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Influence of Matrices on Oxygen Sensing of Three Sensing Films with Chemically Conjugated Platinum Porphyrin Probes and Preliminary Application for Monitoring of Oxygen Consumption of *Escherichia coli* (*E. coli*)

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Abstract

Oxygen sensing films were synthesized by a chemical conjugation of functional platinum porphyrin probes in silica gel, polystyrene (PS), and poly(2-hydroxyethyl methacrylate) (PHEMA) matrices. Responses of the sensing films to gaseous oxygen and dissolved oxygen were studied and the influence of the matrices on the sensing behaviors was investigated. Silica gel films had the highest fluorescence intensity ratio from deoxygenated to oxygenated environments and the fastest response time to oxygen. PHEMA films had no response to gaseous oxygen, but had greater sensitivity and a faster response time for dissolved oxygen than those of PS films. The influence of matrices on oxygen response, sensitivity and response time was discussed. The influence is most likely attributed to the oxygen diffusion abilities of the matrices. Since the probes were chemically immobilized in the matrices, no leaching of the probes was observed from the sensing films when applied in aqueous environment. One sensing film made from the PHEMA matrix was used to preliminarily monitor the oxygen consumption of *Escherichia coli* (*E. coli*) bacteria. *E. coli* cell density and antibiotics ampicillin concentration dependent oxygen consumption was observed, indicating the potential application of the oxygen sensing film for biological application.

Keywords

Oxygen sensor; chemical conjugation; platinum porphyrin; matrix influence; *E. coli*

1. Introduction

Oxygen sensors have attracted great attention because of their significant applications in aerospace, oceanography, biology, meteorology, agricultural industry, environmental science, and life science [1-11]. Various types of oxygen sensors based on different mechanisms including pressure, electrochemistry, and fluorescence quenching – an optical approach - have been developed [1-11]. The most common systems for oxygen sensing are

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based upon either amperometric electrochemical devices (e.g. the Clark electrodes [4]) or optical sensing devices [1,2,10]. Electrodes are reliable with long term stability and do not suffer the problem of environmental interferences by ion strengths, heavy metals, or media. However, the electrode cannot be used for non-invasive study in biological samples nor are they suitable for small volumes (a few tens pico-liters) for single cell study. Fluorescence based oxygen sensors have been intensively studied and developed recently as they are non-invasive, disposable, easily miniaturized (down to sub-micrometer), and simple to process (as a coating or solid layer on optical fibers and some surfaces for remote measurement of chemical and biochemical parameters) [12-17]. These optical oxygen sensors allow for direct imaging of oxygen concentration over a surface or in a volume. The long-term stability and environment interference on the sensing performance is the weaknesses and needs to be improved.

Ruthenium(II) polypyridyl complexes, platinum (II) and palladium (II) porphyrins, and cyclometalated complexes of platinum (II) and iridium (III) [18-24] are typical chemical materials acting as fluorescence-based oxygen sensors because of their long triplet state lifetime lasting from a few to tens of microseconds and sufficient triplet-triplet energy transfer to oxygen molecules, resulting in a decrease of the oxygen sensors' emission intensities and a shortening of their lifetimes by oxygen. Usually, the emission-based oxygen sensors are physically trapped into various polymer matrices including silicon rubbers/gels, polystyrene, cellulose derivatives, and polyurethane-type hydrogels [12-29]. However, when applied in liquid environments, the sensors easily leach out. This problem may result in inaccuracy of the measurements of dissolved oxygen (DO) in liquid and toxicity to biosamples. In order to alleviate the leaching problem, sensing films with chemically conjugated oxygen probes using silica gel [30], star-polystyrene [31], crosslinked polystyrene (PS) and poly(2-hydroxyethyl methacrylate) (PHEMA) [32] as the matrices were previously reported by other scientists and us. We previously evaluated the sensing performance of two new platinum porphyrin derivatives (compound **1** and **2**, Scheme 1) in PS and PHEMA matrices for DO [32]. Compound **2** has four methacrylate moieties, enabling this material to be polymerized and crosslinked with other monomers to form sensing films or membranes for oxygen sensing. It was demonstrated that the sensing films with chemically immobilized sensors alleviated the leaching problems of those with physically doped sensors when applied in aqueous solutions for DO sensing [32]. Compound **1** has four hydroxyl groups, endowing a further chemical modification with other materials. Herein, we use compound **1** to react with triethoxy(3-isocyanatopropyl)silane to form a new silane derivative (compound **3**) for silica gel membrane preparation and to study the sensing behaviors for gaseous oxygen (GO) and DO. The influence of polymer matrices (PS, PHEMA, and Silica gel) on the oxygen sensing performance for GO and DO, including response, response time, and photostability was investigated and discussed. The physical leaching of chemically immobilized probes from their matrices when applied in aqueous solutions was also evaluated.

