Talanta 84 (2011) 1000–1003

Contents lists available at ScienceDirect

Talanta

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

Short communication

Development of analytic microdevices for the detection of phenol using polymer hydrogel particles containing enzyme–QD conjugates

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ARTICLE INFO

Article history:
Received 13 November 2010
Received in revised form 9 February 2011
Accepted 15 February 2011
Available online 22 February 2011

Keywords:
Microfluidic channels
Hydrogel microparticles
Enzyme biosensors
Phenol
Tyrosinase–quantum dot conjugates

ABSTRACT

We present the fabrication of a microdevice for the detection of phenol by combining microfluidic channels and poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel microparticles containing tyrosinase–quantum dot conjugates. PHEMA hydrogel microparticles containing conjugates of enzyme (tyrosinase) and quantum dot (QD) were prepared by dispersion photopolymerization and entrapped within a microfilter-incorporated reaction chamber in a microfluidic channel. The fluorescence change, due to the fluorescence quenching effect caused by the enzyme reaction between phenol and tyrosinase, was used to detect phenol. The fluorescence intensity of PHEMA hydrogel microparticles containing tyrosinase–QD conjugates at 585 nm decreased with phenol concentration. In conclusion, the microfluidic channels fabricated in this study entrapping PHEMA hydrogel microparticles containing enzyme–QD conjugates show the potential to be used as an analytic microdevice for the detection of phenol.

1. Introduction

Phenolic compounds are a class of polluting chemicals easily absorbed by animals and humans through the skin and mucous membranes. A considerable number of organic pollutants, widely distributed throughout the environment, have a phenolic structure. Their presence in surface and ground water poses a potential hazard to human health. Therefore, there is a growing interest in selective and sensitive detection of phenolic compounds. However, the conventional techniques for the detection of phenolic compounds, such as chromatographic, fluorimetric, and spectrophotometric methods, are expensive, time-consuming, need skilled operators, and sometimes require preconcentration and extraction steps that increase the risk of sample loss. Thus, much effort has been devoted to the development of simple, sensitive, accurate, and portable devices to determine the environmental presence and concentration of phenolic compounds [1–4]. Among these, biosensors based on tyrosinase, a copper-containing monooxygenase enzyme, are simple and convenient tools for phenol assays due to their high sensitivity, effectiveness, and simplicity [5,6]. However, despite recent efforts to develop tyrosinase-based biosensors for the detection of phenol, few studies have been reported for developing optical biosensors at the micrometer scale. The miniaturization of biosensors has various advantages over conventional analytical devices, including a smaller dead volume and sample consumption, lower cost, greater sensitivity, higher reproducibility and precision, and the potential to create portable diagnostic tools for on-site analysis. In addition, miniaturized devices provide a safe environment in terms of operator handling of toxic and reactive compounds since nano/micro-volumes of sample are sufficient for analysis [7–9].

In this study, we have developed microdevices for the detection of phenol by combining microfluidic channels and poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel microparticles containing tyrosinase–quantum dot conjugates. In general, for biosensors using enzymes, enzymes are immobilized on hard and dry surfaces such as glass, silica, or magnetic surfaces. However, the main problem associated with enzyme immobilization on a hard surface is that the conformational state of many proteins is very labile and unable to withstand the patterning process conditions, eventually losing their native structure and function [10]. In order to solve this problem, we immobilized enzymes within hydrogel microparticles. Immobilization of enzymes within hydrogel microparticles provides a higher density of enzyme than can be achieved via surface immobilization, as well as a protective environment for the enzyme, resulting in maintenance of the stability of the enzyme due to the features of hydrogels, such as hydrophilicity, biocompatibility, permeability and highly cross-linked networks [11–13]. In addition, microparticles are able to be incorporated into microanalytic devices because of their comparable size to that of microchannels [14]. Conjugates of tyrosinase and quantum dot (tyrosinase–QD conjugates) were prepared and encapsulated in PHEMA hydrogel microparticles by a dispersion

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doi:10.1016/j.talanta.2011.02.021

