Biosensors and Bioelectronics 53 (2014) 268–274

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

On-line protein capture on magnetic beads for ultrasensitive microfluidic immunoassays of cancer biomarkers

Brunah A. Otieno [a], Colleen E. Krause [a], Alina Latus [a,b], Bhaskara V. Chikkaveeraiah [a], Ronaldo C. Faria [a,c], James F. Rusling [a,d,e,f,*]

[a] Department of Chemistry, University of Connecticut, 55 North Eagleville Road, Storrs, CT 06269, United States
[b] Institute of Physical Chemistry "I. Murgulescu", Romanian Academy, Splaiul Independentei 202, Bucharest 060021, Romania
[c] Departamento de Quimica, Universidade Federal de Sao Carlos, SP 13565-905, Brazil
[d] Department of Cell Biology, University of Connecticut Health Center, Farmington, CT 06232, United States
[e] Institute of Material Science, University of Connecticut, CT 06269, United States
[f] School of Chemistry, National University of Ireland at Galway, Ireland

ARTICLE INFO

Article history:
Received 16 August 2013
Accepted 24 September 2013
Available online 8 October 2013

Keywords:
Protein detection
Cancer biomarkers
Magentic beads
Immunoarray
Microfluidics

ABSTRACT

Accurate, sensitive, multiplexed detection of biomarker proteins holds significant promise for personalized cancer diagnostics. Here we describe the incorporation of a novel on-line chamber to capture cancer biomarker proteins on magnetic beads derivatized with 300,000 enzyme labels and 40,000 antibodies into a modular microfluidic immunoarray. Capture and detection chambers are produced from PDMS on machined molds and do not require lithography. Protein analytes are captured from serum or other biological samples in the stirred capture chamber on the beads held in place magnetically. The beads are subsequently washed free of sample components, and wash solutions sent to waste. Removal of the magnet and valve switching sends the magnetic bead–protein bioconjugates into a detection chamber where they are captured on 8 antibody-decorated gold nanoparticle-film sensors and detected amperometrically. Most steps in the immunoassay including protein capture, washing and measurement are incorporated into the device. In simultaneous assays, the microfluidic system gave ultralow detection limits of 5 fg mL^{-1} for interleukin-6 (IL-6) and 7 fg mL^{-1} for IL-8 in serum. Accuracy was demonstrated by measuring IL-6 and IL-8 in conditioned media from oral cancer cell lines and showing good correlations with standard ELISAs. The on-line capture chamber facilitates rapid, sensitive, repetitive protein separation and measurement in 30 min in a semi-automated system adaptable to multiplexed protein detection.

1. Introduction

Molecule-based early cancer diagnoses promise to improve treatment outcomes and patient survival rates (Etzioni et al., 2003; Rusling et al., 2010). Current cancer diagnostics often rely on biopsies, observing symptoms or lesions, or in vivo imaging. These approaches depend on finding a tumor, making early detection difficult and possibly compromising therapy outcomes. Screening for cancer without detecting tumors can be based on assays of body fluids for cancer biomarker proteins to provide an instantaneous record of a patient's disease status (Hanash et al., 2008; Kulasingam and Diamandis, 2008; Lilja et al., 2008; Rusling et al., 2010). For translation to the clinic, measurement devices for biomarker proteins should be accurate, sensitive, cheap and preferably capable of point-of-care (POC) use. For reliable diagnoses of cancers, it will be essential to measure panels of biomarker proteins rather than single proteins for the best prediction efficiency (Gubala et al., 2012; Rusling et al., 2010).

Existing methods for measuring protein biomarkers including enzyme linked immunosorbent assay (ELISA) (Kingsmore, 2006), magnetic bead-based assays (Beveridge et al., 2011; Rusling et al., 2010) and liquid chromatography–mass spectrometry (LC–MS) (Hawkridge and Muddiman, 2009) are currently too expensive, time consuming, and technically complex for multiplexed POC protein determinations in clinical samples. Arrays based on optical (Chin et al., 2011; Lee et al., 2008), electrochemical (Chikkaveeraiah et al., 2011; Rusling, 2012; Rusling et al., 2013; Wang, 2007; Wei et al., 2009) or nanotransistor (Patolsky et al., 2006) detection have been developed to overcome some of these limitations (Chin et al., 2012; Gubala et al., 2012). In reality, selected detection approaches can already achieve the high sensitivity and accuracy necessary for

* Corresponding author at: Department of Chemistry, University of Connecticut, 55 North Eagleville Road, Storrs, CT 06269, United States.
E-mail address: James.Rusling@uconn.edu (J.F. Rusling).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.09.054

clinical applications, but complexity, cost and to a lesser extent multiplexing issues hold back clinical applications.

Microfluidics can improve immunoassay speed, cost and multiplexing (Chin et al., 2012; Gervais et al., 2011; Manz et al., 1992; Pan et al., 2010; Wang et al., 2010; Whitesides, 2006). For example, an integrated microfluidic system recently reported for clinical diagnosis of HIV and syphilis detects antibodies to the disease vectors at clinical levels (Chin et al., 2011). This chip used optical detection to analyze 1 μL of whole blood within 20 min in clinics in the developing world. However, improvements in integrated microfluidic systems still need to address multiplexing and other complexity issues.

