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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.

Keywords

sensor; oxygen sensor; glucose sensor; dual sensor; ratiometric

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, 4, 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:

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Supplementary Data: The chemical structure of a polymerizable monomeric glucose probe.

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 (^3H , ^{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), fluorodeoxyglucose (^{18}F -FDG), and 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) [2, 14–16]. 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 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, Figure 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, Figure 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, Figure 1) and glucose probe (GS) were prepared according to known procedures [26, 30]. Precursor for built-in internal reference probe (Rhod-OH, Figure 2) was prepared according to a published procedure [36]. 6-Aminoethyl 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, MA) were cut into squares of 13 mm × 13 mm using a dicing saw (Microautomation, Billerica, MA).

2.2 Instruments

A Varian liquid-state NMR operated at 400 MHz for ^1H NMR was used for NMR spectra measurements. MALDI-TOFF mass was performed by the ASU Mass Spectrometry Laboratory. An oxygen plasma cleaner (Harrick Plasma, Ithaca, NY) was used for quartz glass surface activation. A Shimadzu UV-3600 UV-Vis-NIR spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) was used for absorbance measurements. A Shimadzu RF-5301 spectrofluorophotometer was used for fluorescence measurements. For easy measurement of the films in liquid solutions, quartz glass was cut with a dicing saw into squares of 13 mm × 13 mm, which can fit diagonally into a quartz fluorescence cuvette to enable the sensing membrane be positioned at an angle of 45° to the excitation light.

2.3 Synthesis of NHS activated glucose probe (GS-NHS)

A mixture containing 500 mg of GS-COOH (0.87 mmol) [26], 303 mg of DCC (1.47 mmol), 201 mg of NHS (1.75 mmol), 213 mg of DMAP (1.75 mmol), and 20 mL of DMSO was stirred at room temperature for 16 hours. Precipitation was removed by filtration. The reaction mixture was diluted with 150 mL of dichloromethane, washed with water three times and with saturated aqueous sodium chloride and dried. The solution was evaporated. 600 mg of the GS-NHS with a yield of 100% was obtained and was used for the next step reaction (immobilization) without further purification.

2.4 Synthesis of the built-in internal reference probe (Rhod-MA)

Rhod-MA was synthesized according to Figure 2. 400 mg (0.86 mmol) of **Rhod-OH** and triethyl amine (261 mg, 2.58 mmol) were dissolved in 20 mL of THF. 135 mg of methacryloyl chloride (135 mg, 1.30 mmol) in 2 mL THF was added into the above mixture slowly at 0 – 5°C. The reaction was stirred overnight at room temperature. The mixture was poured into water. Organic materials were extracted into methylene chloride. The organic phase was washed with water three times and then dried over MgSO_4 . The product of **Rhod-MA** was purified by column chromatography with methylene chloride/methanol (95:5 by volume). Yield: 300 mg (66%). ^1H NMR (δ , ppm, CDCl_3): 7.63 (m, 3H), 7.38 (d, 1H), 6.96

(m, 6H), 5.64 (s, 1H), 5.38 (s, 1H), 4.92 (s, 2H), 3.60 (m, 8H), 1.96 (s, 3H), 1.16 (t, 12H).
MOLDI-TOF (m/z): 497.77 (calc: $C_{32}H_{37}N_2O_3^+$, M-Cl⁻, 497.28).