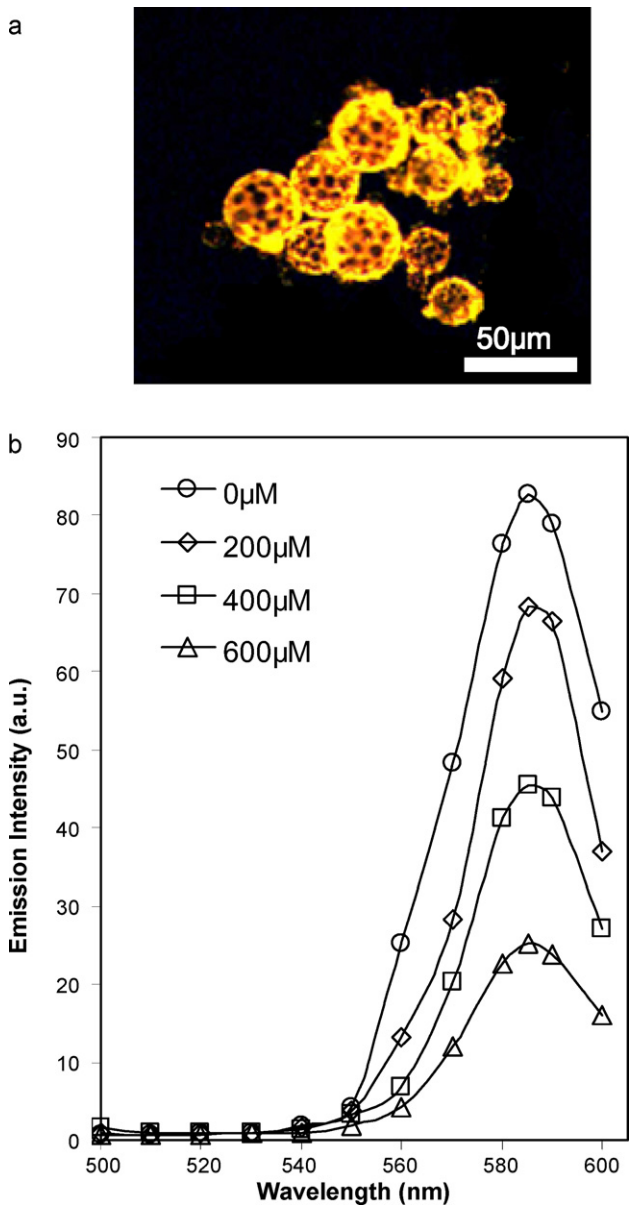

Fig. 1. PHEMA hydrogel microparticles containing tyrosinase–QD conjugates: (a) fluorescence microscope image of PHEMA hydrogel microparticles containing tyrosinase–QD conjugates (excitation at 315 nm) and (b) emission spectra of PHEMA hydrogel microparticles containing tyrosinase–QD conjugates as a function of phenol concentration; phenol concentration (μM) = 0(○), 200(◊), 400(□), and 600(△) (excitation at 315 nm).

photopolymerization. These microparticles were then entrapped within the microfluidic channels fabricated with PDMS in order to make an analytic microdevice. Lastly, the device was tested by investigating the fluorescent change according to the phenol concentration to demonstrate the potential application as an analytic microdevice for the detection of phenol.

2. Experimental

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA, MW 130), poly(ethylene glycol) dimethacrylate (PEGDMA, MW 550), tyrosinase from mushrooms (5370 units/mg solid), silicon oil, and N-(3-dimethylamioproryl)-N-ethyl carbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Carboxyl quantum dots (QD, Qdot® 585 ITK™) were obtained from Invitrogen (Eugene, Oregon, USA). N-hydroxysuccinimide (NHS) and polyethylene glycol trimethylnonyl ether (Tergitol® TMN6) were purchased from Fluka Chemicals (Milwaukee, WI, USA). 1-Hydroxy cyclohexyl phenyl ketone (Irgacure® 184) was obtained from Ciba Specialty Chemicals (Taneatown, NY, USA). Poly(dimethylsiloxane) (PDMS) elastomer was purchased from Dow Corning (Sylgard 184, Midland, MI, USA), which was composed of prepolymer and curing agent.

2.2. Preparation of tyrosinase–QD conjugates

1 mL of tyrosinase solution (2.325 mg/mL in PBS, pH 7.0) and 1 mL of QD solution (0.1 μM in PBS, pH 7.0) were mixed with 1 mL of 20 mM EDC and 1 mL of 40 mM NHS, which were dissolved in PBS. The mixture was then incubated at room temperature for 24 h. During the incubation, the carboxyl groups of QD bonded with lysine residues on the side chain amino groups of tyrosinase through the EDC/sulfo-NHS coupling reaction [15].

2.3. Preparation of PHEMA hydrogel microparticles containing tyrosinase–QD conjugates

PHEMA hydrogel microparticles containing tyrosinase–QD conjugates were synthesized via dispersion photopolymerization of an aqueous monomer mixture in a continuous phase of silicon oil. The formation of PHEMA hydrogel microparticles was based upon the UV initiated free-radical polymerization of methacrylate groups of PHEMA and PEGDMA. Since PEGDMA was used as a cross-linking agent, highly cross-linked PHEMA networks formed. This network represents a three-dimensional structure, capable of entrapping a sensing element and a transducer such as tyrosinase–QD conjugates. The monomer mixture was prepared by mixing 1.4 g of HEMA, 0.072 g (5.0 wt% of monomer) of PEGDMA, 0.036 g (2.5 wt% of monomer) of Irgacure® 184 as an initiator, 0.132 g (9 wt% of monomer) of Tergitol® TMN6 as a dispersion stabilizer, and 1 mL of tyrosinase–QD conjugate solution. The monomer mixture was then added to 20 mL of silicon oil. The mixture of oil and monomer were purged with nitrogen gas for 5 min to remove dissolved oxygen that would act as an inhibitor of the reaction and then stirred using a homogenizer (Ultra Turrax T18 basic, ITK®, Wilmington, NC, USA) at 11,000 rpm for 2 min to form a suspension. For the polymerization, the suspension solution was exposed to 1000 mW/cm^2 of UV light for 300 s. The synthesized particles were separated from the oil by repeated dilution with deionized water and centrifugation at least five times. The washed microparticles were stored in deionized water until future use. The shape and size of the synthesized microparticles were observed using a fluorescence microscope (BX51, Olympus® Co., Japan).

2.4. Fabrication of an analytic microdevice

An analytic microdevice for the detection of phenol was fabricated by combining microfluidic channels and PHEMA hydrogel microparticles containing tyrosinase–QD conjugates. Microfluidic channels in PDMS were obtained by curing a 10:1 mixture of PDMS prepolymer and curing agent against a Si master that had a negative pattern of the desired microchannels defined with SU-8 negative photoresist (MicroChem Co., Newton, MA, USA) [16]. After curing for 5 h at 60 °C, the PDMS replica was removed from the master and oxidized in an oxygen plasma (Harrick Scientific Co., Ossining, NY, USA) with a glass slide for 1 min. Bringing the oxidized PDMS and glass slide into conformal contact resulted in an irreversible seal and thus formed an enclosed microchannel. To make inlet and outlet ports in the microfluidic channel, several holes were punched