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Portable smartphone quantitation of prostate specific antigen (PSA) in a fluoropolymer microfluidic device

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ABSTRACT

We present a new, power-free and flexible detection system named MCFphone for portable colorimetric and fluorescence quantitative sandwich immunoassay detection of prostate specific antigen (PSA). The MCFphone is composed by a smartphone integrated with a magnifying lens, a simple light source and a miniaturised immunoassay platform, the Microcapillary Film (MCF). The excellent transparency and flat geometry of fluoropolymer MCF allowed quantitation of PSA in the range 0.9 to 60 ng/ml with < 7% precision in 13 min using enzymatic amplification and a chromogenic substrate. The lower limit of detection was further improved from 0.4 to 0.08 ng/ml in whole blood samples with the use of a fluorescence substrate. The MCFphone has shown capable of performing rapid (13 to 22 min total assay time) colorimetric quantitative and highly sensitive fluorescence tests with good %Recovery, which represents a major step in the integration of a new generation of inexpensive and portable microfluidic devices with commercial immunoassay reagents and off-the-shelf smartphone technology.

1. Introduction

Decentralisation of diagnostic testing to near the patient sites is both a trend and a need in clinical diagnostics. Immunoassay platforms (the most common laboratory bioanalytical tool) and detection systems must therefore be adapted for point of care (POC) testing, which requires the ability to design affordable, portable, and user-friendly immunoassay systems capable of rapid and sensitive detection using well established immunoassay chemistries (von Lode, 2005).

Diagnostic tests are routinely used to diagnose and select treatment options for many critical health conditions, including cardiovascular diseases, sepsis, ovarian and prostate cancer, demand quantitation of one or multiple analytes (Altintas et al., 2014; Coelho and Martins, 2012; Loeb and Catalona, 2007; Zhang et al., 2011). Agglutination and lateral flow assays are the most widely used POC immunoassay tests, however these formats are usually qualitative or semi-quantitative, lacking both the sensitivity for many important biomarkers and the ability to perform multiplex analysis (von Lode, 2005). This has driven the development of microfluidic immunoassay platforms (Gervais et al., 2011), combining minimal diffusion distances and high surface-area-to-volume ratios for improved performance, with the use microchannels or beads for rapid and sensitive detection of analytes from small sample volumes (Gervais and Delamarche, 2009; Ikami et al., 2010; Park et al., 2012).

Optical detection is often preferred in POC testing, as it can rapidly and simply provide high-resolution microscopic and macroscopic information (Pierce et al., 2014; Zhu et al., 2013). The recent fall in cost of optoelectronic components now offers cost benefits for portable detection systems. Two most common optical detection techniques used in microfluidic immunoassays are fluorescence and chemiluminescence, due to their excellent sensitivity (Lin et al., 2010). However, the readout equipment used to detect these signals is complex and expensive, typically requiring a fluorescence or confocal microscope or high-sensitivity optical sensors, and therefore not portable or cost-effective for POC use (Myers and Lee, 2008). The opportunity for using simple portable optical detection in microfluidic diagnostics has recently arisen because of the rapid expansion in consumer electronics such as high-performance smartphones cameras that are now ubiquitous, and have driven down the price of high performance digital image sensors combined with portable computers (Zhu et al., 2013). Smartphones are portable, widely available, user-friendly and low cost, and are therefore suitable for integration into a microfluidic

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platform for POC diagnostics.

Examples of microfluidic diagnostic tests based on smartphone measurement include colorimetric detection of *Salmonella* from an immunoagglutination assay (Park et al., 2013); measurement of urine, saliva and sweat pH (Erickson et al., 2014; Shen et al., 2012); quantitation of vitamin D measured using a competitive lateral flow immunoassay (Lee et al., 2014); and prostate specific antigen (PSA) quantitation from a sandwich microfluidic immunoassay with a lower limit of detection (LoD) of 3.2 ng/ml PSA in serum samples (Adel Ahmed and Azzazy, 2013). Most smartphone detection systems reported so far are based on colorimetric detection (Oncescu et al., 2013). Although colorimetric detection is usually more cost-effective, easy-to-use and rapid (Lin et al., 2010), fluorescence detection should present higher sensitivity for quantitative POC diagnostics, allowing low analyte cut off values in small sample volumes which is vital for many clinical biomarkers. Smartphone fluorescence detection has been reported in some bioassays, such as quantification of albumin using a dye based assay (Coskun et al., 2013), in a lateral flow assay (Lee et al., 2013) and finally to detect bacteria using a lateral flow assay with fluorescence nanoparticles (Rajendran et al., 2014). However, smartphone fluorescence detection has not yet been reported in sandwich ELISA systems for accurate quantitation of analytes, and this would bring POC microfluidic diagnostics to a new level of portability and sensitivity.