2.5 Preparation of sensor films

2.5.1 Glucose only sensor thin films—

Sensor film was prepared through two steps. The first step is to prepare a film with amino-groups on the surface (Film-NH₂). The second step is to graft the GS-NHS onto the amino-containing film to form glucose sensor film (F1 – F5). Preparation of the film of Film-NH₂ was followed our published protocol [30, 38]. A typical example was given below. Briefly, AM (165 mg), PEGDMA (35 mg), MAHA (16.5 mg), and AIBN (6 mg) were dissolved in 1mL DMF as a stocking solution. 15 μ L of the stocking solution were added onto the surface of TMSPA treated quartz glass and covered with a clean but untreated cover slip to make a sandwich structure. The modification of TMSPA on the surface of quartz glass was to enable the sensor and matrices to be chemically grafted onto a quartz substrate [30]. To produce the polymer thin film with good mechanical stability, PEGDMA was used as a cross-linker. MAHA was applied to introduce active reaction group for the immobilization of glucose sensor onto the thin film later on. The thickness was controlled by using a 25- μ m Kapton tape (DuPont, Wilmington, DE). The sandwich set-up was placed into a vacuum oven, which was then evacuated and refilled with nitrogen three times. Polymerization was carried out under nitrogen at 80°C for 90 minutes in the oven. The quartz glasses with polymer membranes were removed from the oven, with Kapton tape and no-surface modified cover slip being removed from the polymerized membrane surface. For an optimization of the matrices, different ratios of AM/HEMA/MAHA (Table 1) were used in the polymerization process to form various films with different amounts of amino groups for tuning the density of the glucose probes in the sensor films. The obtained amino group-containing films were put in a flat bottom bottle containing 1.0 mL of DMSO, 3.0 mg of DMAP and 0.45 mg of GS-NHS to graft glucose probe onto the films. 16 Hours later, the films were taken out and washed with methanol to remove any remaining non-reacted compounds and residual solvent. The films were dried and stored in the dark at room temperature.

2.5.2. Triple color glucose-oxygen dual sensor—Films were prepared following the above described procedure for F5 (Table 1), while adding OS and Rhod-MA in the mixture of monomers for film preparation. Ratios of OS and Rhod-MA were fixed at 0.32% and 0.1 % of total material weight, respectively. Triple color glucose-oxygen dual sensing film (F10) was formed by grafting glucose probe onto the film with amino moieties.

2.6 Sensor characterization

The sensor performances were characterized using a spectrofluorophotometer. A sensor film with a 1.3 $\times$ 1.3 cm dimension was put diagonally in a cuvette with 2 mL of liquid (either a buffer or a medium) with a 45°facing angel to the excitation light. All the probes used herein could be excited efficiently at 390 nm and their emissions were collected from 410 to 700 nm. During the titration at each condition, the film was equilibrated for 30 second and then the data were collected. Glucose concentrations were varied from 0 to 100 mM in solutions and the concentrations of the dissolved oxygen were tuned from 0 to 41 mg/L, or 0 to 41 ppm, corresponding to oxygen partial pressures of 0 to 100% of atmospheric pressure by bubbling gas oxygen into the liquid for 2 minutes. Note at 23°C, the dissolved oxygen concentration in water is 8.6 mg/L.

2.7 Culture of *E. coli* and *B. subtilis* for extracellular sensing

E. coli (JM109) or *B. subtilis* (168) were cultured in Luria-Bertani broth overnight at 37°C with vigorous shaking at 200 rpm. The concentrations of bacteria in culture were estimated

by measuring the optical density at 600 nm (OD_{600}). OD_{600} value of 1 indicates 5.0×10^8 cfu $\cdot$ mL $^{-1}$ (colony-forming units per milliliter) for *E. coli* and indicates 2.25×10^8 cfu $\cdot$ mL $^{-1}$ for *B. subtilis* [38, 39]. Bacteria in 1 mL of culture was collected by spin-down and resuspended in 10 mL of testing medium containing 7.0g K_2HPO_4 , 3.0g KH_2PO_4 , 1.0g $(NH_4)_2SO_4$, 0.5g sodium citrate, 0.1g $MgSO_4 \cdot 7H_2O$, 5.0mg $CaCl_2$, 0.25mg $FeSO_4$, and 0.2% Casamino acids (BD Diagnostic Systems, Sparks, MD) in 1.0 liter of medium [41, 42]. After vigorous shaking at 37°C for 2 hours, the cell concentration of culture was determined. According to the amount of cells expected for experiments, bacteria were harvested from the appropriate volume of culture by spin-down followed by washing once with testing medium without glucose. The final pellet was re-suspended into testing medium with 10 mM of glucose to get the required concentration for experiments.

2.8. Culture of HeLa cells and J774 for extracellular sensing

Both HeLa and J774 cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin and incubated at 37 °C in a 5% CO_2 atmosphere. Cells were harvested and washed by KRH buffer (50 mM of HEPES, 137 mM of NaCl, 4.7 mM of KCl, 1.85 mM of $CaCl_2$, 1.3 mM of $MgSO_4$ and 0.1% BSA) for three times [2, 14]. Fluorescence assays were started immediately after cells were re-suspended into KHR buffer containing 10 mM of glucose.

