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A dual sensor for real-time monitoring of glucose and oxygen

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ABSTRACT

A dual glucose and oxygen sensor in a polymer format was developed. The dual sensor composed of a blue emitter as the glucose probe, a red emitter as an oxygen probe, and a yellow emitter as a built-in reference probe which does not respond to either glucose or oxygen. All the three probes were chemically immobilized in a polyacrylamide-based matrix. Therefore, the dual sensor possesses three well separated emission colors and ratiometric approach is applicable for analysis of the glucose and oxygen concentration at biological conditions. The sensor was applied for real-time monitoring of glucose and oxygen consumption of bacterial cells, *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*), and mammalian cells of mouse macrophage J774 and human cervical cancer HeLa cell lines. On the other hand, in order to achieve satisfactory sensing performance for glucose, compositions of the matrices among poly(2-hydroxyethyl methacrylate), polyacrylamide, and poly(6-aminohexyl methacrylamide) which is a linker polymer for grafting the glucose probe, were optimized.

1. Introduction

Glucose metabolism not only is the main energy source for cells, but also provides essential biomass for proliferating cells, including cancer cells [1]. Many diseases are associated with glucose transport and metabolic disorders, such as myocardial ischemia, type 2 diabetes and cancer [2]. In proliferating cells, especially cancer cells, the glucose metabolism is reprogrammed (Warburg Effect) to cater for the unconstrained proliferation and invasion [1,3–5]. Therefore, monitoring glucose metabolism of cells can provide important information that reflects cell responses to stimuli and proliferative states, which are extremely useful to cancer therapeutic diagnoses, wound healing diagnoses and for fundamental understating of biological processes of the metabolism.

Glucose metabolism composes of hundreds of reactions and metabolites; however, it can be simplified as below:

$$\text{Glucose} + O_2 \rightarrow \text{Metabolites} + \text{ATPs} + nH_2O$$

Focusing on these metabolites and enzymes, many assay kits and techniques have been developed to detect the metabolic changes that occurred in cells, tissues or living bodies [6]. Some traditional assay techniques have also been applied to the detection of metabolic changes, such as high-performance liquid chromatography (HPLC), mass spectrometry (MS) and NMR spectroscopy [7–10]. For measuring glucose uptake, one available method is radiometric assay, which is based on radiolabeled (^{3}H, ^{14}C) glucose [11,12]. Due to the rapid metabolism of glucose in cells, the assay should be finished in a short time to avoid transporting the radiolabeled final products (H_2O and CO_2) out of cells. Therefore, researchers now prefer to use nonmetabolizable analogs, such as 3-o-methylglucose, 2-deoxyglucose (2-DG), fluoro-deoxyglucose (^{18}F-FDG), and 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-dexoyglucose (2-NBDG) [2,13–16]. These nonmetabolizable analogs of glucose will form metabolic stress in the cells, which will induce cell death [17]. None of these methods have been successful in the attempt to complete a real-time direct assay for glucose metabolism in living cells or organisms. Developing a sensor that can dynamically detect the real-time metabolic changes in living organisms is very necessary and should significantly facilitate metabolism related diagnostics and researches.

Innumerable glucose sensors and devices have been developed by researchers in this field, including electrochemical glucose sensors [18], optical (fluorescence and absorbance) glucose sensors [19,20] and glucose selective polymeric sensing fluid based on direct binding [21]. According to the method for recognition of glucose, Steiner et al. classified these sensors into five fundamental types [22]: type I based on the specific binding of glucose to enzymes/coenzymes, type II based on the detection of glucose metabolites produced by certain enzymes, type III based on the

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interaction between glucose and organic boronic acids, type IV based on concanavalin A (Con A) and type V based on other glucose binding proteins. Organic boronic acids can interact with 1,2- or 1,3-diols to form a complex of five or six membered cyclic esters in aqueous solution [22–27]. The interaction is reversible which is ideal to "true sensor" design [22]. The reversible complexation is required for a sensor that can monitor the continuous change of target molecules. Shinkai and his colleagues developed organic boronic acids by a modification of anthracene with a bis-phenylboronic acid (GS-COOH, Fig. 1) and its derivatives, which possess photo-induced electron transfer (PET) effect [25,26]. Because of the unique cleft-like structure, the compound of GS and its related hydrogels showed high selectivity and sensitivity to glucose [25,28,29].

In this study, we used the sensing moiety in GS-COOH as the glucose probe by a chemical immobilization of the derivative of GS-COOH (GS-NHS, Fig. 1) into polyacrylamide-co-poly(2-hydroxyethyl methacrylate) (PAM-co-PHEMA) matrices to prepare new polymer film based glucose sensors. After an optimization of the glucose sensor films, we further chemically immobilized the glucose probe with an oxygen probe [30] to form a dual glucose and oxygen sensor. For getting accurate data for the analyses of glucose and oxygen in complicated biological environment, we integrated the dual sensor with a built-in internal reference probe, which does not respond to either glucose or oxygen. Therefore, ratiometric approach [31–35] could be applied for getting accurate glucose and oxygen concentrations when the films were used for analysis. The dual glucose and oxygen sensor was used to simultaneously monitor glucose and oxygen concentrations and their changes during the growth and respiration processes of bacteria, i.e. *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*), and mammalian cells of mouse macrophage J774 and human cervical cancer HeLa cell lines.