In this study we present a flexible smartphone based colorimetric and fluorescence detection system, termed the MCFphone, capable of detecting PSA from whole blood in the relevant clinical range in 13 min using colorimetric detection and 22 min using fluorescence detection. PSA is the mostly widely used prostate cancer biomarker, and continuous monitoring of PSA levels in patients with prostate cancer is a vital diagnostic tool. PSA blood levels determination in conjugation with digital rectal examination was approved by the Food and Drugs Administration to test asymptomatic men aged 50 year old with a cut off value of 4 ng/ml of PSA (De Angelis et al., 2007; Institute, 2012). Many studies suggested that prostate cancer mortality can be decreased by early detection, and so screening programs have been proposed utilising PSA quantitation in blood. We propose that a quantitative whole blood PSA sandwich assay in a rapid, sensitive, and portable test device would allow POC prostate cancer monitoring and screening even in remote areas of developing countries where laboratory facilities are limited. The MCFphone detection system could easily be combined with the "Lab-in-a-briefcase" assay platform reported recently (Barbosa et al., 2014), replacing the flatbed scanner readout system and increasing portability, flexibility and sensitivity.

2. Materials and methods

2.1. Materials and reagents

Enzymatic chromogenic and fluorescence products 2,3-diaminophenazine (DAP) and fluorescein isothiocyanate (FITC), dimethyl sulfoxide (DMSO), streptavidin alkaline phosphatase, SIGMA-FASTTM OPD (o-Phenylenediamine dihydrochloride) tablets and FDP (fluorescein diphosphate) were sourced from Sigma-Aldrich (Dorset, UK). High sensitivity streptavidin-HRP was supplied by Thermo Scientific (Lutterworth, UK).

Human kallikrein 3/ Prostate Specific Antigen (PSA) ELISA kit was purchased from R&D Systems (Minneapolis, USA). The kit contained a monoclonal mouse Human Kallikrein 3/PSA antibody (capture antibody), a Human Kallikrein 3/PSA polyclonal biotynilated antibody (detection antibody) and recombinant Human Kallikrein 3/PSA (standard). Phosphate buffered solution (PBS) and Bovine Serum Albumin (BSA) were sourced from Sigma Aldrich, Dorset, UK. PBS pH 7.4, 10 mM was used as the main immunoassay buffer. Carbonate buffer 50 mM pH 8 and Tris Buffer 50 mM pH 9.2 were used for fluorescence detection with alkaline phosphatase. The blocking solution consisted in 3% w/v protease-free BSA diluted in PBS buffer. For washings, PBS with 0.05% v/v of Tween-20 (Sigma-Aldrich, Dorset, UK) was used. The whole blood used was obtained from donation system at Loughborough University, Sports Department, and collected into 5 ml tubes with citrate phosphate as anticoagulant.

The MicroCapillary Film (MCF) platform is fabricated from fluorinated ethylene propylene co-polymer (FEP-Teflon®) by melt-extrusion process by Lamina Dielectrics Ltd. (Billinghurst, West Sussex, UK). The number and internal diameter of the microcapillaries is easily controlled by the design of the die and the operational conditions set during the continuous melt-extrusion process. The two primary light sources used include an Auraglow AG166 Blue LED bulb, from Argos UK and an Ultraviolet Mini Lantern UV Fluorescent purchased from Mapplin UK. A 60x magnification attachment for iPhone® 4/4S purchased from Amazon (Slough, Berkshire) and a 50 mm square dichroic additive green filter sourced from Edmund Optics (York, UK).

2.2. MCFphone – system overview

The MCFphone detection system is composed by a 10 bore fluoropolymer MCF strips pre-coated with immobilized capture antibody and blocked with BSA protein (1), smartphone (iPhone® 4S, 8 megapixels camera) (2) integrated magnifying lens (3), light source (blue LED, with peak wavelength of 450 nm for chromogenic detection) (4), or UV black light for fluorescence detection (5), and a dichroic additive green filter (6) for fluorescence detection (Fig. 1A).

The MCF used consisted of a fluoropolymer melt-extruded plastic film with 10 embedded parallel microcapillaries and a mean 200 μm internal diameter. Each strip has 4.5 ± 0.1 mm width and 0.6 ± 0.05 mm depth (Fig. 1B). This platform was first presented as a cost-effective microfluidic immunoassay platform by Edwards et al. (2011); the hydrophobicity of FEP material allows simple yet effective immobilisation of antibodies by passive adsorption on the plastic surface of the microcapillaries, and the transparency of the MCF material results in high signal-to-noise ratios (Edwards et al., 2011) which is fundamental for sensitive signal quantitation.

The MCFphone working principle consists in illuminating the MCF test strip sample with a light source (blue LED for chromogenic detection and UV black light for fluorescence detection) and capturing the signal (digital image) with a smartphone camera attached with a magnifying lens (Fig. 1C). The digital images were then analysed with *Image J* software (NIH, Maryland, USA) for colourimetric or fluorescence signal quantitation.

2.3. PSA sandwich ELISA (enzyme linked immunosorbent assay)

2.3.1. Fabrication of MCF test strips

A solution of 40 μg/ml of Human Kallikrein 3/PSA capture antibody in PBS buffer was aspirated into a 100 cm lengh MCF and incubated for two hours at room temperature in a petri dish covered with a wet tissue to avoid evaporation of solution in the microcapillaries. A 3% BSA solution in PBS buffer was then aspirated and incubated in the MCF for an additional two hours at room temperature to block any additional binding sites in the microcapillaries. The MCF strip was then washed with 0.05% Tween in PBS, and stored in the fridge at 4 °C or used immediatetly. The MFC was then trimmed into 3 cm long test strips and interfaced with a single 1 ml syringe using a short 3 mm i.d.