Biosensors and Bioelectronics 64 (2015) 43–50

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Detecting multiple cell-secreted cytokines from the same aptamer-functionalized electrode

Ying Liu, Ying Liu [1], Zimple Matharu, Ali Rahimian, Alexander Revzin *

Department of Biomedical Engineering, University of California, Davis, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 27 May 2014
Received in revised form
30 July 2014
Accepted 15 August 2014
Available online 22 August 2014

Keywords:
Aptasensor
Electrochemical detection
Cytokine
Cell secretion
Microfluidics

ABSTRACT

Inflammatory cytokines are secreted by immune cells in response to infection or injury. Quantification of multiple cytokines in parallel may help with disease diagnosis by illuminating inflammatory pathways related to disease onset and progression. This paper describes development of an electrochemical aptasensor for simultaneous detection of two important inflammatory cytokines, interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α). To enable multiplexing, IFN-γ and TNF-α aptamers were labeled with anthraquinone (AQ) and methylene blue (MB) redox reporters respectively. Random immobilization of two aptamer on gold exhibited redox peaks at −0.37V (AQ) and −0.15V (MB) vs. Ag/AgCl reference. When challenged with either IFN-γ or TNF-α, redox signal of the appropriate reporter changed in concentration dependent manner. To demonstrate one possible application of this sensing approach, electrodes were integrated into microfluidic devices and used to dynamically monitor cytokine release from immune cells. Two cell types, primary human CD4 T-cells and U937 monocytic cells, were used to compare differences in cytokine secretions upon stimulation. These cells were infused into the microfluidic devices and stimulated to commence cytokine production. Release of IFN-γ and TNF-α was monitored concurrently from the same small group of cells over the course of 2 h. The strategy of encoding specific aptamer types with unique redox reporters allows sensitive and specific detection of multiple protein biomarkers from the same electrode.

1. Introduction

Cytokines are signaling proteins secreted by immune cells in order to regulate a multitude of immune responses, including proliferation, migration or activation of cells. Outside of keeping infections at bay, production of inflammatory cytokines is closely tied to cancer (Moss and Blaser, 2005), atherosclerosis (Sack, 2002), rheumatoid arthritis (Sarzi-Puttini et al., 2005), Alzheimer's disease, Parkinson's disease and multiple sclerosis (Lee et al., 2010). Interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) are two common pro-inflammatory cytokines produced by immune cells upon stimulation with antigen, and they have been found to play an important role in multiple pathological conditions. For example, in HIV infected individuals, robust production of IFN-γ by CD4 T-cells has been associated with non-progression of the disease. In another example, detection of IFN-γ release by T-cells is supplanting a century old skin test for detection of tuberculosis (TB). In fact, in an effort to identify blood-based correlates/markers of TB it was noted that T-cell production of only IFN-γ was associated with latent form of this disease whereas T-cell secretion of both IFN-γ and TNF-α correlated to active form of TB. Therefore, multiplexed detection of cytokines from immune cells is highly significant.

Cytokines are typically detected using antibody-based immunoassays. These immunoassays are sensitive specific and robust, however, they are time-consuming; requiring multiple washing and handling steps to achieve the readout. Also, the need for multiple washing and labeling steps makes antibody-based assays suboptimal for dynamic monitoring of cellular secretions.

Aptamers are emerging as an effective alternative to antibodies, offering the advantages of high thermal/chemical stability, regenerability and ease of modification. One of the most attractive features of aptasensors is the simplicity with which an oligonucleotide can be designed into a beacons that produce signal directly upon binding of target analyte, without the need for labeling and washing steps. Simplicity of getting the readout makes aptamer-based biosensors particularly suitable for point of care testing. The fact that the signal comes on without the need for additional labeling or washing steps, positions aptasensors well for dynamic monitoring of cellular activity.

* Corresponding author.
E-mail address: arevzin@ucdavis.edu (A. Revzin).
[1] Current address: ReLIA Diagnostic Systems Inc., Burlingame, CA 94040, USA.

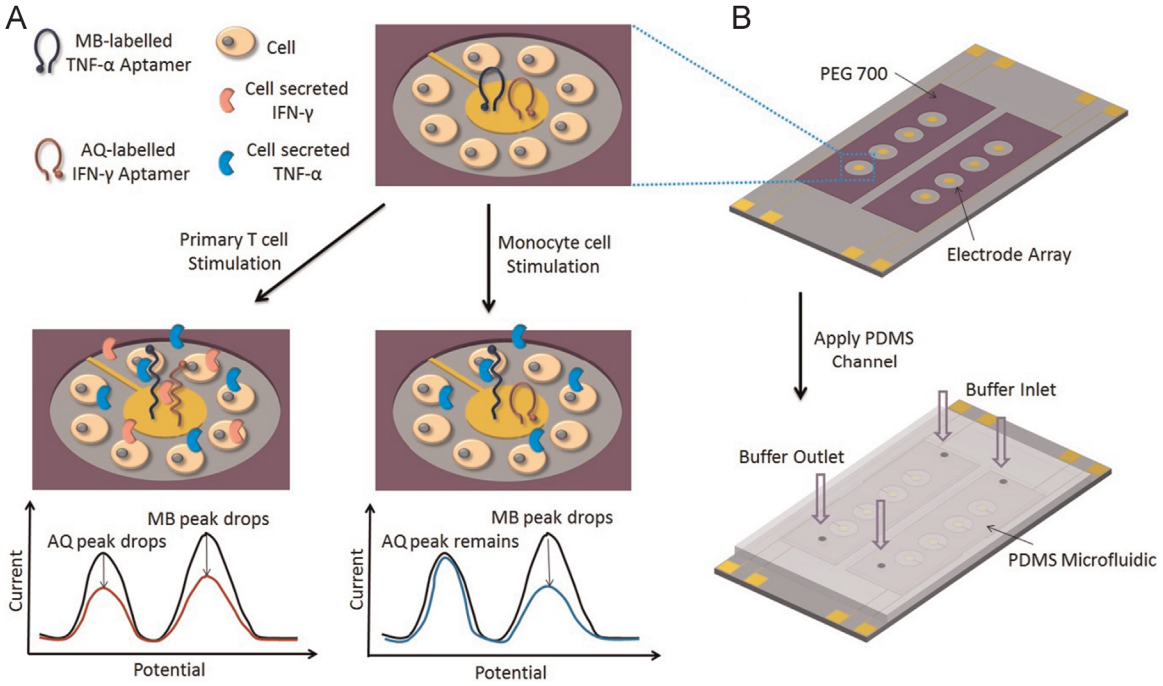
http://dx.doi.org/10.1016/j.bios.2014.08.034
0956-5663/

