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Solid-state sensing tip for zinc ion with double parallel optical fibers embedded in fluorescent hydrogel

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ABSTRACT

A tip-shaped zinc ion solid-state sensor is made by two parallel optical fibers embedded closely in a sensing hydrogel film. The film is made of poly(2-hydroxyethyl methacrylate) (poly HEMA)hydrogel mixed with the selective fluorescent probe meso-2,6-Dichlorophenyltripyrrinone (TPN-Cl_2) with weight ratio of 0.025 wt%. A 405 nm laser output is sent from one fiber and the 622 nm fluorescence of the doped hydrogel is collected by the second fiber. Each fiber diameter is 370 μm (core is 300 μm), whose sum is roughly the tip diameter. The 0.4 cm by 0.5 cm tip has real-time response for zinc ion concentration over 10^{-6} M, with marginal signal for 10^{-7} M. The tip is inserted inside an oyster and successfully detects the zinc ions, showing that the sensor works in complex body fluid and tolerates certain mechanical stress. To show the potential application for medicine, the sensing film is applied for primary neuronal cultures. We report for the first time zinc ions release at concentration levels 10^{-6}–10^{-7} M to the medium under stress conditions of ischemia, inflammation, and intoxication. Furthermore, this correlates with the zinc levels detected by biochemical assay. Such sensing tip has great potential for biomedical monitoring ex vivo or in vivo.

1. Introduction

There is currently a great effort to develop solid-state real-time sensor for the aqueous chemicals in living organisms [1–3]. The functions of animals and plants are regulated by various biochemical messengers. The real-time monitoring of those chemicals is of vital importance for the biology and medicine. Most of the solid-state biochemical sensors are made in the form of a chip, i.e. a planar structure with a sensing surface whose physical properties respond to the surrounding chemical conditions [4,5]. Such sensing chips are convenient to use for open samples like cultured cells or explant tissues, their size and geometry are however difficult for detections inside the body or organ tissues. There has been an increasing demand to acquire the dynamical chemical status inside the living body such that the doctors will have the real-time information on the organs of patients at severe failure conditions. Furthermore, the real-time chemical information in situ inside the body is of crucial importance for the surgeons during operation. In order to meet this demand for in situ biochemical sensing, a sensor with tip shape is much better than the more common ship shape. Specifically, if the tip diameter can be made less than 1.3 mm (~18 gauge needle), it can be readily implantable via subcutaneous, intravascular or even intrathecal route in the body for true real-time monitoring. So far there is little report on such tip-shaped real-time solid-state sensor for one of the key ions [6–10].

In this work we study a tip-shaped zinc ion sensor based on two parallel optical fibers embedded in a fluorescent sensing film. The film is made of a mixture of hydrogel and a specific chemical probe

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whose fluorescence respond to the surrounding zinc ion concentration in real time, partly due to the high water permeability of hydrogel. One of the optical fibers sends the excitation light to the sensing film, whereas the other optical fiber collects the fluorescence signal. Because of the small diameter of the fibers the sensor can be made in a tip shape with total diameter below 1.3 mm in principle. The question is whether this double-fiber tip is able to collect the small fluorescence change at low zinc ion concentration. This concept can be extended to other key metal ions, neurotransmitter or biological relevant substances in the body. Zinc ion however is a good example as it plays vital roles in both the physiology and pathophysiology of brain function [11,12].

Using poly(2-hydroxyethyl methacrylate) (poly HEMA) as the hydrogel host and meso-2,6-Dichlorophenyltripyrrinone (TPN-Cl_2) as the fluorescent zinc ion probe 13 doped in the host, the sensing film is able to detect zinc ion at concentration as low as 10^{-7} M in deionized water (DI water) and 10^{-6} M in the cell culture aqueous medium Dulbecco's modified Eagle medium (DMEM). When the excitation fiber and collection fiber are embedded inside the sensing film, the sensing tip detects clearly the zinc ion at 10^{-6} M whereas the signal for 10^{-7} M is marginal. Large signal is obtained when this sensing tip is inserted into an oyster. In addition, the sensing film is shown to be able to detect the low concentration of zinc ion released by primary neuronal cells under various stress models for ischemia, inflammation, and intoxication. This highlights the potential of these sensors for future biomedical and clinical applications.

