Biosensors and Bioelectronics 99 (2018) 259–267

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

M_{kit}: A cell migration assay based on microfluidic device and smartphone

Ke Yang[a,b,1], Jiandong Wu[b,1], Hagit Peretz-Soroka[b], Ling Zhu[a], Zhigang Li[a], Yaoshuo Sang[a], Jolly Hipolito[b], Michael Zhang[c], Susy Santos[d], Craig Hillier[c], Ricardo Lobato de Faria[c], Yong Liu[a,2], Francis Lin[b,e,f,g,*,2]

[a] Institute of Applied Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, Anhui, PR China
[b] Department of Physics and Astronomy, University of Manitoba, Winnipeg, MB, Canada
[c] Seven Oaks General Hospital, Winnipeg, MB, Canada
[d] Victoria General Hospital and River Heights/Fort Garry Community areas, Winnipeg, MB, Canada
[e] Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada
[f] Department of Immunology, University of Manitoba, Winnipeg, MB, Canada
[g] Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

ARTICLE INFO

Keywords:
Microfluidic device
Smartphone
Cell functional assay
Cell migration
Chemotaxis

ABSTRACT

Mobile **s**ensing based on the integration of **m**icrofluidic device and **s**martphone, so-called **MS^2** technology, has enabled many applications over recent years, and continues to stimulate growing interest in both research communities and industries. In particular, it has been envisioned that MS^2 technology can be developed for various cell functional assays to enable basic research and clinical applications. Toward this direction, in this paper, we describe the development of a MS^2-based cell functional assay for testing cell migration (the M_{kit}). The system is constructed as an integrated test kit, which includes microfluidic chips, a smartphone-based imaging platform, the phone apps for image capturing and data analysis, and a set of reagent and accessories for performing the cell migration assay. We demonstrated that the M_{kit} can effectively measure purified neutrophil and cancer cell chemotaxis. Furthermore, neutrophil chemotaxis can be tested from a drop of whole blood using the M_{kit} with red blood cell (RBC) lysis. The effects of chemoattractant dose and gradient profile on neutrophil chemotaxis were also tested using the M_{kit}. In addition to research applications, we demonstrated the effective use of the M_{kit} for on-site test at the hospital and for testing clinical samples from chronic obstructive pulmonary disease patient. Thus, this developed M_{kit} provides an easy and integrated experimental platform for cell migration related research and potential medical diagnostic applications.

1. Introduction

Mobile **s**ensing based on the integration of **m**icrofluidic device and **s**martphone, so-called **MS^2** technology, is an emerging and fast-developing research area in recent years (Erickson et al., 2014; Yang et al., 2016). It has been used as a mobile laboratory for a wide range of applications, which include biochemical detection and analysis such as water and food quality analysis, routine health test and disease diagnosis (Yang et al., 2016; Zhang and Liu, 2016). The core components of MS^2 are lab-on-chip (LoC) based analytical technologies in a portable and miniaturized manner, and the mobile sensing and data processing functions offered by the new generation of smartphone. Effective integration of the two key technologies critically empowers MS^2 for many mobile sensing applications. Current applications of MS^2 cover detection of various environmental and health indicators such as pH (Lopez-Ruiz et al., 2014), nitrite (Wang et al., 2015), heavy metal (Chen et al., 2014b; Wang et al., 2014), bacterial contamination (Hutchison et al., 2015; San Park et al., 2013; Zhu et al., 2012), blood glucose (Chun et al., 2014), proteins (Chan et al., 2015; Lillehoj et al., 2013; Preechaburana et al., 2012; You et al., 2013) and other pathogen-associated biomarkers (Fronczek et al., 2014; Stemple et al., 2014; Yeo et al., 2016). Some complicated assays such as enzyme-linked immunosorbent assay (ELISA) (Chen et al., 2014a; Wang et al., 2011) and polymerase chain reaction (PCR) (Jiang et al., 2014; Liao et al., 2016; Stedtfeld et al., 2012) were successfully implemented with the MS^2 systems. Furthermore, MS^2 systems offers advantages in test speed, self-containment and sample to result assay operation, which are required for in field test and point of care (PoC)