2.9 The use of the triple color dual glucose and oxygen sensor for simultaneously monitoring glucose and oxygen consumptions

The sensor film F10 was put in a 4 mL cuvette containing 2 mL of cell culture media with different species and densities. In order to prevent the exchange of oxygen in the media with air, 0.5 mL of mineral oil was added on the top of the media. Time dependent fluorescence change was monitored. According to the calibration curve of the same sensor, glucose and/or oxygen concentrations were calculated.

3. Results and discussion

3.1 Design of sensor

The sensor investigated here is in the thin film state and the probes were chemically immobilized in the matrices, where there is no leaching problem of the probes from the matrices. Further, the matrices were chemically grafted on the substrates, i.e., quartz glasses. Thus the sensor is robust, reversible, and stable. Initially, we synthesized a polymerizable glucose probe (GS-MA, supplementary S-Figure 1) and polymerized this probe in PHEMA-co-PAM matrixes. However, the sensitivity of the glucose sensor made this way was found to be very low (data not shown). While, after GS-NHS was chemically grafted (Figure 1) onto the amino-containing polymer film, we found that the sensitivity of the glucose sensor was improved significantly. Therefore, the sensor synthesis in this research followed the procedure as illustrated in Figure 1. For achieving high selectivity to glucose, the glucose probe with two boronic acid moieties was chosen because its known high selectivity to glucose [25]. For oxygen sensing, we chose the modified perfluoro-platinum porphyrin, OS, with four crosslinkable/polymerizable methacrylate moieties, which was demonstrated to be an excellent oxygen probe in various matrices [30, 43]. To enable simultaneous analyses of both the glucose and oxygen concentrations, dual glucose and oxygen sensor was synthesized. To achieve accurate analyses of the glucose and oxygen concentrations in biological conditions, the dual glucose and oxygen sensor possesses an internal built-in probe (Rhod-MA, Figure 1), which does not respond to either glucose or oxygen. Therefore, ratiometric approach can be used for accurate analyses of glucose and oxygen concentrations. Ratiometric approach has been demonstrated to be an efficient approach for

biological analysis with less interference by sensor's local concentrations, non-uniformity, biological backgrounds, and environments [31–35]. We chose the biocompatible poly(2-hydroxyethyl methacrylate) (PHEMA), polyacrylamide (PAM), and their composites as the matrices [44, 45]. A dimethacrylate moieties-containing poly(ethylene glycol) (PEGDAM) was used as a crosslinker to further make the film robust.

3.2 Optimization of the glucose sensor films

3.2.1 Influences of matrices (the ratio of PHMA to PAM) on sensor performances

—Five films (F1 – F5) with variable weight ratios of PHEMA to PAM (Table 1) were prepared with a fixed percentage (7.5%) of PMAHA in the films. Figure 3A shows the typical sensor responses of the film F5. The absorption spectra of the sensor have no changes before and after interactions with glucose (Figure 3A). The emission intensity increases with increasing glucose concentration (Figure 3B), showing the sensing mechanism is due to photo-induced electron transfer (PET). Fluorescence intensity ratio at 445 nm was plotted in Figure 3C. It could be found that the sensor film had a sensitive dynamic range to glucose from 1 mM to 30 mM, indicating the sensor is suitable for cultured cell glucose metabolism assay [46], as well as for blood glucose concentration monitoring for diabetes [47].

Figure 4A shows the sensor responses of F1–F5 to glucose. It was found PHEMA had strong negative effect on the glucose response of sensing films. The films with more PHEMA have low sensitivity to glucose. Without PAM, the sensing film F1 only had the sensitivity (I_{100mM}/I_{0mM}) of 3.2-fold. The sensing film (F5) with a ratio of PHEMA/PAM=0/100 showed the highest sensitivity (9.8-fold) (Figure 3A and Table1). Therefore, after the optimization, we chose the polyacrylamide (PAM) as the matrices for sensor film preparation.