Some oxygen sensing films have been applied for biological studies on eukaryotic and prokaryotic cells [33-40]. To name a few, O'Riordan et al. studied the viability of yeast cells [33]. Kocinocova et al. studied pH and oxygen change induced by *Pseudomonas putida* bacteria growth [34]. Thomas et al. investigated the oxygen consumption of human umbilical vein endothelial cells [35]. We are aiming to integrate the optical sensors with lab-on-a-chip devices for oxygen consumption and cell metabolism investigations at multi- and single-cell levels [10,11]. Along this line, beside the above sensing performance evaluations, we report the preliminary application of a typical sensing film made of the chemical immobilization of compound **2** in a PHEMA matrix for *Escherichia coli* (*E. coli*) oxygen consumption.

2. Experimental section

2.1 Materials and reagents

Compounds **1** and **2** were prepared as carried out in our previous publication [32]. Other chemicals and solvents were of analytical grade and were used without further purification. Britton-Robinson (B-R) buffers composed of acetic acid, boric acid, phosphoric acid, and sodium hydroxide were used for tuning pH values. Calibration gases (nitrogen and oxygen, each of 99.999% purity) were purchased from AIR Liquide America, LP (Houston, TX). Exact gas percentage was controlled using a home-made gas manipulator (measuring error: $\pm 1\%$). All measurements of sensing behaviors were carried out at atmospheric pressure (760 mmHg, or 101 kPa) at room temperature ($22 \pm 1^\circ\text{C}$). Quartz glasses from University Wafer (South Boston, MA) were cut to squares of $1.31\text{ cm} \times 1.31\text{ cm}$ using a dicing saw (Microautomation, Billerica, MA) for fluorescence measurement so that the quartz glass could fit well in the quartz fluorescence cuvettes to enable the sensing membrane be positioned at an angle of 45° to the excitation light.

2.2 Instruments

An oxygen plasma cleaner (Harrick Plasma, Ithaca, NY) was used for quartz glass surface activation. A Shimadzu RF-5301 spectrofluorophotometer (Shimadzu Scientific Instruments, Columbia, MD) was used for fluorescence measurements.

2.3 Preparation of silica gel membranes

A mixture of compound **1** (7.6 mg), triethylamine (5 μL), isocyanatopropyltriethoxysilane (100 μL) in dimethyl sulfoxide (DMSO) (215 μL) was heated at 80°C for 15 hours to *in-situ* generate compound **3**. 0.33 mL of tetraethyl orthosilicate (TEOS) and 0.60 g of dimethoxydimethylsilane (DMDMS) were mixed with 0.5 mL of 0.01 M HCl at 60°C for 5 hours to generate silica gel. 100 μL of *in-situ* generated compound **3** in DMSO solution was added into the gel mixture, continuing to heat the mixture for three more hours. 20 μL of the mixture was removed and placed on plasma activated quartz glass to dry at room temperature overnight. The silica film was then washed with methanol to remove non-crosslinked silane precursor and solvents. The film was then stored at room temperature in the dark.

