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Bioluminescence biosensing platforms for One Health: from paper sensors to thread-based analytical devices

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Abstract. Bioluminescence is a fascinating phenomenon in which photons are emitted as byproduct of a chemical reaction occurring in living organisms, including bacteria, fireflies, and several marine species. Here we report the development of novel bioluminescent tools and strategies which can be used to improve the analytical performance of paper-based smartphone biosensors. In particular two new applications are reported based on paper and thread-based bioluminescent biosensors.

1 Introduction

Bioluminescent systems possess remarkable features, such as high quantum yield and no need for an external light source, that render them highly valuable bioanalytical tools for developing portable biosensors for monitoring molecules, cells, and bioactivities with applications spanning from agro-food to clinical fields. In addition they can be used in combination with smartphones as detectors [1]. Use of smartphones, paper sensing, and 3D printing are important factors that lower cost, promote sustainability, and democratize access to these instruments [2]. Therefore, bioluminescent biosensors have a great potential to support the "One Health" approach, to guarantee health to humans, pets, wildlife and our environment. Here we present two new applications of paper and thread-based bioluminescent biosensors for One Health.

2 Materials and Methods

Paper-based biosensing. A paper-based biosensor was developed by immobilizing naturally bioluminescent *A fischeri* bacteria on a paper support. To this end circular wells (7.0 mm diameter) were created by depositing wax ink on the paper, using a Phaser 8400 office wax printer (Xerox, Norwalk, CT, USA). A very simple analysis procedure was implemented, requiring 50 µl of sample with very short incubation times (from 3 min to 30 min). An Android-based app was developed in Python IDE (integrated development environment) using the Kivy framework.

Thread Biosensing. A bioluminescence thread biosensor in which luciferin and luciferase are immobilized onto two separate cotton threads was obtained. The preliminary tests were performed for detecting ATP, used as an indicator for microbial contaminants in food or hygiene monitoring. In parallel, a thread-based chemiluminescent (CL) device was obtained. In this case, preliminary tests were performed exploiting the enhanced luminol/H_2O_2/horseradish peroxidase (HRP) CL system.

3 Results

Here we report the development of novel bioluminescent tools and strategies which can be used to improve the analytical performance of paper-based smartphone biosensors. BL showed suitable to develop highly sensitive biosensing paper platforms enabling the detection of target analytes down to the ppm and ppb levels in complex biological matrices such as environmental and clinical samples. Cell biosensors, were implemented on paper using the smartphone as detector. The implementation of artificial intelligence algorithms to smartphone-based bioluminescence detection is also reported for the first time. The paper-based toxicity smartphone biosensor which provides, thanks to an Android AI mobile app, quantitative and user-friendly information. A representative picture of the biosensor is shown in Figure 1.

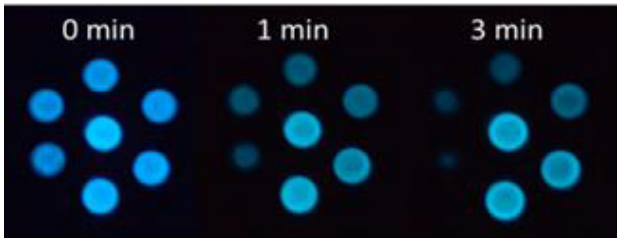

Fig. 1. Dose-response curve for NaClO in drinking water.

We also explored the combination of bioluminescence and chemiluminescence biosensing with microfluidic thread-based analytical devices (µTADs), which represent a sustainable and low-cost alternative to paper based biosensing, especially to handle very low volumes of samples (less than 5 µl), showing great potential also for multiplex analysis in combination with bio-chemiluminescence. We report a proof-of-principle

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application of bio-chemiluminescence biosensing on cotton threads and, to prompt future applications in point-of-care and point-of need settings, we exploited smartphone detection enabling easy detection of the bio-chemiluminescent signal directly on the thread (Figure 2).

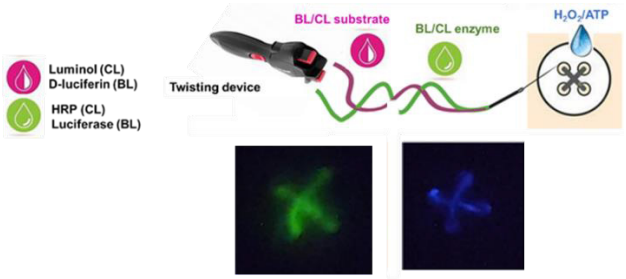

Fig. 2. Schematic representation of the optimized thread-based bio-chemiluminescent biosensor and pictures of luminol/H2O2/horseradish peroxidase and luciferin/ATP/luciferase system obtained with OnePlus 5 smartphone.

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