3.2.2. Influences of MAHA on the sensor performance—Films F5–F9 were prepared using PAM as the matrices, with different weight ratios of PMAHA. Figure 4B shows the comparisons of the sensor performance. It was found that amounts of probe linker (MAHA), from 5% to 22% by weight, did not show much effect on the sensitivities to glucose. Taking the sensitivity and quality of sensing films into account, the matrix composition of the sensor film F5 was selected as the only recipe for dual sensor preparation.

3.3 Evaluation of the selectivity to glucose

The selectivity of sensor film was studied by determining fluorescent response to different saccharides, i.e. glucose, fructose, mannose and galactose. As shown in Figure 5, the sensing film had the highest selectivity to glucose.

3.4 pH effect on the glucose thin film sensor

Due to metabolites produced during cell metabolism, the pH of cell environment always changes in a narrow range [38, 48]. It is important to know if the change is going to affect the performance of sensing film. The sensing film (F5) was titrated from pH3 to pH9 with or without glucose. As shown in Figure 6, at the condition without glucose, the sensor suffered seriously from pH changes. But with the existence of 5 mM or 20 mM glucose in the media, fluorescent responses to pH decreased significantly, especially in the physiological pH range, i.e. from 6.5 to 8. The glucose sensor has almost no responses to pH from pH 6.5 to 8, indicating that the glucose sensor is applicable to analyze glucose in physiological conditions without the interferences by pH changes.

3.5 Dual glucose-oxygen sensor

A dual sensor (film F10) was prepared by using a weight ratio of the matrix precursors of AM:MAHA:PEGDMA of 77.5:7.5:15. Weight ratios of OS, GHS-NS, and Rhod-MA in the matrix are of 0.32%: 0.6%: 0.1%. Figure 7 shows the responses of the dual sensor to glucose and oxygen. It can be found that the internal built-in reference probe of Rhod-MA with an emission maximum at 580 nm and the oxygen probe with an emission maximum at 650 nm did not respond to glucose (Figure 7A). Rhod-MA and GS did not respond to oxygen (Figure 7C). This observation showed the Rhod-MA is a suitable internal reference probe, enabling the application of the ratiometric approach for the glucose and oxygen concentration calculation. Further, there are no cross-talks between the oxygen probes and glucose probes. All the probes can be excited efficiently using a single wavelength, simplifying optical operational condition when used for bioanalysis. Figure 7B shows the glucose responses calculated using the ratiometric approach by I_{445}/I_{580} of the dual sensor film. The sensor responds to glucose in a range of 1 to 20 mM, which is narrower than the glucose sensor film of F5. This may be due to the fact that the film F10 contains more crosslinkers, e. g. the oxygen probe molecules, to make the network tight, which probably affected the glucose permeability in the films as well as the dynamic range of glucose. Other possibilities such as the fluorescence resonance energy transfer (FRET) among the three fluorophores in the thin films may also affect the sensor performance. Figure 7D shows the Stern-Volmer plot of the dual sensor to oxygen using the ratiometric approach. The responses to oxygen are not linear, indicating the non-uniform distributions of the oxygen probes in the films and/or some possible FRET effects among the three fluorophores.

3.6 Application of the dual sensor film to monitor extracellular glucose and oxygen changes during bacteria growth

Sugar concentration and dissolved oxygen are two important factors that affect microbial growth, and vice versa, consumption rates of sugar and oxygen reflect the physiological state of the microbial culture [48]. Many methods and tools have been developed to monitor the consumption rates of glucose and oxygen. For glucose consumption assay, most commonly used assay methods include [$U-^{14}C$] glucose-labeling experiment [49], fluorescent glucose analog, 2-NBDG [50, 51] and other indirect methods like phosphorus-31 nuclear magnetic resonance (^{31}P -NMR) spectroscopy [52]. As we know, well trained and experienced operators are required to handle those radioactive materials. Glucose analogs, 2-NBDG, actually are metabolism inhibitors by which metabolic stresses are going to be produced during experiments. The results obtained from these analogs most likely cannot reflect the real cell metabolism level.

