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Short communication

A microfluidic device with integrated coaxial nanofibre membranes for optical determination of glucose

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ABSTRACT

In this work, a multifunctional material with a core–shell structure containing an inner optical oxygen transducer (PdTFPP) has been successfully used for the immobilization of glucose oxidase on its outer surface and the subsequent determination of glucose. The material was fabricated by co-electrospinning and immobilizing the enzyme by physical adsorption. The sensing mechanism is based on glucose oxidase oxidation of glucose that creates a localized decrease in the dissolved oxygen amount and consequently produces a measurable increase in the luminescence intensity of the inner oxygen transducer. The material was applied to detect glucose at room temperature, and exhibited a good luminescent response.

Furthermore, this coaxial material was integrated into a microfluidic chip and its sensitivity to glucose was tested (LOD of 35 μM and LOQ of 105 μM), obtaining higher sensing properties than using the membrane alone under ambient conditions. This improvement in the sensing response can be explained by considering that the chip limits the oxygen transfer from the ambient air to the coaxial membrane, creating a more controlled environment in where to carry out the measurements. Therefore, the combination of this core–shell material with microfluidic devices could have great potential in the fabrication of oxygen dependent optical biosensors.

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1. Introduction

Microfluidic devices have shown their high potential as micro- and nano-scale platforms with applications in many areas such as biology, physics, chemistry and biomedicine [1–7]. The use of these devices often allows a decrease in sample and reagent volumes and faster analyte detection, and provides a well-controlled microenvironment, automation and high portability [8–10]. Moreover, the integration of different detection techniques into microfluidic devices can create device systems for multiplexed sensing and detection, and makes it possible to achieve higher sensitivity using very small sample volumes [11–14].

The fast development of nanotechnology has provided a wide variety of possibilities in the design and fabrication of new nanomaterials with different and tuneable properties, which generally exhibit enhanced performance compared to their large scale counterparts [15–18]. More precisely, the use of new nanomaterials in the field of sensing and biosensing has meant a high boost for the development of new multifunctional devices with improved features such as high sensitivity and selectivity, improved resolution, fast response, biocompatibility, and reusability [19–21]. In addition, these new sensors can take an assortment of forms depending on the particular application, from carbon nanotubes used for the detection of DNA or the delivery of small drug molecules [22–26], to nanoparticles and nanofibres employed in bioimaging and for the enzymatic or non-enzymatic determination of a high variety of biomolecules [27–33].

The integration of nanomaterials into microfluidic devices has allowed the combination of the excellent advantages and properties of both the nanomaterials with the unique and distinctive characteristics of microfluidic technology [34–38].

Among the materials used in the fabrication of microfluidic chips, silicon, glass and polymers are the most popular [39–43]. The selection of the material is a key step for the performance and function of the chips, and has to be based on the requirements and features of the specific application. Thus, properties of the materials such as stability, compatibility with solvents, chemi-

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cal inertness, non-toxicity, biocompatibility, optical transparency, and high-pressure resistance are often taken into account for the fabrication of microfluidic chips [44–47].

In this study, we have used a core–shell nanofibre membrane with an inner optical oxygen dye as a support and transducer material for the immobilization of glucose oxidase which has been successfully used for the optical determination of glucose. We have previously demonstrated the capability of this nanofibre membrane in the development of an optical oxygen transduction biosensor for the analysis of uric acid in serum [33]. Here we demonstrate that the nanofibre based biosensor can be implemented into a microfluidic chip for the detection and quantification of glucose as well as that this implementation provides an improvement in its sensitivity and range.

2. Experimental

2.1. Instrumentation

A Varioskan® Flash spectral scanning multimode reader with temperature control was used in all the luminescence measurements. The luminescence intensity was measured at an excitation wavelength of 405 nm, emission wavelength of 668 nm, bandwidth of 5 nm, delay time of 200 μs, and a measurement time of 100 ms.

2.2. Fabrication of coaxial membranes

The preparation of the coaxial membrane was made following the procedure previously described by our research group [33]. Briefly, two different solutions were used: a solution of PolymBlend® (10%, w/w) in DMF was used for the outer fibre and PMMA (6% w/w) with PdTFPP (1%, w/w) in DMF was used for the inner fibre. These solutions were independently driven by two syringe pumps (Cole Parmer 74900 Series) using flow-rates of 1.3 and 0.3 mL h^{-1} for the outer and inner solutions, respectively, establishing a potential difference between the injector and the collector, of 8.9 kV and −1.3 kV with respect to ground, respectively. The process was run for 8 h to obtain membranes with thickness about 150 μm.

