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Portable, one-step, and rapid GMR biosensor platform with smartphone interface

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ABSTRACT

Quantitative immunoassay tests in clinical laboratories require trained technicians, take hours to complete with multiple steps, and the instruments used are generally immobile–patient samples have to be sent in to the labs for analysis. This prevents quantitative immunoassay tests to be performed outside laboratory settings. A portable, quantitative immunoassay device will be valuable in rural and resource-limited areas, where access to healthcare is scarce or far away. We have invented Eigen Diagnosis Platform (EDP), a portable quantitative immunoassay platform based on Giant Magnetoresistance (GMR) biosensor technology. The platform does not require a trained technician to operate, and only requires one-step user involvement. It displays quantitative results in less than 15 min after sample insertion, and each test costs less than US$4. The GMR biosensor employed in EDP is capable of detecting multiple biomarkers in one test, enabling a wide array of immune diagnostics to be performed simultaneously. In this paper, we describe the design of EDP, and demonstrate its capability. Multiplexed assay of human immunoglobulin G and M (IgG and IgM) antibodies with EDP achieves sensitivities down to 0.07 and 0.33 nanomolar, respectively. The platform will allow lab testing to be performed in remote areas, and open up applications of immunoassay testing in other non-clinical settings, such as home, school, and office.

1. Introduction

Laboratory health diagnostic platforms have been continuously advancing, taking advantage of a wide variety of transducing mechanisms such as optics (Fan et al., 2008; Haes and Van Duyne, 2002; Kneipp et al., 2010), magnetics (Llandro et al., 2010; Wang and Li, 2008), and electric field effects (Allen et al., 2007; Chen et al., 2011). A lot of these technologies are complicated to use at point-of-care: they require sophisticated laboratory equipment and laboratory technicians to operate. The biological samples measured by these platforms also often need extra processing, for example separating serums from whole blood samples. These requirements contribute to relatively expensive diagnostic cost and slow turnaround time, in addition to being bulky and immobile (Kricka, 1998; Tudos et al., 2001). These barriers limit the applications of emerging new platforms in resource-limited settings, such as in Africa and rural Asia, and also in non-clinical settings such as in homes, offices, or schools. On the other hand, commercially available strip tests, such as home pregnancy tests or Hepatitis tests, are simple to use and relatively inexpensive. However, the assay results are often only binary, or qualitative in nature, limiting its usefulness for in-depth disease analysis or disease monitoring. In addition, some researchers have raised concerns regarding the accuracy of these tests (Adeyemi et al., 2013; Cole et al., 2004; Doshi, 1986).

A diagnostic platform that combines the convenience of a strip test and the quantitative nature of clinical laboratory tests will be crucial in areas where access to healthcare is scarce. It can also reduce the burden of healthcare facilities where laypeople are able check their own health status, without having to visit hospitals or clinics. In this paper, we introduce Eigen Diagnosis Platform (EDP): a portable, quantitative immune diagnostic platform that employs Giant Magnetoresistance (GMR) biosensors. It has a smartphone interface that is simple to operate, and has a result turnaround

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time of only less than 15 min. The simple operation of EDP makes it user-friendly, eliminating the need for a trained laboratory technician to operate the device. EDP employs one-step immunoassay; to operate the test, only one-time user involvement at the beginning of each test is necessary. The platform is also designed to be affordable; each test costs less than US$4 (Table S1). The use of a smartphone allows test results to be recorded, monitored, and transferred to the cloud seamlessly.

The GMR biosensors used in EDP can be customized to detect multiple biomarkers for a range of applications. In this paper, to demonstrate the performance of EDP we measured total human immunoglobulin G and M (IgG and IgM) concentrations simultaneously in one chip. IgG antibodies are found in all bodily fluids (Hoffbrand and Moss, 2015; Kindt et al., 2006). They are the most common antibodies (80% of all antibodies) in the body. IgG antibodies are essential in fighting bacterial and viral infections. IgM antibody is the first type of antibodies made in response to an infection (Hoffbrand and Moss, 2015; Kindt et al., 2006). They also cause other immune system cells to destroy foreign substances. IgM antibody makes up about (5–10%) of all the antibodies in the human body. Monitoring of IgG and IgM antibody levels in the body are important to help diagnose health conditions such as immunodeficiency, infection, autoimmunity, and hypergammaglobulinemia (Buckley, 1987; Dispenzieri et al., 2001; Driessen and van der Burg, 2011).