2.4 Response time study

Sensing films were placed diagonally into quartz cuvettes. Emission measurements were taken every four seconds at the sensor's peak emission at 650 nm, excited at 405 nm. To vary the oxygen concentration, a gas manifold was used to control gas flow through a tube and needle to the cuvette. Holes were installed in the cuvette cap to allow the needle into the cuvette and gas flow out. The needle was positioned so the gas and gas bubbling would occur just above the sensor film to decrease the possible interference by the gas flow, which may affect the fluorescence measurements. Oxygen and nitrogen flow rates were set at 66 standard cubic centimeters per minute (sccm). In all trials, the film was first saturated with oxygen before beginning the measurements. Measurements began with the change from 100% oxygen flow into the cuvette to 100% nitrogen, and then continued as the gas flow composition was switched between oxygen and nitrogen. The intervals of gas flow depended on the type of film.

2.5 Culture of *E. coli*

E. coli were cultured in liquid Lysogeny Broth (LB) medium from a single colony. The LB medium was made of 10 g tryptone, 5 g yeast extract and 10 g NaCl in 1 L distilled water. The cell density of *E. coli* in the culture medium was estimated by measuring the optical

density at 600 nm (OD_{600}), which is linearly proportional to *E. coli* cell density when OD_{600} is between 0.1 and 1.0. A value of 1 indicated a cell density of 5.0×10^8 colony-forming unit per milliliter (cfu/mL), based on calibrations of our UV-Vis spectrophotometer. After *E. coli* cell density of the overnight culture was determined, appropriate dilutions were made using fresh LB to achieve desired initial *E. coli* cell densities for experiments.

3. Results and discussion

3.1 Results of sensors' performances

3.1.1 Sensing film preparation using PS and PHEMA as the matrices—The preparation of the polymer sensing films is described in detail in our previous publication [32]. The sensor-conjugated thin films were grafted on a quartz substrate with the thickness of the thin film controlled to approximately 25 μm . Films prepared by chemical immobilizations of **2** in PHEMA and in PS were named F1 and F2, respectively. Our study indicates that the probe concentration of 0.1 and 1 mg/g in matrices didn't obviously affect the sensing performance [32]. However, at high concentration of 10 mg/g, the quenching velocity was slower than those with 0.1 and 1.0 mg/g in matrices. Herein, concentrations of sensors in matrices for F1 and F2 were 1 mg/g.

3.1.2 Sensing film preparation using silica gel as the matrix—For a chemical conjugation of the probe with the silica gels, compound **1** with four hydroxyl groups was first reacted with triethoxy(3-isocyanatopropyl)silane to generate compound **3**, a silane-containing compound. Compound **3** (Scheme 1) was then used *in-situ* without a further purification. The silica gel film F3 (approximately 42 μm) was prepared by an acid catalyzed condensation of the silane precursors [9,22-24]. The concentration of sensor in the silica gel matrix for this study was 2 mg/g.

3.1.3 Sensing performance for gaseous oxygen—A mixture of oxygen and nitrogen gas was used to control the oxygen concentration. Figure 1 shows the typical oxygen response curves of F2 (A) and F3 (B). F1 had almost no response to gaseous oxygen (data not shown). F2 had a larger response, and F3 had the strongest response to oxygen gas. The I_0/I_{100} among the three films for gaseous oxygen were $F1 \ll F2 \ll F3$ (Table 1), where I_0 is the intensity at 650 nm under nitrogen and I_{100} is the intensity under oxygen gas.

The fluorescence intensity of F2 decreased with an increase in oxygen concentration. The intensity ratio (I_0/I) curve for F2 (Figure 1C) follows the Stern-Volmer equation,

$$\frac{I_0}{I} = 1 + K_{SV} [pO_2] \quad (1)$$

where K_{SV} is the Stern-Volmer quenching constant, I_0 and I are the steady-state luminescence signals measured in the absence of oxygen and presence of different oxygen concentrations, and $[pO_2]$ is the oxygen partial pressure in the nitrogen and oxygen mixed gas. The linear response to oxygen indicates a uniform distribution of the probes in the matrix.

