AN ENZYME-FREE DIGITAL BIOSENSOR FOR DETECTION OF REACTIVE OXYGEN SPECIES

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ABSTRACT

This work presents the design, fabrication and testing of a novel plastic biosensor which utilizes a stimuli responsive polymer for early detection of circulating hydroperoxides in blood. The system, is composed of interdigitated microelectrodes (IDME) coated with a thin film of a responsive polymer which is sensitive to reactive oxygen species (ROS). In presence of hydroperoxides such as lipid hydroperoxide, an early biomarker for atherosclerosis, the morphology of polymer on the surface of the IDME changes as a result of ROS generated from the decomposition of hydroperoxides. This generates a measurable electrical signal that correlates with the amount of hydroperoxides present in the sample.

KEYWORDS: Reactive oxygen species, Interdigitated microelectrodes

INTRODUCTION

Hydroperoxides such as lipid hydroperoxides are the primary biomarker of lipid oxidation, which lead to cell damage, inflammation and accumulation of lipid-loaded macrophages, key mediators in development of atherosclerosis [1]. Studies have clearly shown that elevated levels of circulating lipid hydroperoxides can predict cardiovascular events in patients with a history of cardiovascular diseases [2, 3]. Conventional fluorescence assays to quantify the amount of hydroperoxides in blood are expensive, require advanced instrumentation and are not suitable for routine clinical monitoring. In this work we have developed an enzyme free impedimetric plastic-based biosensor, termed ROC, which incorporate a novel ROS responsive polymer for the detection of circulating hydroperoxides in a point of care (POC) manner. ROC is composed of interdigitated microelectrodes coated with a thin film of a novel ROS responsive polymer. The backbone of ROS responsive polymer is cleaved in presence of ROS generated from the decomposition of hydroperoxides in presence of iron. Upon interaction of ROS molecules with the ROS sensitive polymer immobilized on the surface of the ROC electrodes, the electrical property of the sensor changes as a result of polymer degradation.

THEORY

The sensor for ROS detection consists of an array of interdigitated microelectrodes coated with ROS responsive polymer and was fabricated using microfabrication technologies. The sensor measures the change in polymer morphology in presence of ROS by performing impedance measurements on the IDME. The schematic for synthesis of ROS responsive polymer is shown in fig. 1a. The degradation of ROS responsive polymer is facilitated through cleavage of the thiocarbamate bonds in the crosslinking molecules in presence of ROS, resulting in decrease in network crosslinking density and polymer degradation. The schematics of fabrication process is shown in figure 1b. In order to fabricate a functional ROS responsive sensor, ROS pre-polymer solution was placed directly onto the sensor array and was allowed to polymerize overnight at room temperature. The sensor configuration allows the direct measurement of any variation in the electrical properties of the system as a result of polymer degradation that can be directly correlated with the amount of ROS present in the sample.
Figure 1: (a) Schematic of the synthesis of 3D ROS responsive polymer. (b) Schematics of sensor fabrication process and photo of the finished sensor.

EXPERIMENTAL

First, the degradation rate of ROS responsive polymer was evaluated by measuring its weight loss in the presence of varying concentrations of ROS generated from fenton reagents. The surface morphology of the polymer after exposure to ROS was also examined via scanning electron microscopy (SEM). Furthermore, the polymer-based ROC sensor described above was utilized to detect known amount of hydroperoxide in PBS in the presence of iron to determine the optimal time point and frequency to increase sensor sensitivity. Finally, the biosensor was utilized to measure known amount of hydroperoxide (H$_2$O$_2$) spiked fetal bovine blood serum (FBS) in presence of electrolyte solution, where the response of the sensor was recorded in optimal frequency and time obtained from previous experiment.

RESULTS AND DISCUSSION

Figure 2a indicates the polymer mass loss after exposure to varying concentration of hydroperoxides. Direct visualization of the polymer morphology by SEM can detail any significant changes in the morphology of the polymer after exposure to ROS. Representative SEM images of the polymer after 20 minutes exposure to electrolyte solution in absence and presence of 1mM H$_2$O$_2$ are presented in figure 2b,c. The results from SEM images clearly shows the appearance of micro/macro pores and significant morphology changes after exposure to ROS compared with that of polymer exposed to buffer solutions that does not contain ROS.

Figure 2: (a) Polymer mass loss after exposure to varying concentration of hydroperoxides. SEM Pictures of the ROS responsive polymer (b) before and (c) after exposure to ROS species.
The results obtained from impedimetric response analysis of ROC sensor indicated that the sensor has higher sensitivity at 10kHz (Figure 3a) and the electrical response of the sensor linearly increased with the concentration of hydroperoxide in FBS (Figure 3b). This results indicate that the change in impedance directly correlated with the amount of H$_2$O$_2$ and linearly decreased as H$_2$O$_2$ concentration increased. The sensitivity of the sensor can be further optimized by decreasing the thickness of the polymer coating on the surface of the interdigitated microelectrodes or increasing the number of electrodes in each array.

**CONCLUSION**

We have developed a novel ROS responsive polymer-based interdigitated plastic biosensor for assay-free detection of hydroperoxide in the blood for early detection and prevention of associated diseases such as atherosclerosis. ROC is an impedance spectroscopy (IS) based sensor, which represents one of the most powerful methods, for POC diagnostics due to their ability to be miniaturized without the need of high-energy sources and ease of output reading. More importantly this technology introduces a new strategy for developing long-lived and cost effective enzyme-free digital biosensors by incorporating biologically responsive polymers. Beside the inexpensive production, these sensors offer enzyme and label-free detection mechanism for fast and reliable measurement without the need for instrumentation or skilled personnel.

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