Sensors and Actuators B 225 (2016) 249–257

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Smartphone-interfaced 3D printed toxicity biosensor integrating bioluminescent "sentinel cells"

Luca Cevenini[a], Maria Maddalena Calabretta[a], Giuseppe Tarantino[a], Elisa Michelini[a,b,*], Aldo Roda[a,b]

[a] Department of Chemistry "Giacomo Ciamician", Alma Mater Studiorum – University of Bologna, Via Selmi 2, Bologna, Italy
[b] INBB, Istituto Nazionale di Biostrutture e Biosistemi, Viale delle Medaglie d'Oro, 305 Rome, Italy

ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form 30 October 2015
Accepted 3 November 2015
Available online 10 November 2015

Keywords:
Smartphone
Bioluminescence
Toxicity assay
Whole-cell biosensor
3D-printing
On-site analysis

ABSTRACT

In this work, we report the design, fabrication, and preliminary assessment of analytical performance of a smartphone-based bioluminescence (BL) whole-cell toxicity biosensor. Genetically engineered human embryonic kidney cells, constitutively expressing a powerful green-emitting luciferase mutant, were used as "sentinel cells" and integrated into 3D printed ready-to-use cartridges, also containing assay reagents. Customizable, low-cost smartphone adaptors were created using a desktop 3D printer to provide a mini-darkbox and an aligned optical interface between the smartphone camera and the cell cartridge for BL signals acquisition. The developed standalone compact device, which also includes disposable droppers for sample and reagents addition, allows the user to perform the toxicity assay within 30 min following the procedure provided by a custom-developed application running on Android (Tox-App). As proof-of-concept we analyzed real samples including ubiquitous products used in everyday life. The results showed good correlation with those obtained with laboratory instrumentation and commercially available toxicity assays, thus supporting potential applications of the proposed device for portable real-life needs.

1. Introduction

The availability of toxicity sensors suitable for rapid field testing is highly valuable also considering the recent global security threats. The routine monitoring of water, food and the environment for chemical and biological threat agents is often hampered by the fact that most of the available techniques, such as those based on high performance liquid chromatography-tandem mass spectrometry, require clean samples and sophisticated equipment, and are thus unsuitable for real-time, cost-effective and on-field testing [1]. Additionally, threats may derive from different sources, therefore conventional analytical methods able to detect one or few analytes are inappropriate [2]. Both enzymatic and microbial biosensors have been developed to detect general toxicity or environmental pollutants including heavy metals, endocrine disruptors, explosives [3–8]. The Microtox® toxicity test, based on the use of bioluminescent *bacteria Vibrio fischeri*, has been considered as the official standard for acute toxicity assay in several countries such as Germany (DIN 38412-1990) and USA (ASTM method D5660-1995) [9]. This test relies on the use of bioluminescent bacteria where the light emission of bacteria after being challenged by a sample is compared to light output of a control sample. The difference between two light outputs is ascribed to the toxic effect of sample. Moreover whole-cell biosensors present the important feature of assessing not the total concentration of a given analyte in a sample but rather its bioavailability, i.e., the fraction of analyte which is able to permeate in the cell membrane and once entered into the cell interacts with specific molecular targets. These systems provide quantitative information about biological effects of a sample such as the case of biosensors for mercury and organic mercury (methylmercury) in water samples [10,11]. All chemical and biological threat agents share the ability to damage in some ways living cells, which can be therefore employed as living "sentinel cells". We previously demonstrated the possibility to integrate microbial cells (including *Escherichia coli*, *Saccharomyces cerevisiae* and *Magnetospirillum gryphiswaldense*) into portable analytical devices relying on the use of charge-coupled device (CCD) detectors [12,13]. The use of mammalian cells, which actually mirror what happens in vivo, would provide a more reliable mean to assess cytotoxicity to humans, as previously demonstrated in proof-of-concept toxicity biosensor devices exploiting

* Corresponding author at: Department of Chemistry "G. Ciamician", University of Bologna, Via Selmi 2, 40126 Bologna, Italy.
E-mail address: elisa.michelini8@unibo.it (E. Michelini).

http://dx.doi.org/10.1016/j.snb.2015.11.017
0925-4005/

eukaryotic cells-lines [14,15]. Recently an automated bench-top mammalian cell-based toxicity sensor was reported incorporating fluidic biochips with endothelial cells and Electric Impedance Sensing (EIS) detection [14]. Such systems, being able to assess the cellular cytotoxic responses, were successfully applied to toxicity test screening and to early warning real-time biomonitor [16]. Despite adequate analytical performance of these biosensors, they still require additional instrumentation and cell culture facilities. Also portable prototype devices require detectors and laptop control computer for data elaboration. In this view, the implementation of an analytical platform requiring only disposable ready-to-use cartridges containing the sensing cells and a smartphone for light detection is extremely appealing. The possibility to run tests that are routinely performed by trained personnel in laboratories with benchtop instrumentation such as microscopes and spectrophotometers with smartphone-interfaced devices offers tremendous potential in those situations in which a rapid and reliable response is needed, for example self-monitoring of chronic pathologies, and for early detection of toxicity and pollutants in water, food and the environment. In contrast to conventional biosensors and point-of-care (POC) systems that require external components like detectors and power supplies, smartphones offer the unique opportunity to have an all-in-one device that integrates a digital camera with portability and wireless data transfer [17]. Ozcan pioneered the concept of cellphone-based devices [18] and applied it to several types of bioassays including lateral flow immunoassay and enzymatic assays for the detection of biomarkers in biological fluids and other bioanalytical applications [19]. Several examples can be cited from the literature, most of them relying on colorimetric or fluorescent assays [20–23]. Interestingly, data connectivity and geotagging capabilities of smartphones can also be exploited for distributed sensing, as demonstrated by Wei et al. who developed a smartphone-based mercury(II) ion sensor platform with ppb sensitivity [24]. We previously demonstrated the feasibility of implementing enzyme-based assays with bio-chemiluminescence detection into smartphones and we fabricated cartridges with facile and low-cost 3D printing technology [25,26].