The intensity ratio curve of F3 (Figure 1D) doesn't follow the linear Stern-Volmer quenching equation (1), especially for high oxygen concentrations. This phenomenon is quite common in many silica gel-based oxygen sensors [15, 17, 22, 30], indicating that the probes reside in different sites in the silica gel with different accessibility to the oxygen molecules. This phenomenon is attributed to a dynamic quenching theory [22, 41] equation,

$$\frac{I_0}{I_0 - I} = \left\{ \sum \frac{f_i K_{SVi} [pO_2]}{1 + K_{SVi} [pO_2]} \right\}^{-1} \quad (2)$$

where f_i is the fraction of probes i with a quenching constant K_{SVi} .

Equation 2 can be simplified as shown in the following equation,

$$\frac{I_0}{I_0 - I} = \frac{1}{f K_{SV} [pO_2]} + \frac{1}{f} \quad (3)$$

where $f = \sum f_i$ is the maximum mole fraction of probes which are quenched by oxygen. If all fluorophores are quenched to the same degree, $f = 1$. According to this modified Stern-Volmer equation, a plot of $I_0/(I_0 - I)$ against $1/[pO_2]$ based on the data, shown in Figure 1D, was reconstructed with the result given in Figure 1E.

A good linear relationship was fit to the data in Figure 1E with an equation of

$$\frac{I_0}{I_0 - I} = 1.0094 + 0.681 \frac{1}{[pO_2]} \quad (4)$$

In equation 4, an intercept of 1.0094 and coefficient constant (R^2) of 0.9955 was obtained. The good linearity over the entire oxygen concentration range facilitates the calibration of the oxygen sensor in practical applications.

3.1.4 Sensing performance for dissolved oxygen in B-R 7.5 buffer—A typical response of sensing film F3 was given in Figure 2. Significant quenching behavior was observed (Figure 2A). Similar to its response to gaseous oxygen, a nonlinear Stern-Volmer quenching effect was observed (Figure 2B). This multi-site quenching phenomenon was reconstructed using equation 3 and plotted in Figure 2C with an intercept of 1.0074 and R^2 of 0.9985. It should be noted here that the concentration of dissolved oxygen $[O_2]$ in liquid is proportional to its partial pressure. A standard atmosphere pressure of 101.3 kPa corresponds to an oxygen partial pressure pO_2 of 21.3 kPa, and the DO in air-saturated B-R buffer at 23 °C under the standard pressure is 8.6 mg/L or 8.6 ppm.

Although the sensing film F1 had no response to gaseous oxygen, it responded well to dissolved oxygen [32]. Film F2 had a good response to DO, similar to its response to gaseous oxygen. F1 and F2 follow linear Stern-Volmer equation in DO. I_0/I_{100} among the three films for dissolved oxygen were $F2 < F1 \ll F3$ (Table 1).

3.1.5 Response time to gaseous oxygen—Figure 3 shows the response time and operation stability of the probes to gaseous oxygen, which was conducted by reading the intensity signal when oxygen and nitrogen gases were switched.

It was found that the response time of F3, t_{95} (i.e. the time for 95% of the total change in fluorescence intensity to occur), for oxygen gas was 12 s switched from nitrogen gas to oxygen gas. The recovery time was slower with a t_{95} of 190 s. For F2, the response time t_{95} was 80 s and the recovery time t_{95} was 330 s (Table 1). It should be noted here, the response time actually is a combination of the response of the sensors to oxygen and the saturation

time of the environment by oxygen; the recovery time actually is a combination of the response of the sensors to nitrogen and the saturation time of the environment by nitrogen.

3.1.6 Response time to dissolved oxygen—Figure 4 shows the response time of the silica gel film F3 to dissolved oxygen. A fast response time (24 s) was still observed, but the recovery time was extremely slow being around 3000 s.

The response times of F1 and F2 to DO were 50 and 80 seconds and the recovery times were 240 and 420 seconds (Table 1), which were reported previously [32].

3.1.7 Leaching and photostability investigation of the sensing membranes in aqueous solution—The membranes were immersed in B-R buffers to study the fluorescence intensity change over time. No clear fluorescence intensity difference was observed before and after 48 hours of soaking in the liquid, showing that there was no leaching of the probes from the matrices because the sensors were chemically bonded with their matrices. Short term photostability was also tested under our experimental conditions. Films were excited at 405 nm (0.2 mW/cm^2) for 5 hours. Fluorescence intensity decays of less than 2% for F3 and less than 1% for F1 and F2 were observed after 150 continuous scans.