Previously, our lab has developed hairpin structure aptamer for IFN-γ detection (Liu et al., 2013, 2010). The hairpin was thiolated, conjugated with a redox reporter methylene blue (MB), and immobilized on a gold electrode. Binding of cytokine caused a conformational change in the aptamer, resulting in a decrease of the redox current. More recently, we have demonstrated an aptasensor for TNF-α detection (Kwa et al., 2014) and fabricated electrodes for simultaneous detection of IFN-γ and TNF-α from the same small group of cells (Kwa et al., 2014; Liu et al., 2011). In this recent study, IFN-γ and TNF-α aptamers were labeled with same redox reporter (MB) and were assembled on specific electrodes through a series of electrode protection and de-protection steps. We could then monitor electrochemical signals from specific electrodes to discern changes in IFN-γ or TNF-α concentrations. However, we were dissatisfied with the complexity of functionalization protocol employed in this previous study and sought to develop a more facile multiplexing strategy. Instead of using the same redox reporter and encoding cytokine specificity based on electrode location, we wanted to encode different aptamer types with redox reporters and then use the same electrode to decode changes in cytokine concentrations (Scheme 1). There have been reports describing the use of nanostructured electrodes for sensing based on electrochemical redox spectra that arose due to multiple redox/electroactive moieties diffusing from solution and reacting on the electrode (Karimi-Maleh et al., 2013, 2014). Plaxco lab recently demonstrated the concept of redox encoding using oligonucleotide sequences as target analytes (Kang et al., 2012). However, to the best of our knowledge, this strategy has not been applied to aptamer-based biosensors and protein detection. To prove the concept, thiolated aptamers against IFN-γ and TNF-α were labeled with anthraquinone (AQ) and methylene blue (MB) respectively. Electrodes functionalized with a mixture of the two aptamers showed redox peaks at −0.37 V (AQ) and −0.15 V (MB) vs. Ag/AgCl reference. Importantly, correct redox peak shifted downwards upon introduction of either IFN-γ or TNF-α (Scheme 1). No change in redox activity of the electrodes was observed after challenges with nonspecific proteins. As the next step in this study, micropatterned electrodes were integrated with microfluidic devices and used for immune cell analysis. Primary T-cells and monocyte cell line were analyzed for production of IFN-γ and TNF-α to demonstrate that secretion pattern was cell type specific. The production of both cytokines from a small group of immune cells was monitored concurrently (from the same microelectrode) over the course of 2 h. In the future, we envision increasing the number of redox reporters and aptamers assembled on the electrode surface to enhance multiplexing power of this strategy.

2. Materials and methods

2.1. Materials

10× phosphate-buffered saline (PBS) without calcium and magnesium, sodium bicarbonate ($NaHCO_3$), anhydrous toluene (99.9%), dimethylformamide (DMF), acetone, bovine serum albumin (BSA), 6-mercapto-1-hexanol (MCH), Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), poly(ethylene glycol) diacrylate (PEG-DA, MW 700), and 2-hydroxy-2-methyl-propiophenone (photoinitiator), T cell and monocyte activation reagents: phorbol 12-myristate-13-acetate (PMA) and ionomycin were purchased from Sigma-Aldrich (St. Louis, MO). Chromium etchant (CR-4S) and gold etchant (Au-5) were purchased from Cyantek Corporation (Fremont, CA). Positive photoresist (S1813) and developer solution (MIF-318) were purchased from Shipley (Marlborough, MA). 3-Acryloxypropyltrichlorosilane was purchased from Gelest, Inc. (Morrisville, PA). Monoclonal purified mouse anti-human CD4 Abs and anti-human CD14 Abs were purchased from Gelest, Inc. (Fullerton, CA). Human recombinant Interferon gamma (IFN-γ), Tumor necrosis factor alpha (TNF-α), Interleukin-12 (IL-12), Interleukin-6 (IL-6), Interleukin-10 (IL-10) were purchased from R&D systems (Minneapolis, MN). Cell culture medium RPMI 1640 with



Scheme 1. Layout of the microfluidic sensing platform. The electrode array is encased in PEG hydrogel layer and integrated into PDMS microfluidic channels (A). Au electrodes are modified with different aptamers-redox reporter constructs. Either T-cells or monocytes are infused into microfluidic channels and are bound next to aptasensors. Upon cytokine binding, aptamer changes conformation resulting in decreased electron transfer efficiency. Two cytokine types released by T-cells are dynamically monitored on the same electrode using square wave voltammetry (SWV) (B).