Herein, we used our non-invasive dual glucose and oxygen sensor to investigate the kinetic real-time cell metabolism. The capability of detecting changes of glucose and oxygen were tested with *E. coli* and *B. subtilis*. Figure 8A shows the typical spectral changes during the cell growth of *B. subtilis* with a cell density of 4×10^7 cfu/mL. Because cells consume glucose and oxygen during growth and respiration, both the glucose and oxygen concentrations decrease, which will result in a decrease of the glucose sensor's intensity at 445 nm, while an increase of the oxygen sensor's intensity at 650 nm. According to the titration curve of the sensor using the ratiometric approach, the real-time concentrations of glucose and oxygen were calculated and plotted in Figure 8B. It can be seen that at such a condition, oxygen was consumed completely by the bacteria at about 15 minutes. According to the time dependent concentrations, the "drawdown" rates of glucose and oxygen by *B. subtilis* in 10 minutes were determined to be 6.92 ± 0.45 fmol $\cdot$ min $^{-1}$ $\cdot$ cfu $^{-1}$ and 0.49 ± 0.05 fmol $\cdot$ min $^{-1}$ $\cdot$ cfu $^{-1}$, respectively (Table 2).

Figure 8C presented the kinetic curves demonstrating the uptakes of glucose and consumptions of oxygen by *E. coli* with the same optical density as *B. subtilis*, i.e. $OD_{600}=0.2$. In this testing condition, the “drawdown” rates of glucose and oxygen by *E. coli* (JM109) in 10 minutes were determined to be $0.66 \pm 0.02 \text{ fmol}\cdot\text{min}^{-1}\cdot\text{cfu}^{-1}$ and $0.17 \pm 0.03 \text{ fmol}\cdot\text{min}^{-1}\cdot\text{cfu}^{-1}$ respectively (Table 2).

It is notable that the “drawdown” rates of glucose and oxygen depended on the time scale. The rates determined using a 30 minute time scale are much slower than those determined in a 10 minute time scale. The comparison was given in Table 2. For the same bacterial species, the observation of the time-dependent oxygen and glucose consumption rates shows the advantage of the glucose-oxygen dual sensor as compared to other traditional approaches. The advantage is that the sensor reported herein can monitor the real-time metabolism level of living organisms without invasion. Those dynamic data may reflect the metabolism of cells at a specific proliferation stage or differentiation state.

3.7 Application of sensing film F10 to monitor the consumption of glucose and oxygen for mammalian cells

In this study, two mammalian cell lines, murine macrophage cell line J774 and HeLa cells were applied to test the glucose uptake and oxygen consumption rates by the film F10 (Figure 9). For both the two cell lines, under our experimental conditions, cells consumed oxygen completely within two hours. However, clear differences of their glucose uptakes were observed. At the same time point, HeLa cells consumed oxygen faster than J774 did. Using the two hour time scale, the oxygen consumption and glucose uptake rates were calculated and given in Table 2. Very similar oxygen consumption rates were determined, i.e. $1.98 \pm 0.3 \text{ fmol}\cdot\text{min}^{-1}\cdot\text{cell}^{-1}$ for HeLa cells and $1.59 \pm 0.2 \text{ fmol}\cdot\text{min}^{-1}\cdot\text{cell}^{-1}$ for J774 cells, while different glucose uptake rates were observed: $12.1 \pm 1.5 \text{ fmol}\cdot\text{min}^{-1}\cdot\text{cell}^{-1}$ for HeLa cells and $4.75 \pm 0.4 \text{ fmol}\cdot\text{min}^{-1}\cdot\text{cell}^{-1}$ for J774 cells (Table 2).

4. Conclusion

New polymer-based glucose sensor and its dual glucose and oxygen sensor were developed. The influence of polymer matrix on the glucose sensing performance was investigated. Results showed polyacrylamide is a good matrix for glucose sensing. The dual glucose and oxygen sensor composed of three fluorophores, a red emitter as an oxygen probe, a yellow emitter as a built-in reference probe which does not respond to either glucose or oxygen, and a blue emitter as the glucose probe. The three probes were chemically grafted in the polyacrylamide matrix. Because of an integration of the built-in reference probe with glucose and oxygen probes, the ratiometric approach was used to measure glucose and oxygen concentrations at biological environments. Results showed that the tricolor dual glucose and oxygen sensor is not only suitable for the determination of glucose and/or oxygen level at the set time point, but also can simultaneously monitor in real-time the glucose and oxygen concentration changes/consumptions by bacteria and mammalian cells. The sensor's capability of monitoring dynamic changes of oxygen and glucose at real-time provides researchers more information than traditional methods do. Further works are in progress to apply this glucose-oxygen dual sensor to determine the metabolism levels of living organism at different growth phases, as well as the metabolism levels at specific differentiation states at not only bulk cell levels but also single cell levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 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.