2. Experimental sections

2.1. Materials and reagents

All chemicals and solvents were of analytical grade and were used without further purification. Glucose, 2-acetyl-9,10-dimethylanthracene, N-bromosuccinimide, triethylamine, dichloromethane, tetrahydrofuran (THF), methanol, methylamine, methacryloyl chloride, hexamethylenediamine, N, N′-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), 2-hydroxyethyl methacrylate (HEMA), acrylamide (AM), N-hydroxysuccinimide (NHS), 4-dimethylaminopyridine (DMAP), N,N′-dicyclohexylcarbodiimide (DCC), 3-(trimethoxysilyl)propyl acrylate (TMSPA), poly(ethylene glycol) dimethacrylate (PEGDMA, M_n = 550), and azobisisobutyronitrile (AIBN) were commercially available from Sigma–Aldrich (St. Louis, MO) and used without further purification. Oxygen probe (OS, Fig. 1) and glucose probe (GS) were prepared according to known procedures [26,30]. Precursor for built-in internal reference probe (Rhod-OH, Fig. 2) was prepared according to a published procedure [36]. 6-Aminohexyl methacrylamide (MAHA) was synthesized according to a modified procedure in literature [37]. Doubly distilled water was used for the preparation of buffer solutions. The pH values were determined with a digital pH meter (Thermo Electron Corporation, Beverly, MA) calibrated at room temperature with standard buffers. For fluorescence measurements, quartz glasses from University Wafer (South Boston,

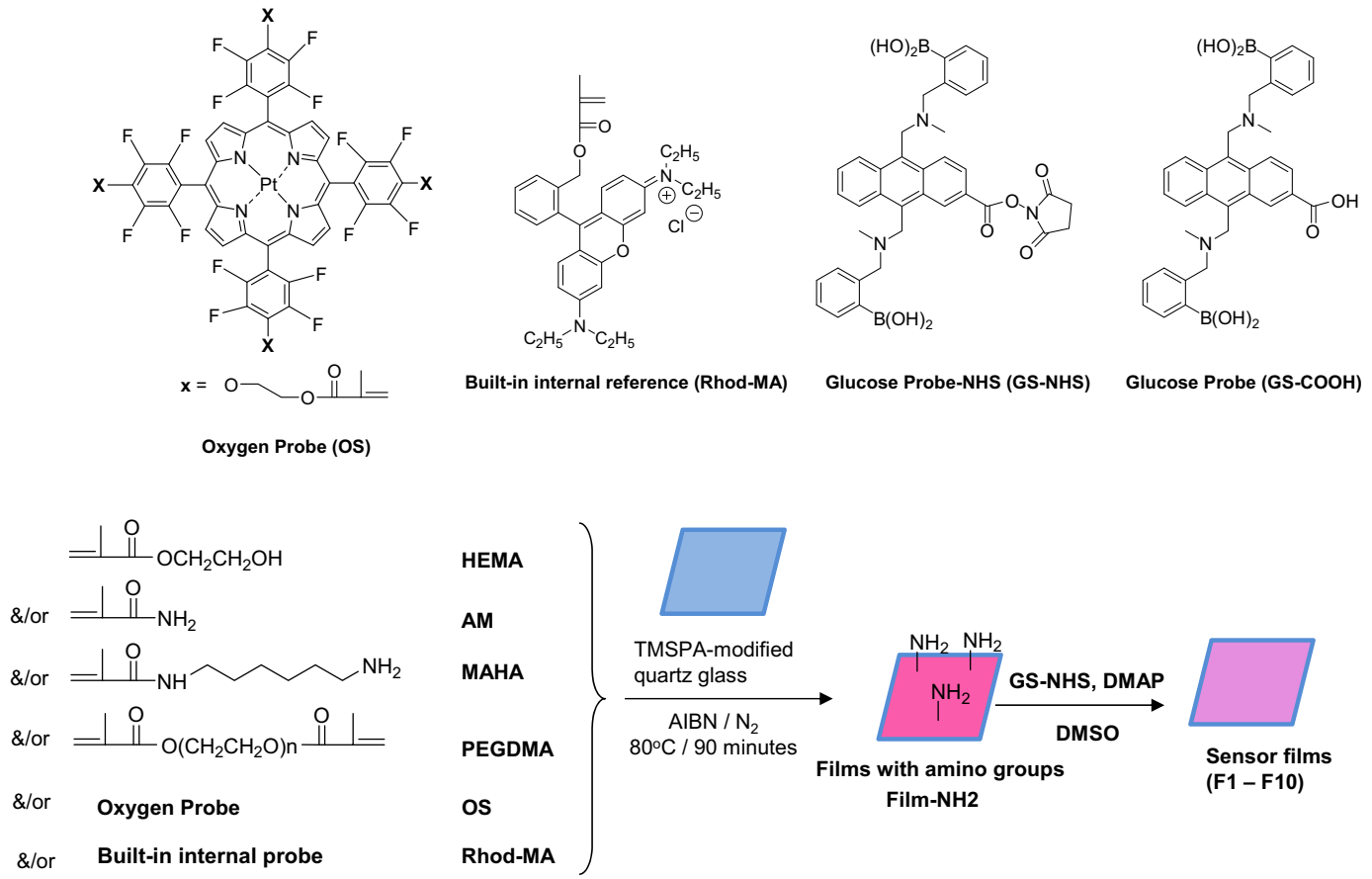

Fig. 1. Chemical structures of the probes and monomers used for the sensor film preparation and a simple schematic drawing for the preparation of sensors in the thin film format.

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