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A smartphone-based colorimetric reader for bioanalytical applications using the screen-based bottom illumination provided by gadgets

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ABSTRACT

A smartphone-based colorimetric reader (SBCR) was developed using a Samsung Galaxy SIII mini, a gadget (iPAD mini, iPAD4 or iPhone 5s), integrated with a custom-made dark hood and base holder assembly. The smartphone equipped with a back camera (5 megapixels resolution) was used for colorimetric imaging via the hood and base-holder assembly. A 96- or 24-well microtiter plate (MTP) was positioned on the gadget's screensaver that provides white light-based bottom illumination only in the specific regions corresponding to the bottom of MTP's wells. The pixel intensity of the captured images was determined by an image processing algorithm. The developed SBCR was evaluated and compared with a commercial MTP reader (MTPR) for three model assays: our recently developed human C-reactive protein sandwich enzyme-linked immunosorbent assay (ELISA), horseradish peroxidase direct ELISA, and bicinchoninic acid protein estimation assay. SBCR had the same precision, dynamic range, detection limit and sensitivity as MTPR for all three assays. With advanced microfabrication and data processing, SBCR will become more compact, lighter, inexpensive and enriched with more features. Therefore, SBCR with a remarkable computing power could be an ideal point-of-care (POC) colorimetric detection device for the next-generation of cost-effective POC diagnostics, immunoassays and diversified bioanalytical applications.

1. Introduction

There is an urgent need for cost-effective point-of-care (POC) instruments with homogenous technical requirements as well as more flexible devices for biomarker diagnostics in clinical settings. This is especially critical for emergency, operating theaters, and intensive care units, when medical personnel with different specialties team up and encounter various POC devices and medical information provided by clinical chemistry. Dealing with different instrument settings for diagnostics on top of direct patients' care is an important stress factor for healthcare professionals. Harmonization of POC devices is expected to improve multimodality, efficiency and data integration. Current smartphones with many striking features provide a promising digital platform for point-of-care (POC) diagnostics, mobile healthcare and bioanalytical needs. Such devices are fully automated and equipped with a high resolution camera, a powerful processor with high storage capacity, wireless connectivity, real-time geo-tagging, secure data management, and cloud computing. Some major providers continue offering more features and decreasing costs for such devices in this extremely competitive and lucrative market with 7 billion cellphone users (http://www.itu.int/en/ITU-D/Statistics/). Indeed, smartphones have been advocated and commercialized for health and fitness (http://www.runtastic.com), diabetes and weight management, and blood pressure/pulse rate (http://www.ihealthlabs.com/). More advanced bioanalytical applications include smartphone-based microscopy (Breslauer et al., 2009; Tseng et al., 2010), fluorescent imaging (Zhu et al., 2013a, 2011a, 2011b), imaging cytometry (Zhu et al., 2013b), electrocardiography (http://www.alivecor.com/home), lateral flow assays (Mudanyali et al., 2012; You et al., 2013), surface plasmon resonance-based sensing (Preechaburana et al., 2012), electrochemical sensing (Lillehoj et al., 2013), immunoassays (IAs) (Coskun

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et al., 2013a; Lu et al., 2009; McGeough and O'Driscoll, 2013; Wang et al., 2011; Zhu et al., 2012), and other applications (Lamel et al., 2012; Martinez et al., 2008; Muaremi et al., 2013; Preechaburana et al., 2014; Vashist et al., 2014c). Therefore, smartphones will have a significant impact on healthcare monitoring and management as it will lead to transformative landscape changes in diagnostics by enabling real-time on-site analysis and telemedicine opportunities in remote settings (Ozcan, 2014).

The microtiter plate (MTP)-based enzyme linked immunosorbent assay (ELISA) has been the gold standard in various bioanalytical settings as evident by over 300,000 peer-reviewed publications in the last five decades. In such settings, the MTP-based BCA protein assay is another routine biochemical procedure for protein analysis and determination. Therefore, these two important assays are the highly prospective targets for the developed smartphone-based colorimetric reader (SBCR).

The development of a portable and affordable MTPR is an essential requirement for in vitro diagnostics (IVD) and bioanalytics as it enables the assays to be performed at decentralized and remote settings, where the commercially-available bulky and expensive MTPR is not suitable. During the last decade, there is an exponential use of smartphones in bioanalytical sciences, and healthcare monitoring and management. Some significant efforts have been devoted to arrive at such a prospective compact system. A standard smartphone with several sophisticated features is sold at US$ 300 and could be below US$150 by 2018. There are over 7 billion cellphone users (http://www.itu.int/en/ITU-D/Statistics/), accounting for 85% of the world population. Moreover, 70% of cellphone users are from developing countries with high population. Therefore, the smartphone-based technologies will have an extensive outreach and tremendous potential in healthcare and bioanalytical sciences. The initial research efforts have demonstrated the use of smartphones for the readout of colorimetric (Coskun et al., 2013b; McGeough and O'Driscoll, 2013), fluorescent (Zhu et al., 2013a, 2011a, 2011b), electrochemical (Lillehoj et al., 2013) and SPR signals (Preechaburana et al., 2012). However, most of these approaches are not validated by conventional procedures, a prerequisite for the adoption of any novel approach for routine bioassays.