2. Method

2.1. GMR biosensor

GMR biosensor chips were fabricated as previously described (Gaster et al., 2009; Osterfeld et al., 2008). The chip has dimensions of 1.2 cm × 1 cm. It has an array of 64 (8 × 8) GMR spin valve sensors. Each sensor occupies an area of 100 μm × 100 μm. In-plane spin valve sensors were deposited on Si/SiO_2 substrate. The thickness of each layer from bottom to top is of the type (thickness in nm): Ta (3)/seed layer (4)/PtMn (15)/CoFe (2)/Ru (0.85)/CoFe (2)/Cu (2.3)/CoFe (2)/Cu (1)/Ta (4). The structure was passivated with SiO_2 (10)/Si_3N_4 (20)/SiO_2 (10) to protect the sensors from direct exposure to fluids. The mean resistances of GMR sensors used in the experiment were 1.86 K Ohms with the standard deviation of 73 Ω. The magnetoresistance ratio of the GMR sensors were 4%.

2.2. Sensor surface functionalization

Surface functionalization was done similarly as previously described (Kim et al., 2013). The GMR biosensor chips were triple washed with acetone, methanol, and isopropyl alcohol solution, then blow dried. The chips were exposed to oxygen plasma (PDC-32G Basic Plasma Cleaner, Harrick Plasma) for 1 min to remove remaining organic contaminants. A solution of 1% poly(allylamine hydrochloride) in deionized water was incubated on the chip for 5 min. The chips were then rinsed with deionized water, and heated up to 120 °C on a hotplate. After letting the chip to cool down to room temperature, a solution of 2% poly(ethylene-alt-maleic anhydride) was incubated on the chip for 5 min. The chip was then washed with deionized water, and a 1:1 mixture of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) was incubated on the chip for one hour, then washed with deionized water.

2.3. Capture probe immobilization

The GMR biosensors were spotted with droplets of IgG or IgM capture antibodies. Bovine serum albumin (BSA) was spotted as a negative control. Biotinylated BSA (biotin-BSA) was spotted as a positive control and as a trigger to start data acquisition. Four groups of sensors in a same chip were spotted with 0.8 mg/ml of goat anti-human IgG Fc polyclonal antibody (RnD Systems, G-102-C), 0.8 mg/ml goat anti-human IgM Fc polyclonal antibody (RnD Systems, G-105-C), 1% bovine serum albumin (BSA) solution in phosphate buffered saline (PBS), and 0.1% biotin-BSA in PBS solution. Robotic nanopipetter machine (Scienion) was programmed to spot about 1.5 nL of solution per individual sensor in the GMR biosensor array. The prepared chip was incubated overnight at 4 °C in a humidity chamber. Subsequently, the sensor chip was washed and blocked with 2% BSA in PBS solution for one hour. After blocking, it was washed before use.

2.4. Detection of GMR biosensor signal

GMR biosensors have flicker noise (Hardner et al., 1993). If a readout signal is located at the low frequency, the signal is disturbed by the noise. To reduce the effect by the flicker noise from the GMR biosensor, the signal of interest is extracted by implementing the double modulation technique (de Boer et al., 2007; Han et al., 2007; T. Aytur, 2002). The double modulation is implemented by applying both sinusoidal electric voltage (fc) to the GMR biosensor and external sinusoidal magnetic field (ff). As the sinusoidal voltage (fc) and external magnetic field (ff) excite the GMR biosensors, the frequency spectrum of the output current contains primarily a carrier tone at fc and side tones at fc ± ff. The flicker noise of the sensor is modulated around fc, and the signal of interest is located at fc ± ff as shown in Fig. S2 (b).

2.5. Sensing scheme and signal acquisition

To detect an analyte of interest, EDP employs one-step magnetic sandwich immunoassay on the surface of the GMR biosensor (Fig. 2). A capture antibody that binds specifically to an analyte of interest is immobilized on the sensor surface. A sample mixture to be analyzed which has been mixed with analyte-specific biotinylated detection antibodies and streptavidin-coated MNPs (Miltenyi) is transferred into the reaction chamber on the sensor. An external magnetic field driven by one-inch Helmholtz coil generates magnetic fringing field from MNPs. When sandwiches of antibody-analyte-antibody-MNPs assemble on the sensor surface, the fringing magnetic field induces changes in the magnetoresistance of the GMR biosensor. GMR biosensor is a proximity-based sensor (Hall et al., 2010a); only the fringing field from MNPs that are immobilized on the sensor surface contributes to the change of resistance.

2.6. One-step wash-free immunoassay

To perform sample measurement, 25 μL of a mixture of diluted human IgG and IgM antibodies in 0.05% Tween 20 in PBS buffer (Sigma Aldrich) was mixed with 25 μL of streptavidin-coated superparamagnetic MNPs (Miltenyi, 130-048-101, 46 nm in diameter) and 5 μL mixture of 20 μg/ml of biotinylated anti-IgG Fc detection antibody (Jackson Immunoresearch) and 20 μg/ml of biotinylated anti-IgM Fc detection antibody (Jackson Immunoresearch). The mixture was mixed well, and then added into the reaction well on the chip. The sandwich between the capture probe, target analyte, detection antibody, and MNPs self-assembled on the sensor surface, giving rise to positive sensor signals (Fig. 1). With the one-step assay approach, no washing step is required, and assay runtime can be significantly reduced.