2.3. Preparation of the microfluidic chip

The microfluidic chip was made out of optically clear chemically inert poly(methyl methacrylate) (PMMA) substrate (2 mm). 8 parallel channels were accommodated in a geometry mimicking 96-well microtiter plates in order to fit the Varioskan® instrument, allowing easy readout of the coaxial membrane. Sensing cell chambers (diameter = 7 mm, depth = 0.2 mm) were positioned 10 mm from one another. Each chamber had an inlet and outlet channel (overall length = 30 mm, depth = 0.1 mm, width = 2.5 mm). Channels and chambers have been CO_2-laser cut out of the double sided medical grade adhesive substrate (125 μm) (LG, South Korea) and secured between two 2 mm thick PMMA plates (one plate had CO_2-laser cut inlet and outlet holes). The chip was assembled from bottom up – first securing the double sided adhesive onto the bottom PMMA plate, followed by the insertion of the coaxial membrane into the dedicated chamber and closing the chip with the top PMMA plate. Finally, pressure was applied to the assembly in order to ensure a tight fit and prevent any slippage or leakages. Fig. 1 shows a schematic representation of the microfluidic chip.

2.4. Immobilization of glucose oxidase

The immobilization of glucose oxidase on to the coaxial membrane was carried out by incubating directly membrane disks (6 mm dia.) with 10 μL of a glucose oxidase solution (1 mg mL^{-1}) in PBS (100 mM, pH = 7.4) for 15 min at room temperature. After this time, the excess solution was rinsed with 10 mL water.

The amount of immobilized GOx onto the coaxial material was determined from the difference between the initial free enzyme amount and the total enzyme amount which is in the washing solution after immobilization. The enzyme concentration in the washing solution was measured by a spectrofluorometric method at 348 nm [48].

The relative activity (%) of the immobilized GOx was calculated by a colorimetric method based on two enzymatic reactions [48]. First, GOx catalyses the oxidation of D-glucose to gluconic acid and hydrogen peroxide. Next, in the presence of peroxidase (HRP), the released hydrogen peroxide reacts with sodium 3,5-dichloro-2-hydroxybenzenesulfonate (DCHBS) and 4-aminoantipyrine (4-AAP), producing a red quinoneimine dye with an absorption maximum at 515 nm. The relative activity of GOx was calculated as a percentage of the immobilized GOx referred to free GOx activities.

In detail, to determine the relative activity of the immobilized GOx, 3 mL PBS (100 mM, pH 7.4) containing 5 μmol D-glucose was incubated with the membrane disks with immobilized GOx for 15 min at 37 °C. Next, 0.5 mL 4-AAP (10 mM), 0.5 mL DCHBS (50 mM) and 0.1 mL HRP (20 U mL^{-1}) in PBS (100 mM, pH 7.4) were added and kept at 37 °C for 5 min to develop the colour.

2.5. Measurements

Two kinds of experiments were carried out. First, the coaxial membrane with the immobilized glucose oxidase was attached to a black vinyl support substrate and then the luminescence measurements were carried out after adding 20 μL of glucose solution in PBS (100 mM, pH = 7.4) to the coaxial membrane. Second, the membrane with the immobilized enzyme was integrated into the microfluidic chip and the measurements were carried out after flowing 50 μL of glucose solution in PBS (100 mM, pH = 7.4) through the channel of the chip. Experimental temperature was maintained at 25 °C during all the measurements.

The detection mechanism for glucose is based on the increased measurement of the luminescence intensity of the embedded oxygen dye due to the decrease in the localized dissolved oxygen concentration as a result of the selective oxidation of glucose catalyzed by GOx. Therefore, an increase in the concentration of glucose produces an increase in the luminescence intensity which is proportionally related with the glucose concentration. To avoid large analysis times, the slope of this signal was used instead of the equilibrium final luminescence signal. Thus, the sensing response was calculated as the increase in the slope (see Eq. (1)) once the glucose solution was added,

$$\text{Sensing response} = \frac{S_x - S_0}{t_x} \tag{1}$$

where S_0 is the luminescence intensity of the material before the addition of glucose, S_x is the signal after the addition of glucose and t_x is the time in which S_x was obtained; t_x = 60 s was used in this study. Although, the sensing response increases when the concentration of glucose increases (due to the oxygen concentration decreasing), this increase is not linear. This is due to the measurement of glucose being made indirectly by measuring the concentration of O_2. It is well known that the luminescence decreasing with the O_2 concentration is not always linear and for this reason is not possible to use the Stern–Volmer equation and a more complex scenario such as Demas or Lehrer two-site models needs to be used [49]. The equation for the Demas model is:

$$\frac{I_0}{I} = \left[\frac{f_1}{1 + k_{SV,1}\text{pO}_2} + \frac{f_2}{1 + k_{SV,2}\text{pO}_2}\right]^{-1} \tag{2}$$

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