Figure 2.
Synthesis of Rhod-MA.

Figure 3.

Typical responses of the film F5 to glucose. A) absorption spectra of the film with and without glucose, B) fluorescence changes by glucose excited at 390 nm, C) glucose concentration dependent fluorescence intensity ratios at 445 nm. I_0 : intensity at 445 nm without glucose, I : intensity at 445 nm with various concentrations of glucose.

Figure 4.

Influence of matrices on glucose responses. A) responses of F1 to F5 with variable weight ratios of HEMA to AM; B) responses of F5 – F9 with tunable MAHA ratios. I_0 : intensity at 445 nm without glucose; I : intensity at 445 nm with glucose.

Figure 5.

Evaluation of selectivity. I_0 : intensity at 445 nm without saccharides; I : intensity at 445 nm with different concentrations of saccharides.

Figure 6. Influence of pH on the sensor's responses. I_0 : intensity at 445 nm at pH 9; I : intensity at 445 nm at various pH.

Figure 7.

Responses of a dual sensor to glucose (A and B) and to oxygen (C and D). A: responses to glucose; B: ratiometric fluorescence changes for glucose using the ratio of fluorescence intensities at 445 and 580 nm; C: responses to oxygen; D: ratiometric fluorescence changes for oxygen using the ratio of fluorescence intensities at 580 and 650 nm.

Figure 8.

A: Emission spectral changes of a film of F10 during the growth of *B. subtilis* (4×10^7 cfu/mL). B: Time dependent glucose and oxygen concentrations and their changes during the growth of *B. subtilis*. C: Time dependent glucose and oxygen concentrations and their changes during growth of *E. coli* detected by the dual sensor (F10).

Figure 9.
Glucose uptake and oxygen consumption assay by HeLa cells (A) and J774 cells (B) detected by the dual sensor (F10).

Table 1

Compositions of thin films of F1 to F9 and their sensitivity

Films	PHEMA ^{a)}	PAM ^{a)}	Percentage of probe linker (MAHA) in total weight of polymer compositions	Sensitivity ^{b)} (I_{100mM}/I_{0mM})
F1	100	0	7.5	3.2
F2	85	15	7.5	4.0
F3	50	50	7.5	7.6
F4	10	90	7.5	8.3
F5	0	100	7.5	9.8
F6	0	100	3.3	10.3
F7	0	100	11	9.2
F8	0	100	15	9.7
F9	0	100	22	9.1

^{a)} Weight ratios of PHEMA/PAM in the thin films;

^{b)} fluorescence intensity ratios between those with 100 mM glucose and without glucose.

Table 2

Glucose uptake and oxygen consumption rates by cells.

	Cell density	Glucose (fmol.min ⁻¹ .cell ⁻¹)	Oxygen (fmol.min ⁻¹ .cell ⁻¹)
<i>E. coli</i>	1×10 ⁸ cfu.ml ⁻¹	0.66 ± 0.02 ^a	0.17 ± 0.03 ^a
		0.28 ± 0.03 ^b	0.08 ± 0.02 ^b
<i>B. subtilis</i>	4×10 ⁷ cfu.ml ⁻¹	6.92 ± 0.45 ^a	0.49 ± 0.05 ^a
		2.51 ± 0.03 ^b	0.21 ± 0.02 ^b
HeLa cells	1×10 ⁶ cells.ml ⁻¹	12.1 ± 1.5 ^c	1.98 ± 0.3 ^c
J774 cells	1×10 ⁶ cells.ml ⁻¹	4.75 ± 0.4 ^c	1.59 ± 0.2 ^c

^a average rate in 10 minutes;^b average rate in 30 minutes;^c average rate in 120 minutes.