Nonetheless, to the best of our knowledge, the exploitation of bioluminescent cells as sensing elements in a smartphone-based platform has not been explored yet.

Here we report a compact stand-alone toxicity sensor incorporating bioluminescent cells into a smartphone-based device. We fabricated 3D printed cartridges to integrate an array of bioluminescent cells into ready-to-use cartridges and demonstrated the feasibility to accurately detect and quantify the BL signals. We used human embryonic kidney cells (Hek293T) constitutively expressing a green-emitting luciferase as “sentinel cells” and an Android app was developed to provide a user-friendly environment. Additionally, we obtained a smartphone accessory including pre-loaded cartridges with immobilized cells, reagents’ reservoirs and droppers to provide a ready-to-use device. The analytical performance of the smartphone-biosensor was evaluated with model and real samples.

2. Materials and methods

2.1. Chemicals and reagents

Human embryonic kidney Hek293T cells were from ATCC (American Type Culture Collection, Manassas, VA, USA) and materials used for culturing of cells were from Carlo Erba Reagents (Cornaredo, Milano, Italy). The enzymes required for cloning were from Fermentas (Vilnius, Lithuania). The kits for plasmid extraction and purification and beetle D-luciferin potassium salt were from Promega (Madison, WI, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA).

The mammalian expression plasmid pCDNA-PpyGRTS expressing the *Photinus pyralis* luciferase green thermostable mutant was obtained from vector pGEX-PpyGRTS [27], kindly provided by Prof. Bruce Branchini (Connecticut College, New London, CT), by standard molecular cloning procedures.

2.2. 3D-printed cell minicartridges and smartphone adaptor fabrication

Minicartridges and smartphone adaptors were fabricated using a desktop 3D printer Makerbot Replicator 2X (Makerbot, Boston MA, USA) using thermoplastic acrylonitrile butadiene styrene (ABS) polymer. Two cartridges were designed in this work: the first one was created for calibration purposes using black and white ABS and contains an array of 16 well of 50 μL each (3.5 mm × 3.5 mm × 4.5 mm). The second cartridge (Fig. 1(b)), printed with white ABS, contains 4 wells (volume of about 150 μL each, size 4.5 mm × 4.5 mm × 7.5 mm) and two reservoirs, one for BL reagent and one for control. The cartridge also includes a sliding-lid created with dual-extrusion of black and transparent ABS. The adaptor, which provides a dark box, was designed to fit the Samsung Galaxy Note II smartphone. The open-source Tinkercad browser-based 3D design platform (Autodesk, Inc) was used to create 3D models. MakerWare v.2.4 software was used to set up printing options.

2.3. Android-based application

We developed a custom application (Tox-App) running on Android using Python (https://www.python.org/) and Kivy Open source Python library (http://kivy.org/#home) to convert the camera images into a quantitative and user-friendly output. The Tox-App functions as follows (see Fig. 2):

(a and b) the user selects the TOX icon and then the “start” button to run the application on the smartphone;
(c) in the home page several tabs can be selected; the user can choose among reading the “Procedure”, analyzing a sample with the “test sample” button, or opening previous data using “Select image”. The “Info” box provide information about App developer “Unibo, Laboratory of Analytical and Bioanalytical Chemistry”;
(d) in the “Procedure” box the user can read the instructions to perform the assay and some images provide a quick view of the steps;
(e) the “Begin” button allows to proceed to the “Checklist” box where preset timers guide the user through the steps following the correct incubation time (30 min) before image acquisition. At the end of the countdown the “Acquire” button activates the cellphone camera and the BL image is taken;
(f) by clicking “Analyze” the BL image is rapidly processed on the smartphone within few seconds;
(g) the result is displayed as percentage of “Cell viability” together with a warning message (“Safe”, “Harmful” or “Highly toxic”). Both BL raw image and results can be saved for downstream applications such as sending the results to a central laboratory or cloud computing.

2.4. Cell culture and transient transfections

HEK293T cells were routinely grown in Dulbecco Modified Essential Medium (DMEM high glucose 4,5 g/L, GE Healthcare) supplemented with 10% fetal bovine serum, L-Glutamine 2 mM, 50 U/μL penicillin, and 50 μg/mL streptomycin. FuGENE HD transfection reagent (Promega) was used for transient transfections