* Correspondence to: University of Manitoba, 30A Sifton Rd, 301 Allen Bldg, Winnipeg, MB, Canada R3T 2N2.
E-mail address: flin@physics.umanitoba.ca (F. Lin).
[1] These authors contributed equally.
[2] Co-senior authors.

http://dx.doi.org/10.1016/j.bios.2017.07.064
Received 3 February 2017; Received in revised form 15 July 2017; Accepted 21 July 2017
Available online 27 July 2017
0956-5663/

diagnosis (D'Ambrosio et al., 2015; Hu et al., 2016; Laksanasopin et al., 2015; Mudanyali et al., 2012). Those MS^2 applications integrated sophisticated assay control accessories and sample-chip-phone interfaces, which demonstrate the potential of MS^2 to enable high-level biological applications.

Among the high-level biological applications, we envisioned that MS^2 technology can be applied for various cell functional assays (Yang et al., 2016). To be more specific, here we refer cell functional assays to the in-vitro assays that can qualitatively or quantitatively measure the presence or level of functional activities of live biological cells (e.g. cell adhesion assay; cell migration assay). Indeed, growing efforts have been made to develop compact imaging systems so that cell functional assays can be performed without requiring specialized microscopy facilities. For example, various incubation microscopes were developed so the microscope can be placed inside a conventional incubator for cell imaging or directly control the temperature of the cell assay within the portable microscope (Jin et al., 2015; Pushkarsky et al., 2014; Walzik et al., 2015; Zhang et al., 2015). A highly integrated portable and robotically controlled live cell imaging system was employed for cell migration assay in a microfluidic device (Saito et al., 2016). In addition, USB microscopes or webcams were also used for functional cell and tissue imaging, which significantly lower the costs while maintaining adequate imaging performance (Isikman et al., 2012; Kim et al., 2011, 2012; Lynch et al., 2014; Walzik et al., 2015). We have previously performed cell chemotaxis test using a USB microscope based portable system and a smartphone for remote data monitoring (Wu et al., 2014). More recently, the new generation of smartphones with advanced hardware and software configurations led to growing development of smartphone-based microscopy applications (Wei et al., 2013; Zhu et al., 2013, 2012). Collectively, these previous works support the concept of integrating mobile sensing devices with microfluidic chips and control systems to enable MS^2-based cell functional assays. To our best knowledge, although smartphone-based imaging systems have been applied to image cells (Liu et al., 2014; Skandarajah et al., 2014; Smith et al., 2011; Zhu et al., 2011), they have not been used for cell functional assays.

In this direction, cell migration and chemotaxis assays represent an attractive target application to realize the potential of MS^2 for advanced cell functional assays. Cell migration and chemotaxis are important for many biological and pathological processes such as host defense (de Oliveira et al., 2016; Kolaczkowska and Kubes, 2013), tissue development (Laird et al., 2008), autoimmune disease (Luster et al., 2005) and cancers (Condeelis and Segall, 2003; Friedl and Wolf, 2003). *In-vitro* real-time visualization assays are widely used for cell migration and chemotaxis research, which typically require sophisticated chemical gradient generation, controlling incubation temperature, single cell imaging and quantitative data analysis (Funamoto et al., 2002; Muinonen-Martin et al., 2010). Over the past near two decades, microfluidic devices have become widely used research tools for quantitative cell migration and chemotaxis studies owing to their ability to better control microenvironments, low reagents consumption, simple and automated fluid handling systems (Huang et al., 2009; Jeong et al., 2010, 2013; Li et al., 2016; Sackmann et al., 2014a; Vargas et al., 2014; Wu et al., 2013). Many of these devices can configure chemical gradients without the requirement of external fluid delivery instruments (Ge et al., 2015; Sackmann et al., 2012; Wu et al., 2016). Recently, new microfluidic methods have been developed to allow rapid immune cell chemotaxis test directly from a drop of whole blood by incorporating on-chip cell isolation module (Agrawal et al., 2008; Hamza and Irimia, 2015; Jones et al., 2016; Sackmann et al., 2012, 2014b; Wu et al., 2016). Several studies have also employed microfluidic cell migration systems for disease orientated applications (Butler et al., 2010; Jones et al., 2014; Wu et al., 2015). These developments provide the general background of microfluidics technology for performing portable cell migration and chemotaxis assays on a smartphone platform. Such a platform will enable easy cell migration experiment for scientific research and rapid on-site cell migration test for potential clinical diagnostic applications (e.g. neutrophil chemotaxis test for lung disease diagnosis) without requiring specialized research facilities and skills.

