Biosensors and Bioelectronics 68 (2015) 245–252

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Portable oxidative stress sensor: Dynamic and non-invasive measurements of extracellular H_2O_2 released by algae

Volodymyr B. Koman [a], Christian Santschi [a,*], Nadia R. von Moos [b], Vera I. Slaveykova [b], Olivier J.F. Martin [a]

[a] Nanophotonics and Metrology Laboratory (NAM), Swiss Federal Institute of Technology (EPFL), 1015 Lausanne, Switzerland
[b] Environmental Biogeochemistry and Ecotoxicology, Institute F.-A. Forel, Earth and Environmental Sciences, Faculty of Sciences, University of Geneva, 10, route de Suisse, 1290 Versoix, Switzerland

ARTICLE INFO

Article history:
Received 22 October 2014
Received in revised form
17 December 2014
Accepted 20 December 2014
Available online 23 December 2014

Keywords:
Optical detection
Multiscattering
Cytochrome *c*
Hydrogen peroxide
Toxicity

ABSTRACT

Reactive oxygen species (ROS) generated by aerobic organisms are essential for physiological processes such as cell signaling, apoptosis, immune defense and oxidative stress mechanisms. Unbalanced oxidant/antioxidant budgets are involved in many diseases and, therefore, the sensitive measurement of ROS is of great interest. Here, we present a new device for the real-time monitoring of oxidative stress by measuring one of the most stable ROS, namely hydrogen peroxide (H_2O_2). This portable oxidative stress sensor contains the heme protein cytochrome *c* (cyt *c*) as sensing element whose spectral response enables the detection of H_2O_2 down to a detection limit of 40 nM. This low detection limit is achieved by introducing cyt *c* in a random medium, enabling multiscattering that enhances the optical trajectory through the cyt *c* spot. A contact microspotting technique is used to produce reproducible and reusable cyt *c* spots which are stable for several days. Experiments in static and microfluidic regimes, as well as numerical simulations demonstrate the suitability of the cyt *c*/H_2O_2 reaction system for the real-time sensing of the kinetics of biological processes without H_2O_2 depletion in the measurement chamber. As an example, we detect the release of H_2O_2 from the green alga *Chlamydomonas reinhardtii* exposed to either 180 nM functionalized CdSe/ZnS core shell quantum dots, or to 10 mg/l TiO_2 nanoparticles. The continuous measurement of extracellular H_2O_2 by this optical sensor with high sensitivity is a promising new means for real-time cytotoxicity tests, the investigation of oxidative stress and other physiological cell processes.

1. Introduction

Micropollutants (e.g. trace metals and inorganic nanoparticles) and abiotic factors (e.g. variations in temperature, salinity, UV irradiation) have been shown to negatively affect the cellular homeostasis of oxidants and antioxidants in aquatic microorganisms by enhancing the generation of reactive oxygen species (ROS) (Cap et al., 2012; Mallick and Mohn, 2000; Sies, 1986; von Moos et al., 2014; Xia et al., 2006). An overproduction of ROS can lead to the oxidative damage of subcellular components, including the membrane and DNA, and ultimately to cell death (Apel and Hirt, 2004; Maynard et al., 2006). Hydrogen peroxide (H_2O_2) is one of the most stable ROS, which can diffuse through the cell membrane (Dröge, 2002; Mallick and Mohn, 2000). Other ROS species are not likely to escape outside the cell due to their extremely short lifetimes: while micromolars of H_2O_2 decompose in about 12 h under normal conditions, $^1O_2$, $O_2^{\bullet -}$ and $OH^{\bullet}$ have lifetimes of 4 µs, 1 µs and 1 ns, respectively (Jin et al., 2010). Thus, tracing the kinetics of H_2O_2 in biological systems provides further insight into the mechanisms of oxidative stress (von Moos and Slaveykova, 2014).