3.2 Discussion about the sensing performances

3.2.1 Matrices influence on responses to oxygen—The matrix significantly affected the oxygen responses. As shown in Table 1, the ratios of I_0/I_{21} , and I_0/I_{100} for gaseous oxygen followed the order of silica gel (F3) $\gg$ PS (F2) $\gg$ PHEMA (F1). The ratios of I_0/I_{21} and I_0/I_{100} for dissolved oxygen followed the order of silica gel (F3) $\gg$ PHEMA (F1) $>$ PS (F2). This matrix influence is most likely related with the oxygen diffusion ability in the matrix. Higher oxygen diffusion may result in a larger oxygen response. The silica gel was reported to have very good response to oxygen as the porous silica gel has high oxygen diffusion constants of $2.9 \times 10^{-4} \sim 1.1 \times 10^{-2} \text{ cm}^2/\text{s}$ [42,43]. The PHEMA films have no obvious response to oxygen gas, showing its non-oxygen diffusion ability for gaseous oxygen. The PHEMA can be significantly swollen by water, therefore, when used for DO measurement, the dissolved oxygen can diffuse well into the sensing membrane with water. As a result, the swollen PHEMA hydrogel has an oxygen diffusion constant of $3.8 \times 10^{-7} \sim 2.2 \times 10^{-6} \text{ cm}^2/\text{s}$ [31,43–46]. The oxygen diffusion constant in PS was known in the range of $1.1 \times 10^{-8} \sim 2.3 \times 10^{-7} \text{ cm}^2/\text{s}$ [47,48].

3.2.2 Matrix influence on the response time—Table 1 also lists the response times of the three sensing films. The silica gel film had the fastest response time to GO and DO, but the recovery time for DO was the longest. The PHEMA film had a faster response time and recovery time to DO than those of PS.

It has been reported that oxygen sensing silica gel had a response time t_{95} for gaseous oxygen in a range 3 seconds to 15 seconds [15,17,23,24,30], depending on sensors, the preparative materials, methods of the silica gels, and film thickness. The response time is also affected significantly by the experimental setup of the measurements. Although different groups may use the same materials both with sensors and matrices, experimental conditions like the gas flow velocity often vary for response time studies, which can affect the response times. However, these differences in experimental conditions can often go unreported in literature.

The silica sensing film also had the fastest response time for DO among the three films. However, the recovery time was extremely slow. Various response times for DO of the silica gel sensing films in the range of 4 s to 2 min were reported [15,17,22].

Platinum(II)-5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorophenyl)-porphyrin (PtTFPP), the precursor for the preparation of compound **1**, dispersed physically in PS films was studied by other scientists. Lee et al. [28] reported that PtTFPP in PS had a response time of 18 seconds and a recovery time of 60 seconds for gaseous oxygen. However, it was not indicated whether the time was t_{90} or t_{95} , nor was a detailed experimental set-up for the response time measurement reported. The increased response time of the polystyrene film F2 for gaseous oxygen and dissolved oxygen over those times reported may be due to: (1) our experimental setup may be different from others. It should be noted that when we increased the gas flow velocity to 1500 sccm, the response time t_{95} of F2 for gas oxygen was shortened to 42 seconds; (2) the crosslinking of the sensing film may possibly delay the response time.

As reported previously, the PHEMA film had a faster response time to DO than that of PS [32], since the PHEMA swells well in water to enable a higher oxygen diffusion constant in PHEMA than in PS.

For each film, when comparing the response times for DO and GO, it was found that the response to DO was slower. This phenomenon was reported for different sensing films and discussed by other scientists previously [15,17,44]. This is most likely because that oxygen has a much lower solubility in water than in gas with the same gas component under ambient conditions.

The above comparison clearly indicated that the matrices significantly influenced the response time to oxygen and provided information that may be useful for evaluating the matrices influences.

