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Development and validation of an immunosensor for monocyte chemotactic protein 1 using a silicon photonic microring resonator biosensing platform

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ABSTRACT

Objectives: We report the development of an optical immunosensor for the detection of monocyte chemotactic protein 1 (MCP-1) in serum samples. MCP-1 is a cytokine that is an emerging biomarker for several diseases/disorders, including ischemic cardiomyopathy, fibromyalgia, and some cancers.

Design and methods: The detection of MCP-1 was achieved by performing a sandwich immunoassay on a silicon photonic microring resonator sensor platform. The resonance wavelengths supported by microring sensors are responsive to local changes in the environment accompanying biomarker binding. This technology offers a modularly multiplexable approach to detecting analyte localization in an antibody-antigen complex at the sensor surface.

Results: The immunosensor allowed the rapid detection of MCP-1 in buffer and spiked human serum samples. An almost 2 order of magnitude linear range was observed, between 84.3 and 1582.1 pg/mL and the limits of blank and detection were determined to be 0.3 and 0.5 pg/mL, respectively. The platform's ability to analyze MCP-1 concentrations across a clinically-relevant concentration range was demonstrated.

Conclusions: A silicon photonic immunosensor technology was applied to the detection of clinically-relevant concentrations of MCP-1. The performance of the sensor was validated through a broad dynamic range and across a number of suggested clinical cut-off values. Importantly, the intrinsic scalability and rapidity of the technology makes it readily amenable to the simultaneous detection of multiplexed biomarker panels, which is particularly needed for the clinical realization of inflammatory diagnostics.

1. Introduction

Major advances in the field of biomolecular detection have recently led to improvements in the sensitivity and specificity achievable for the detection of a myriad of biomarker targets. Many of these technologies have been applied to clinical diagnostics, where the analysis of biomarker signatures can be utilized to identify and monitor a wide spectrum of human diseases and disorders [1–5]. Due to their important roles in modulating the immune response and inflammation, cytokines are attracting increasing attention for clinical diagnostics [6–8]. Cytokines, a broadly defined category of small (~5–20 kDa) proteins that include chemokines, interleukins, interferons, lymphokines, and tumor necrosis factors, regulate many types of cellular interactions in response to both self- and non-self antigens. Not surprisingly, alterations of cytokine levels can be diagnostic for a wide variety of maladies, including autoimmune disorders, cancer, and pathogenic infections. Importantly, the low basal concentrations and overlapping functions of cytokines, coupled with the acute nature of many inflammatory conditions conspire to demand high analytical specifications for cytokine-based clinical diagnostics. To fully realize the promise of cytokine-based diagnostics, new technologies that deliver robust and cost effective performance with high sensitivity (pg/mL), specificity, and rapid time-to-result are needed.

Of particular relevance to this manuscript is monocyte chemotactic protein 1 (MCP-1, also known as CCL2). MCP-1 is a low molecular weight (~13 kDa), 76-amino acid protein, that belongs to the CC chemokine family. MCP-1 is implicated in pathogeneses of several diseases such as ischemic cardiomyopathy [9], fibromyalgia syndrome [10], or systemic lupus erythematosus [11]. It is also related to the rare neurological disorder Miller Fisher syndrome [12], and proposed as a biomarker for ovarian cancer [13]. The concentrations of clinical interest of this cytokine vary considerably depending on its clinical application; however, studies have defined MCP-1 cut-off values of 130 pg/mL for fibromyalgia syndrome [10], 187 pg/mL in neonates with hypoxic-ischemic encephalopathy [14], and 718 pg/mL for prognosing ovarian cancer [13].

As an alternative to plate- and bead-based immunoassays, our group has investigated a silicon photonic microring resonator technology that leverages robust and cost effective semiconductor fabrication

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techniques to create a sensitive and modularly multiplexable biomolecular detection platform. Microring resonators are chip-integrated optical microcavities that support the propagation of optical modes that are extremely sensitive to the local refractive index environment. Specifically, photons of a particular wavelength will only propagate in the microring under a tightly held resonance condition.

$$m\lambda = 2\pi r n_{eff} \quad (1)$$

In Eq. (1), m is a non-zero integer, λ is the wavelength of light, r is the radius of the resonator, and n_{eff} is the effective refractive index sampled by the optical mode. Importantly, biomolecular binding events at the microring surface lead to a local change in refractive index, which in turn leads to a shift in the resonance wavelengths supported by the device. The shifts in particular resonance wavelengths can then be tracked for individual sensors and utilized to quantitate unknown amounts of biomolecular targets. Our group has previously demonstrated the applicability of this technology to detect a several different classes of biologically-relevant targets, including proteins, nucleic acids, viruses, and biotoxins [15–18]. We have also demonstrated several different signal enhancement strategies on the silicon photonic platform [19–21] that deliver limits of detection comparable with many commercial immunoassays.

In this manuscript we describe the development of a high-performing silicon photonic immunosensor for MCP-1. Using an enzymatically-enhanced, sandwich immunoassay, we were able to sensitively detect this representative cytokine at sub-pg/mL levels with a relatively rapid (71 min) time-to-result. We demonstrate the ability to quantitate MCP-1 almost 2 orders of magnitude linear range in both buffer and human serum samples. We find minimal matrix effects when detecting in serum with full signal recovery for samples within the assay working range achieved by a simple 10-fold dilution of the sample. Importantly, we show the ability to clearly detect MCP-1 concentrations at the previously defined, clinically-relevant cut-off values for the biomarker. The biologically-relevant performance metrics of this technology, coupled with the capability to perform multiplexed detection, position this technology as an attractive platform for inflammatory cytokine-based clinical diagnostics.