ROS in biological systems are commonly detected using endpoint bioassays based on chemoluminescence and fluorescence (Chen et al., 2010, 2012; Jin et al., 2010; Miller et al., 2005). These methods, especially chemoluminescence, are very sensitive but continuous measurements are problematic because of their limited stability and deactivation by photobleaching (Kalyanaraman et al., 2012; Resch-Genger et al., 2008). Moreover, such probes can interfere with intracellular proteins and redox-cycling mechanisms (Kalyanaraman et al., 2012). Quantum dots (Qdots) are an interesting alternative to fluorescent labels thanks to their nanometer size and photostability (Gill et al., 2008; Resch-Genger et al., 2008). However, their limited biocompatibility is a drawback to their application in biological systems (Hoshino et al., 2004;

* Corresponding author.
E-mail address: christian.santschi@epfl.ch (C. Santschi).

http://dx.doi.org/10.1016/j.bios.2014.12.044
0956-5663/

Resch-Genger et al., 2008). The few available examples of real-time measurements mostly involve electrochemical biosensors (Suárez et al., 2013; Wightman, 2006). Yet, these suffer from electrode fouling, lack of long-run stability, and poor selectivity with respect to the analytes (Putzbach and Ronkainen, 2013). Alternatively, we have recently developed a novel optical tool for continuous measurements (Suarez et al., 2012, 2013). A difficulty associated with this approach lies in the fabrication of the sensing elements in a controlled and repeatable manner and the bulky nature of the optical setup.

Here, we present a portable oxidative stress sensor (POSS) for the non-invasive and continuous measurements of H_2O_2. This compact POSS was specifically designed for efficient and easy field analysis. We achieve a limit of detection (LOD) in the nanomolar range by tracing the optical response of spots with a few pmol of cyt *c*. Printing such spots in the porous substrate shows excellent repeatability and reproducibility and enhances their absorption thanks to multiscattering. During measurements, cyt *c* remains in the substrate and does not react directly with the living organisms present in the solution. This configuration has a non-invasive nature, enabling continuous measurements, which can be used to study the underlying dynamics. For calibration purposes, the kinetics of the biosensor response to different H_2O_2 concentrations is studied both in diffusion and flow regimes. For the latter case, we develop a reusable microfluidic device. Numerical simulations reveal no H_2O_2 depletion in the vicinity of the cyt *c* spot, indicating that diffusion effectively repletes H_2O_2 in the measurement chamber.

To demonstrate the potential use of the POSS in biological experiments, we study stress-related H_2O_2 release from the green microalga *C. reinhardtii*, a widespread microorganism present in soil and freshwater throughout the world. It is a primary producer at the base of the food chain and thus highly relevant from an ecotoxicological perspective (Grossman, 2007). To induce oxidative stress, algae were either treated with functionalized CdSe/ZnS core shell Qdots that are widely used as fluorescent labels (Dabbousi et al., 1997) or with TiO_2 nanoparticles, which are common constituents of everyday consumer products (Park et al., 2008). Elevated levels of H_2O_2 were successfully detected by means of the POSS. Hence, it is a promising new approach for the study of the mechanisms of oxidative stress in particular and of the interactions between nanomaterials and living organisms in general.

2. Materials and methods

2.1. Preparation of sensing spots

A microarray robot (QArray2, Genetix) was used to spot 4 mM aqueous cyt *c* (C2037, Sigma Aldrich) from a 384-well plate into the substrate – a filtration membrane (GSWP 220 nm, Millipore) – by a spotting pin (946MP8XB, Arrayit) with a delivery volume of 5 nl. Cyt *c* was partially crosslinked by exposing the freshly printed spots to glutaraldehyde (G5882, Sigma Aldrich) vapor for 1 h. The prepared samples were stored in water at 4 °C. To fully reduce the spots prior utilization, they were immersed into 1 ml of 10 μg/ml aqueous ascorbic acid (AA) solution for 30 min. The spots were subsequently washed 5 times for 10 min each in 1 ml distilled water.