3.2.3 Matrix influence on the leaching and photostability—Under our experimental conditions, no influence of the matrices on probe leaching was observed. Since the sensors were chemically conjugated with their matrices, no leaching was found. Also, all the sensing films showed good photostability under continuous illumination using 405 nm.

3.2.4 Advantages and disadvantages of each material studied in this manuscript—The silica gel sensing film is very sensitive to oxygen in a low oxygen concentration range, but not sensitive for high concentrations of oxygen when the oxygen component is >50% in the gas mixture. This suggests that it is an excellent material for low oxygen concentration detection. Among the three materials for DO detection, it had the slowest recovery time after absorbing oxygen, which may possible limit its application for DO detection.

The linear response of the PS film to oxygen makes its applicable for all oxygen-containing environments. However, its sensitivity is small, which may possibly affect the measurement accuracy.

The PHEMA film was not sensitive to gaseous oxygen, indicating its non-applicability for GO. However, the swollen PHEMA film in liquid had reasonable sensitivity, suggesting a good avenue for DO sensing.

Therefore, by using the chemical immobilization approach and varying the probes and matrices, suitable sensing films with different applicability can be prepared to meet the practical requirements.

3.3 Preliminary application of F1 for dissolved oxygen monitoring during the growth of *Escherichia coli* (*E. coli*) JM109 under different conditions

3.3.1 Biocompatibility test—Through the above comparison of the three sensing films (F1, F2 and F3), we found F1 had the fastest response from oxygenated solution to deoxygenated solution (Table 1), e.g. the recovery time, therefore, we chose F1 as a typical example to monitor the oxygen consumption of *E. coli*. For all the studies herein, a strain of ampicillin-sensitive *E. coli* JM109 was used. In order to apply the sensing films, one requirement is whether the sensors are biocompatible. Two parallel experiments under the same experimental conditions were performed at room temperature (22 ± 1 °C) and 37 ± 1 °C. The variable is the presence and absence of the sensing film F1 (a trial with F1, another trial without). Results showed that time dependent OD₆₀₀ in one day cell culture with and without the sensing film F1 was the same (Figure 5), showing the sensing films' biocompatibility. It is noted here that this biocompatibility test was under the same experimental condition for oxygen consumption measurements. The sensor thin film F1 was immersed into 2.5 mL of sterilized LB medium in a quartz cuvette diagonally and a layer of mineral oil was added on the top of the LB medium to prevent the introduction of O₂ from the air into the medium. The use of mineral oil to prevent gas diffusion for bacteria studies was reported in literatures [33,49].

3.3.2 Long term stability test under *E. coli* JM109 environment—Long term stability and photostability of the sensing films against *E. coli* JM109 under different conditions were also carried out. A thin film F1 was put in a cuvette with 3 mL of LB medium with *E. coli* JM109 cell density of 10^8 cfu/mL. The cuvette was kept under dark. Emission intensity was measured at certain time intervals. It was found the fluorescence intensity of the film remained no change after the initial depletion of the oxygen by *E. coli* JM109. The intensity remained at the same value for 12 hours, showing the stability of the sensing film F1 against *E. coli* JM109. Another film F1 was put in the cuvette with the same condition as above, except that it was with continuous exposure to 405 nm light (0.2 mW/cm²) for 5 hours. The emission intensity at 650 nm was measured with an interval of 3 minutes. It was found that after reaching the maximum of the fluorescence intensity due to the consumption of oxygen by *E. coli* JM109, 5% total fluorescence decay was observed from the maximum fluorescence intensity. The photostability of the film in the *E. coli* JM109 culture condition was lower than that in the B-R buffer and medium, showing the influence of the bacteria on the films stability under continuous light exposure. Therefore, for accurate determination of dissolved oxygen concentration, the experiment design needs to avoid the long term continuous light irradiation, or reduce the light intensity when the long term continuous expose cannot be avoided. This weakness in long term photostability is common for organic materials-based optical sensors, which was mentioned in the introduction part.