2. Zinc ion sensing film

2.1. Film fabrication

The probe molecule TPN-Cl_2 has high selectivity to the zinc ion Zn^{2+} [13]. After capturing zinc ion there is a strong increase in the red fluorescence in solution as shown in Fig. 1(a and b), together with its absorption spectrum in methanol solution. TPN-Cl_2 has been previously mixed with hydrogel with fiber structure, made by electro-spinning to enhance the surface area [13]. Here TPN-Cl_2 is mixed with the hydrogel polymer poly HEMA to form the solid film rather than electro-spun fibers for robust integration with optical fibers below. Despite of the high surface area, the electro-spinning fibers do not have enough hardness for a reliable sensing tip. The absorption and fluorescence spectrum of the solid poly HEMA film containing the TPN-Cl_2 probe are shown in Fig. 1c. The poly HEMA with molecular weight of 300 kDa is purchased from Sigma–Aldrich. It is dissolved together with the probe TPN-Cl_2 in dimethylformamide (DMF). The weight ratio of TPN-Cl_2 to poly HEMA is 2.5×10^{-2} wt%, the total solid weight concentration in the DMF solution is 30 wt%. Magnetic stirrer is applied to agitate the host solution at 80 °C for 12 h. Then TPN-Cl_2 powder is added with an additional stirring for 20 min at 80 °C. The mixed DMF solution is dropped into the Teflon mold, followed by annealing at 80 °C for 12 h to form a solid hydrogel.

2.2. PL results

The solid sensing film alone is studied first. The changing red fluorescence of the solid sensing film peaked around 622 nm is shown in Fig. 2(a–e). The photoluminescence (PL) is taken by a F-4500 fluorescence spectrophotometer. The film is immersed in DI water with a plastic container. The solid film size is about 14 mm × 14 mm × 0.4–0.5 mm. Before the addition of the zinc ion the hydrogel sensing film is immersed in DI water for 2 h for stabilization. This period is necessary as the water permeation through the dry hydrogel film takes a long time. Without such initial stabilization time the PL data will change due to the swelling of the sensing film. After this period, zinc ions at various concentrations are added to the DI water in the form of Zinc acetate salt. Before the zinc ion addition the remaining liquid in the plastic container are sucked away by a syringe and the new liquid with fixed zinc ion concentration is added to the container by another syringe. In between two zinc ion concentrations DI water is added as a reference. As shown in Fig. 2f, there is an increase in the PL peaked at 622 nm for zinc ion as low as 10^{-7} M. The PL spectra at various times are shown for all zinc ion concentrations and the intermediate DI water period. In Fig. 2f, the peak value at 622 nm is plotted against time. Even though the slopes for 10^{-7} M and 10^{-6} M are small, the difference to DI water is certain. The slope becomes large for higher concentration like 10^{-5} M and 10^{-4} M. The pictures of the sensing film in the container before and after the total experimental time of 290 min are shown in Fig. 2f. The different colors reflect some changes in not only the fluorescence but also the absorption spectrum after the zinc ion reaction. The reaction of the probe TPN-Cl_2 with zinc ion is irreversible. The film however can be reused as long as the reaction is not saturated. For example the film still responds to 10^{-6} M of zinc ions after a detection of 10^{-6} M ions as shown in Fig. 3. Furthermore, the film also has good stability of heat. As shown in Figure S1, while the film is working at 37 °C, the responsibility is similar to it is working at room temperature.

2.3. Film in cell culture medium

To check the bio-compatibility, the sensing film is immersed in the cell culture medium high-glucose DMEM with serum-free supplement (N_2, Gibco), indicated by DMEM for simplicity below. The cell culture medium provides the living cell the necessary ingredient to stay alive. Therefore in order to be applied to cell study the sensing film has to work in not only DI water but also the aqueous cell medium. In other words, the culture medium should not interfere the key chemical reaction of the probe TPN-Cl_2 with the zinc ion. Such compatibility with DMEM has been proved for TPN-Cl_2 in electro-spinning hydrogel film [13]. Here we demonstrate this for the solid hydrogel film. The results are shown in Figure S2(a–f). The PL peak values at 622 nm are plotted against time for the sensing film in high-glucose DMEM with N_2 in Fig. 4. The typical PL spectra at zinc ion of 10^{-4} M are also shown. The spectral shape does not change as DI water is replaced by DMEM. In the time-dependence 10^{-6} M zinc ion is detected only after about 100 min of reaction, slower than the case of DI water where reaction is seen after 10 min of reaction as shown in Fig. 2(a–f). This difference in response speed suggests that the complex composition of DMEM still block the zinc ion reaction to some extent. There are many salts in DMEM. Some of the metal ions from the salt like Magnesium ion may have a small background fluorescence signal and interfere with the zinc ion signal at low concentration. In addition, the cell culture medium like DMEM contains many organic ingredients and has a higher viscosity, which may also block the diffusion of the zinc ions through the poly HEMA film. In particular, 10^{-7} M zinc ion concentration is no longer detected in case of DMEM. For higher concentration as 10^{-5} M and 10^{-4} M the blocking effect of DMEM is not significant. The sensitivity of the sensing film in various aqueous media is determined by the reaction rate k, i.e. probability per unit time, of a zinc ion to be captured by the probe TPN-Cl_2. The fluorescence signal is proportional to the concentration of the captured zinc ion, which is in term proportional to the product of the free zinc ion and k. In DI water the lower limit of free zinc ion is 10^{-7} M, whereas in DMEM medium the lower limit becomes 10^{-6} M. Since they give the same marginal captured zinc ion concentration, the k in DI water is expected to be larger than the k in DMEM by a factor of 10.