2. Materials and methods

2.1. Instrumentation

Resonance wavelength shifts were monitored using the Maverick Detection System (Genalyte, Inc., San Diego, CA). The pH of all buffers and solutions were measured with an Orion 3-star benchtop pH meter (Thermo Scientific). Data analysis was performed using OriginPro 9.1.0 (OriginLab Corporation, Northampton, MA) and calibration curves were fit with a four-parameter logistic equation using GraphPad Prism 5 for Windows (GraphPad Software, San Diego, CA). Data presented corresponds to the average of at least 16 on-chip technical replicates per concentration of MCP-1.

2.2. Chemical and biochemical reagents

Dulbecco's phosphate buffered saline packets were purchased from Sigma-Aldrich (St. Louis, MO). 3-aminopropyltriethoxysilane (APTES) (cat. num. 80,370), bis[sulfosuccinimidyl] suberate (BS3, cat. num. 21,585), streptavidin-HRP conjugate (cat. num. 21,130), 1-step 4-chloro-1-naphthol (4-CN) solution, and StartingBlock (PBS) blocking buffer (cat. num. 37,538) were purchased from Thermo Scientific. DryCoat assay stabilization reagent was purchased from Virusys (cat. num. AG066-1) and glycerol (cat. num. BP229-1) from Fisher BioReagents. The capture antibody (anti-Human MCP-1 (CCL2), cat. num. 14-7099), detection antibody (biotinylated anti-MCP-1 (CCL2), cat. num. 13-7096), and the target analyte (recombinant human protein MCP-1 (CCL2), cat. num. 14-8398) were purchased from eBioscience (San Diego, CA). The non-specific adsorption control antibody (Mouse IgG, cat. num. ab37355) was purchased from abcam (Cambridge, MA).

2.3. Buffers and solutions

PBS buffer (10 mM) was reconstituted from Dulbecco's phosphate buffered saline packets (D5773 Sigma) and the pH was adjusted to 7.4. The MCP-1 capture antibody was buffer exchanged to 10 mM PBS, followed by addition of glycerol to a final 5% (*v*/*v*) glycerol in PBS. The assay running buffer was 0.5% BSA in 10 mM PBS. All buffer solutions were prepared with purified water (ELGA PURELAB filtration system; Lane End, UK).

2.4. Human serum samples

Certified pooled human serum from healthy individuals was obtained from Innovative research (Novi, MI). The test material was aliquotted upon receipt and stored at −20 °C until to use.

2.5. Silicon photonic microring resonators: sensor substrates and read-out instrumentation

The Maverick M1 optical scanning instrumentation utilized to measure shifts in microring resonance wavelengths and sensor substrates were obtained from Genalyte, Inc. The fabrication of the sensor chip and scanning instrumentation operation used has been described previously [22,23]. The 4 mm × 6 mm silicon-on-insulator chips each contain 128 individually-addressable microrings. Four additional microrings in the fluidic channel, but covered by a fluoropolymer cladding layer, serve as controls to correct for thermal drift. An additional 4 exposed microrings (no cladding layer) lie outside the fluidic channel and serve as leak sensors.

Each individual microring is located next to an adjacent linear waveguide, such that interference between photons circulating the microring and passing down the linear waveguide creates a resonant microcavity that supports optical modes only at specific wavelengths [23]. The configuration of the fabricated chip allows for division of the 128 microrings in two fluidically-addressable flow channels, as defined by a laser cut Mylar gasket that is sandwiched between the chip and Teflon lid. This fluidic design allows two unique samples to be assayed simultaneously.

To measure the resonance wavelength shift associated with the steps of the immunoassay, a tunable external cavity diode laser centered at 1550 nm serially probes each microring individually as a function of time. Resonances were determined as dips in the intensity of light propagating down the linear waveguide past the microring as the laser wavelength is scanned across a suitable spectral window. Relative shifts in resonance wavelength, presented in units of picometers (pm), were recorded as a function of time during each of the immunoassay steps. The data acquisition software enables real-time control subtraction and averaging of active sensor responses.

2.6. Surface functionalization

Before functionalization, sensor chips were briefly rinsed with acetone to remove a protective photoresist coating. Sensor chips were immersed into a 5% (*v*/*v*) solution of APTES (2 mL in acetone, 4 min, with stirring) and then sequentially rinsed in acetone (2 min), isopropanol (2 min), and water. Silanized chips were gently dried under a N_2 stream, reacted with a 5 mM solution of the BS3 crosslinker (20 μL, 2 mg in 700 μL 2 mM acetic acid) for 3 min, and again dried with N_2.

Solutions of capture and control antibodies (0.3 mg/mL, in 10 mM PBS with 5% glycerol) were immobilized onto specific regions in both channels of the chip via microspotting (0.2 μL per drop). Antibody solutions were reacted for 1 h at RT. The chips were then immersed in the blocking solution (600 μL, StartingBlock) for 1 h at RT. After blocking,