3.3.3 Monitoring of oxygen consumption of *E. coli* JM109—It is well known that generation time of prokaryotic *E. coli* is short, usually in the range of 20 to 90 minutes, depending on culture conditions including temperature and cell density [50,51]. This short growth time facilitates the use of *E. coli* for preliminary monitoring of extracellular dissolved oxygen concentration changes. *E. coli* can grow either in the presence or absence of oxygen because of its ability to use different metabolic pathways. During the growth process, the aerobic metabolism of *E. coli* consumes oxygen. To efficiently monitor oxygen concentration changes, a layer of mineral oil was added on the top of the LB medium to prevent the introduction of O₂ from the air into the medium [33,49]. *E. coli* JM109 cell densities of 1×10^6 , 1×10^7 , 5.0×10^7 , and 1.0×10^8 cfu/mL were used. Time dependent emission intensity at 650 nm was measured continuously, which was excited using 405 nm. Interval of each measurement was 30 seconds. Experimental duration was 90 minutes,

performed at room temperature (22 ± 1 °C). As *E. coli* was cultured in LB medium, the sensor thin film F1 was titrated in the same medium before the bio-experiment. Intensity at 650 nm against oxygen partial pressure and the Stern-Volmer curve fitting were plotted and were used as calibration curves for the oxygen concentration determination. Fluorescence intensity changes during *E. coli* JM109 growth were given in Figure 6.

When the *E. coli* cell density was relative low (1×10^6 cfu/mL), only a small fluorescence intensity change was observed during the growth process as the consumption of oxygen was not enough to change the oxygen concentration of the LB medium significantly. With the increase of initial *E. coli* cell densities, significant oxygen consumption was observed during the growth periods. For the initial *E. coli* cell density of 1×10^8 cfu/mL, the dissolved oxygen was completely consumed within 4 minutes. The emission intensity of about 64 a.u. corresponds to an oxygen partial pressure of zero. Although the oxygen was consumed, the cells continued to grow (Figure 5) under the oxygen deficient condition.

According to the rate of oxygen depletion by *E. coli* and initial cell densities, oxygen consumption rate of single cell was calculated to be from 1.02×10^{-19} to 2.05×10^{-19} mol $\cdot$ cfu $^{-1} \cdot$ s $^{-1}$, slightly affected by the initial cell densities. It is known oxygen consumption rate depends on particular bacteria and experimental conditions including temperature and time. Geckil et al [52] reported that the oxygen consumption rate of *E. coli* was in a range of 1.21×10^{-19} – 1.78×10^{-18} mol $\cdot$ cfu $^{-1} \cdot$ s $^{-1}$. Kaya et al [53] reported single *E. coli* oxygen consumption rate of $\sim 0.9 \times 10^{-20}$ mol $\cdot$ cfu $^{-1} \cdot$ s $^{-1}$ in cone-shaped microwell, however of $\sim 4.4 \times 10^{-19}$ mol $\cdot$ cfu $^{-1} \cdot$ s $^{-1}$ in cylindrical microchip.

3.3.4 Antibiotics influence on the oxygen consumption—Influence of antibiotics on the cell metabolic activity, herein oxygen consumption, was investigated by using ampicillin as an example. Ampicillin is a beta-lactam antibiotic that has been used extensively to treat bacterial infections since 1961. Ampicillin acts as a competitive inhibitor of the enzyme transpeptidase, which is needed by bacteria to make their cell walls [54]. Ampicillin inhibits the third and final stage of bacterial cell wall synthesis in binary fission, which ultimately leads to cell lysis. For this study, the ampicillin-sensitive *E. coli* JM109 cells were cultured with an initial OD₆₀₀ of 0.1 under various concentrations of ampicillin under aerobic condition at 37 °C with a shake rate of 200 rpm. After 12 hours, OD₆₀₀ was measured (Figure 7A). As shown previously in Figure 6, the oxygen consumption with an initial cell density of 5×10^7 cfu/mL, corresponding to an OD₆₀₀ of 0.1, showed an oxygen depletion time of 16 minutes. In order to measure the oxygen consumption at a reasonable time frame, the cells were diluted using fresh LB medium with various ampicillin concentrations to initial OD₆₀₀ of 0.1. In the cases of the cell cultures with OD₆₀₀ less than 0.1 (when the ampicillin concentration was higher than 5 µg/mL), there was no dilution of the cell culture media. Figures 7B showed the antibiotics influence on the oxygen consumption. No significant influence of the oxygen consumption by an ampicillin concentration of 1 µg/mL was observed. Clear slowdown of the oxygen consumption by the ampicillin concentration of 2 µg/mL and higher was observed. Ampicillin concentration with 5 µg/mL and higher completely stopped the oxygen consumption. The slowly continuous fluorescence intensity decrease of the film in the cell culture medium with 50 µg/mL ampicillin (Figure 7B) probably indicated the photodecomposition of the sensing film under this condition. Most likely the high concentrated cell lysis products by the antibiotics increased the film photo-instability.