We have developed modular microfluidic systems to facilitate fast multiplexed detection of proteins in biomedical samples (Chikkaveeraiah et al., 2011; Krause, et al., 2013; Malhotra et al., 2012). These devices feature a sensor array coated with gold nanoparticle (AuNP)–antibody conjugates in a poly(dimethylsiloxane) (PDMS) microchannel interfaced to a syringe pump and sample injector. Paramagnetic beads loaded with multiple detection antibodies and horseradish peroxidase (HRP) enzyme labels are used to capture protein analytes from sample solutions in small vials to provide detection of biomarker proteins in serum down into the low fg mL^{-1} range (Malhotra et al., 2012). Accuracy and diagnostic utility of these microfluidic arrays was demonstrated by measuring four biomarker proteins in oral cancer patient serum samples.

While useful for diagnostics, the above system would benefit from simpler operation for clinical and POC screening. Herein we report incorporation of a new on-line protein capture chamber into a modular microfluidic system. We used magnetic beads coated with ~40,000 antibodies and ~300,000 HRP labels, and validated the new system for simultaneous immunoassays of two proteins. The capture chamber features an oval PDMS channel equipped with a tiny stir bar sandwiched between two transparent poly(methyl methacrylate) (PMMA) plates (Fig. 1 and S1). The bioconjugated magnetic beads and protein samples are incubated in the chamber for on-line protein capture. After washing the beads and sending the wash to waste, the protein–magnetic beads are directed into the microfluidic detection chamber housing the 8-sensor AuNP array. This new design allows semi-automated ultrasensitive assays to be completed in the microfluidic device within 30 min. Nanostructured sensors combined with massively labeled magnetic detection beads provided simultaneous assays with detection limits (DLs) of 5 fg mL^{-1} for IL-6 and 7 fg mL^{-1} for IL-8 in serum, similar to DLs for off-line manual protein capture. Accuracy was demonstrated by measuring these proteins in conditioned media for oral cancer cell lines, with good correlations with standard ELISA.

2. Experimental section

2.1. Chemicals and materials

Biotinylated horseradish peroxidase (HRP, MW 44,000, 250–330 unit mg^{-1}), L-glutathione reduced (GSH, 99%), gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%), sodium borohydride (99%), poly(diallyldimethylammonium chloride) (PDDA, MW 100,00–200,000, 20%), bovine serum albumin (BSA), 1-(3-(Dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (NHSS) were from Sigma. Hydrogen peroxide (H_2O_2, 30%) was from Fisher. The poly(dimethoxy)silane (PDMS) kit was from Dow Corning. Buffer pH 7.4 phosphate saline (PBS) was 0.01 M in phosphate, 0.14 M NaCl, 2.7 mM KCl. Tween-20 and hydroquinone (HQ, ≥ 99%) were from Sigma-Aldrich. Streptavidin-coupled superparamagnetic beads (1 μm, Dynabeads) were from Invitrogen. Monoclonal Human IL-6 Antibody (Ab_1, clone no. 6708), human IL-6 biotinylated polyclonal antibody (Ab_2, goat IgG), monoclonal human CXCL8/IL-8 antibody (Ab_1, clone no. 6217), human CXCL8/IL-8 biotinylated polyclonal antibody

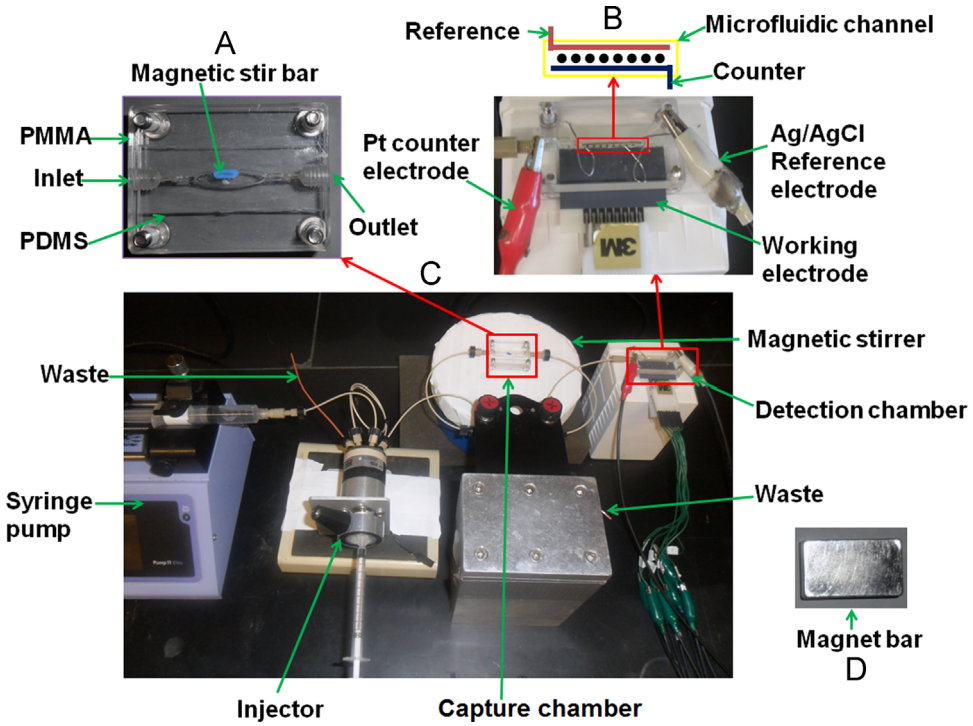

Fig. 1. Photographs of microfluidic system for on-line protein capture and detection using magnetic beads. (A) Capture chamber in which target proteins are captured on-line from the sample by heavily labeled HRP-antibody-magnetic beads to form protein–bead bioconjugates. These are washed, and then flowed into the detection chamber (B) in the modular microfluidic system (C). The magnet (D) traps bioconjugate beads in the channel during injection of sample and washing, and is removed for transfer of beads to the detection chamber.