produced from the binding event can be concentrated in a small volume within the sensor to increase the sensitivity of the assay. Third, labels such as nanoparticles, quantum dots, enzymes, and metal precipitation can provide effective strategies for signal amplification. Detailed description of each of these strategies and representative examples are provided in following sections.

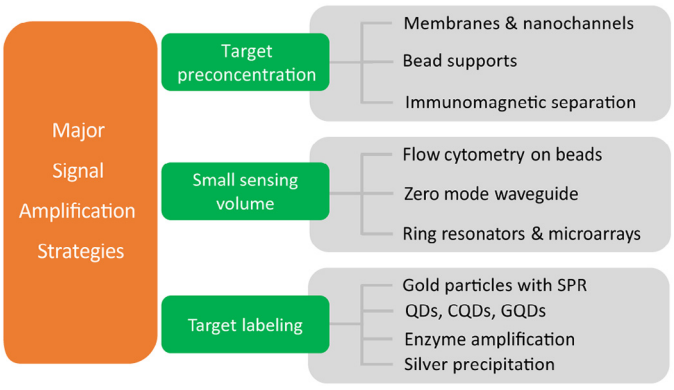

Fig. 1. Major signal amplification strategies for microfluidic immunoassays.

3. Target preconcentration

3.1. Membranes and nanochannels for target concentration

Physical trapping of target analytes based on target size is one approach for target preconcentration. Target analytes can be physically trapped using a porous membrane or nanochannel prior to the immunoassay. The use of membranes in a microchannel restricts the passing of target molecules or cells upstream of the immunosensor [6]. Even though this technique is popular for preconcentrating proteins, DNA, and dye molecules [7], there are few examples of its applications in immunoassays. An example of preconcentration of a target biomarker using a membrane prior to an immunoassay was demonstrated by Yanagisawa and Dutta [8]. Analyte molecules were electrokinetically trapped in front of a semi-permeable photopolymerized membrane in a microchannel that had already been modified with capture molecules. This strategy resulted in 2 orders of magnitude improvement in the LOD for measuring the tumor marker cancer antigen 19-9 and antibody to Blue Tongue Virus compared to the assays without analyte preconcentration. Reichmuth et al. [9] integrated a photopolymerized membrane to concentrate virus particles to up to 4-fold compared to a system without a membrane. The *in situ* polymerized polyacrylamide membrane was used to concentrate swine influenza viral particles and separate the virus-antibody complex from the unbound antibody. The porous (~10 nm) polymer membrane allowed the transport of large proteins including antibodies but did not allow the larger viral particles (~80 nm). The filtration effect of the polymer membrane also removed interfering sample components and eliminated the need for washing, commonly required with surface-based immunoassays, increasing the speed of the assay. Han et al. [10] reported a new electrokinetic concentration method to enhance the sensitivity of a competitive immunoassay utilizing highly charge-selective polyelectrolyte polymer plugs made up of poly-2-acrylamido-2-methyl-1-propanesulfonic acid on a microfluidic chip. This polymer membrane served as an effective charge-selective preconcentrator that resulted in a 2000-fold enhancement for detecting biotin within 3 min. Immunoassays for the sensitive determination of a number of biotoxins were demonstrated using glass microchips [11]. This chip contained polymeric gels with larger pores to perform electrophoretic separation of antibody-antigen complexes and excess antibody, whereas a size-exclusion membrane with a molecular weight (MW) cut-off of ~10 kDa concentrated proteins bigger than the MW cut-off of the membrane. The on-chip preconcentration and mixing method resulted in a ~30-fold improvements in the detection limit.

In addition to polymeric membranes, nanochannels and Nafion® membranes have also been developed to trap target molecules to enhance the assay signal in microfluidic devices. When charged molecules are transported through a nanochannel by applying electrical current, a sudden change of flux density at the interface of the nanochannel and the surrounding bulk solution gives rise to a concentration buildup of molecules on one side of the nanochannel and a depletion of molecules on the other. This phenomenon is called *ion concentration polarization* (ICP) [12], and it has been usefully applied for analyte preconcentration. The concentration using ion-exchange membranes such as Nafion® has also been explained on the basis of ICP. Application of nanochannel and Nafion® preconcentration for immunoassays is relatively new. The Han research group from MIT has demonstrated a 500-fold enhancement in the immunoassay sensitivity for measuring R-phycoerythrin with 30-min concentration using nanofluidic preconcentrator combined with polystyrene beads [13]. The polyacrylamide gel plug in a nanofluidic device can also be combined with magnetic bead-based immunoassay as shown by Xu et al. [14]. In their device, magnetic beads loaded with antibodies were trapped into the immunoassay region just in front of the porous