Therefore, in this study we were motivated to construct a MS^2-based cell migration test kit (we name it the M_{kit}) that integrates a new microfluidic device, a smartphone-based portable live cell imaging platform, reagents and accessories for cell migration assay and custom smartphone apps for image acquisition and data analysis. This M_{kit} is the first microfluidic platform coupled with smartphone for cell migration and chemotaxis test. We successfully validated this new M_{kit} by testing chemotaxis of both purified human blood neutrophils and breast cancer cell line. Moreover, the M_{kit} allows rapid chemotaxis test of neutrophils from a drop of whole blood with red blood cell (RBC) lysis. Finally, we demonstrated the effective use of the M_{kit} for on-site test at the hospital and testing patient samples for potential clinical diagnostic applications. Thus, this effective M_{kit} demonstrates the potential of MS^2-based cell functional assays.

2. Materials and methods

2.1. Assembly of M_{kit}

The components of the M_{kit} and the method of cell migration test are illustrated in Fig. 1.

2.1.1. Reagents and supplies

BSA, RBC lysis buffer, FITC-Dextran, N-Formyl-Met-Leu-Phe (fMLP), DME/F12, epidermal growth factor (EGF), Paraformaldehyde (PFA) and Rhodamine 6 G were purchased from Sigma-Aldrich. The EasySep Direct Human Neutrophil Isolation Kit was purchased from STEMCELL Technologies Inc. RPMI-1640, DMEM, Fetal Bovine Serum (FBS) and DPBS were purchased from Fisher Scientific. Pipettors or squeeze pipettes were used for adding reagents and samples to the microfluidic device.

2.1.2. Smartphone-based imaging platform

The smartphone-based imaging platform is illustrated in Fig. 2A–B. A commercial brand smartphone (H30-T100, HUAWEI, China) was

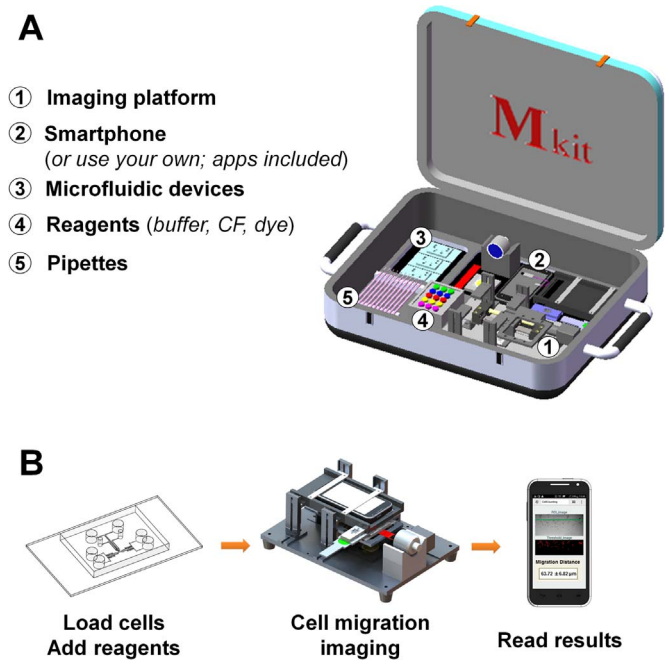

Fig. 1. *Illustration of the* M_{kit}. (A) Components of the M_{kit}; (B) Operation flow of the M_{kit}. CF indicates chemotactic factor.