The manuscript describes an extremely low-cost SBCR using three different commercial gadgets (iPAD mini, iPAD4 and iPhones 5s) integrated with an inexpensive dark hood and a base holder assembly. It will be demonstrated for three model assays: rapid sandwich ELISA for human C-reactive protein (CRP) (Vashist et al., 2014a), direct ELISA for horse radish peroxidase (HRP) and the commercially-available BCA protein estimation assay. The analytical performance of SBCR in terms of dynamic range, sensitivity, precision, and detection limit will be evaluated and compared with that of MTPR.

2. Experimental

2.1. Materials

Tosylated Dynabeads® M-280 (2.8 μm diameter; concentration 30 mg mL^{-1}) and phosphate buffered saline (0.1 M PBS, pH 7.4) were procured from Invitrogen, Germany, while Tween 20 was from Carl Roth GmbH, Germany. The BCA protein assay kit and the Nunc microwell 96-well polystyrene plate (Cat# 12-565-311) were bought from Thermo Fisher Scientific, Germany. The human CRP Duoset kit's (DY1707) components consisting of anti-human CRP capture antibody (Ab), recombinant human CRP and biotinylated anti-human CRP detection Ab, were procured from RnD Systems, USA. The 3,3′,5,5′-tetramethylbenzidine (TMB) enzyme substrate, the stop solution, bovine-serum albumin (BSA), streptavidin-conjugated horseradish peroxidase (SA-HRP), HRP and monoclonal anti-peroxidase were from Sigma-Aldrich, Germany. The magnetic holder (Quadermagnet) containing 24 magnets, each magnet spaced in the center of four MTP wells, was from Supermagnete, Germany; the MTP shaker was from VWR International, Germany; the autoclave was from Systec GmbH, Germany; the MTP reader used was Perkin Elmer Wallac VICTOR 1420 Multilabel Counter. The human whole blood (HQ-Chex Level 2) was purchased from Streck, USA, while the CRP-free human serum was procured from HyTest Ltd., Finland. The anonymized ethylenediaminetetraacetic acid (EDTA) plasma samples of patients were provided by University Hospital Ulm, Ulm, Germany. The smartphone was Samsung Galaxy SIII Mini. The 24-well MTP were made from the 96-well MTP by CO_2 laser cutting (40 W, laser pulses: 850 pulses/in, laser speed: 0.3 in/s, Universal Laser Systems PLS3.60, Vienna, Austria). All buffers and solutions were prepared in autoclaved ultrapure water—DNase and RNase free (Gibco, Germany). The binding and washing buffers used in the developed CRP immunoassay were PBS—0.1% BSA and PBS—0.05% Tween 20, respectively. The working aliquots of commercial lyophilized human CRP were prepared in 20 mM Tris–HCl, pH 8.0 with 0.1% BSA as described in the product brochure. The CRP-spiked human whole blood and serum were prepared by spiking various CRP concentrations in a fixed dilution (1:100) of the respective sample matrix. The EDTA plasma samples of patients were diluted 1:1000 and 1:4000 so that the CRP concentration in such samples ranging from 0.3 to 81 μg mL^{-1} or above falls within the detection range of the developed IA.

The anti-CRP capture Ab was bound to the tosylated Dynabeads® using the standard immobilization procedure provided by the Dynabeads® manufacturer (Invitrogen). The resulting stock solution was stored at 4 °C until needed. The biotinylated anti-CRP detection Ab conjugated to SA-HRP was prepared by adding 1 μL of biotinylated anti-CRP detection Ab (0.5 mg mL^{-1}) and 1 μL of SA-HRP to 2998 μL of the binding buffer followed by incubation for 20 min at room temperature (RT). The resulting bioconjugate was highly stable and could be stored at 4 °C for up to 4 months. The concentration of biotinylated anti-CRP detection Ab used was 0.17 μg mL^{-1}, while SA-HRP dilution employed was 1:3000.

2.2. One-step kinetics-based sandwich ELISA to detect human CRP

The MTP wells were pre-blocked by incubating with 300 μL of 5% BSA for 30 min at RT and subsequently washed five times with 300 μL of the washing buffer. The pre-blocked MTPs could be stored in 0.1 M PBS, pH 7.4 at 4 °C for up to 2 months. They were sequentially dispensed with 2 μL of the diluted stock solution of anti-CRP capture Ab-bound Dynabeads® (diluted 1:10 in binding buffer), 38 μL of the binding buffer and 40 μL of biotinylated anti-CRP detection Ab (0.17 μg mL^{-1}) pre-conjugated to SA-HRP (diluted 1:3000). Finally, 40 μL of CRP (varying concentrations; 0.3–81 ng mL^{-1}) in the PBS-diluted whole blood/serum was added to the respective MTP wells and incubated for 15 min at RT (performed in triplicate). The MTP was then placed on the shaker at 250 rpm for 1 min and on the magnetic holder for 3 min, respectively. The magnetically captured the Dynabead®-bound sandwich immune complex and the excess reagents were obtained by sucking the solution with a 300 μL multi-channel pipette. The magnetically-captured sandwich immune complex-bound Dynabeads® were then washed twice with 300 μL of the washing buffer using the same washing procedure and subsequently suspended in 50 μL of the binding buffer. Thereafter, 100 μL of TMB was added to each MTP well and the enzyme–substrate reaction was allowed to proceed for 4 min followed by adding 50 μL of the stop solution to terminate the reaction. The

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