2.2. Experimental design

For measurements in the static regime, a small chamber, delimited by an O-ring (9×1 mm^2, volume = 60 μl, No. 860110.0525, BRW), was filled with the solution of interest and the membrane containing the printed spot (Fig. 1a). The chamber was sealed with a cover slip and any excess liquid was removed. For every measurement a new O-ring and a new spot were used.

In the flow regime, the sensor spots were integrated into a microfluidic channel (0.3×1 mm^2 cross-section area and 4 mm length) fabricated in PDMS by molds prepared using standard photolithography (Fig. 1b). To guarantee an optimal tightness of the microfluidic channels, an additional PDMS layer (100 μm thickness) containing a hole to accommodate the porous membrane was added. These two PDMS layers along with the glass substrate were then clamped between two metallic plates. In order to optimize the performance of the sensor, the spot was aligned with respect to the center axis of the channel.

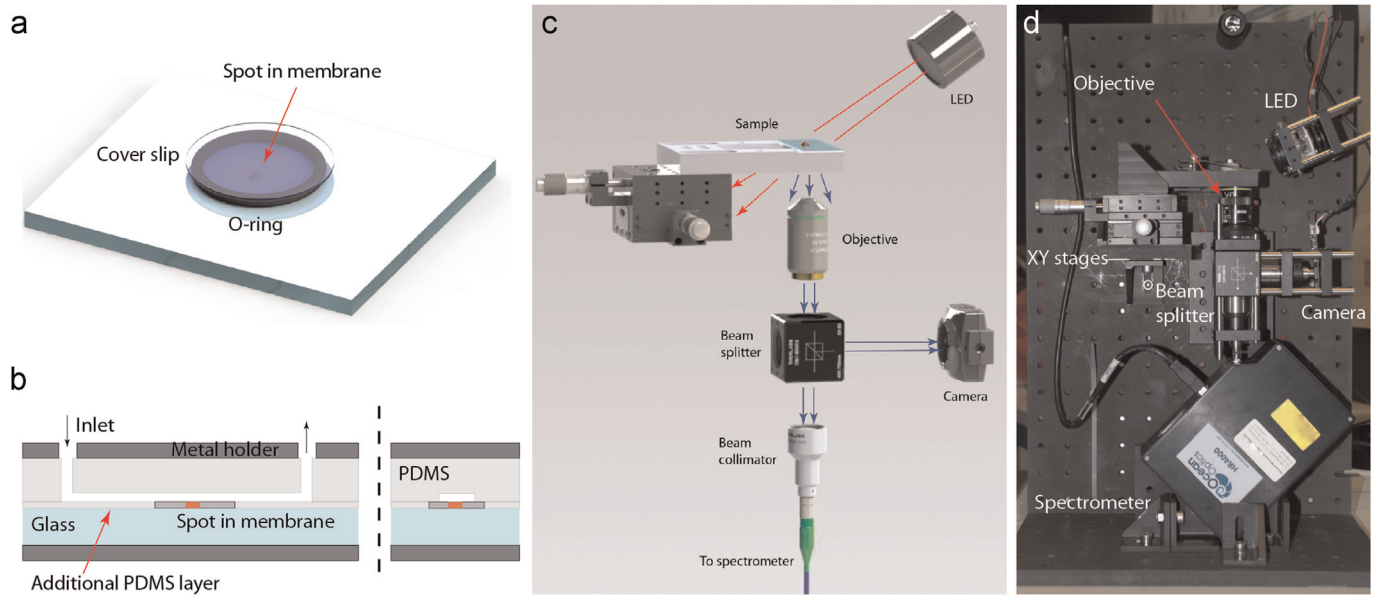

Fig. 1. Setup of the POSS. (a) Schematic illustration of the static regime configuration: the membrane containing the spot is placed onto the glass substrate in the O-ring. (b) Schematic illustration of the microfluidic configuration in the flow regime (side and front views). (c) Schematic drawing of the portable setup. For the sake of clarity, only the main elements are shown. Red arrows represent the light from the LED whereas blue arrows indicate the light scattered by the sample. (d) Picture of the portable setup (front view). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)