According to the results shown in Figure 7, the minimum inhibitory concentration (MIC) is in between 1 and 2 µg/mL. Kaya et al reported that the MIC of the ampicillin to *E. coli* JM109 was 1.56 µg/mL [55]. The MICs of the ampicillin to other ampicillin-sensitive *E. coli* strains were reported in a range of 1.1 to 5.7 µg/mL [56]. Therefore, our results are in

accordance with those reported in literature. Also our studies indicated our sensing materials have potentials for understanding of the antibiotics influence on cell metabolic activities and the evaluation of the antibiotics activities.

4. Conclusion

A new crosslinkable silane-containing oxygen sensor was prepared and was used in a new silica-gel oxygen sensing film preparation. Sensing behaviors among the silica gel sensing films, the polystyrene films, and the poly(2-hydroxyethyl methacrylate) films were compared. Matrices significantly affected the oxygen sensing behaviors, including sensitivity and response time. The silica film had the fastest response time and was most sensitive to oxygen when the oxygen fraction was below 50% by volume. The PHEMA sensing films were not sensitive to gaseous oxygen, but were more sensitive to dissolved oxygen and responded more rapidly than those of the PS films for dissolved oxygen sensing. A successful preliminary application of the PHEMA film was performed, which was used to monitor dissolved oxygen concentration changes induced by the growth of *E. coli*.

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Figure 1.

Response to gaseous oxygen using sensing films F2 (A and C) and F3 (B, D and E). A and B: fluorescence intensity of the probes quenched by oxygen. C: linear Stern-Volmer quenching constant of F2 to oxygen. D: nonlinear Stern-Volmer quenching constant of F3 to oxygen. E: linear quenching constant of F3 using modified Stern-Volmer equation (3).

Figure 2.

Response to dissolved oxygen using sensing film F3. A: fluorescence intensity of the probes quenched by oxygen. B: nonlinear Stern-Volmer quenching constant of F3 to oxygen. C: linear quenching constant of F3 using modified Stern-Volmer equation (3).

Figure 3.
Response time of F2 (A) and F3 (B) to gaseous oxygen.

Figure 4.
Response time of F3 to dissolved oxygen.

Figure 5. Time dependent OD₆₀₀ of 5×10^7 cfu/mL of *E. coli* with and without F1 film at 37 °C and 22 °C.

Figure 6.
Oxygen consumption monitored using F1 at different initial *E. coli* cell densities.

Figure 7. Ampicillin concentration dependent OD_{600} (A) and oxygen consumption (B) in the emission intensity model.

Scheme 1.

Table 1

Responses of the three sensing films to gaseous oxygen (GO) and dissolved oxygen (DO) in B-R buffer.

Intensity ratios of I_0/I_{100} and I_0/I_{21}						
			Response Time (t_{95})		Recovery Time (t_{95})	
I_0/I_{100} (GO)	I_0/I_{100} (DO)	I_0/I_{21} (GO)	I_0/I_{21} (DO)	GO	DO	
F1	1.1	9.8 [32]	1.0	3.0 [32]	-----	240 s [32]
F2	5.8	5.0 [32]	2.1	1.9 [32]	80 s	84 s [32]
F3	70	60	24.6	15.9	12 s	24 s
						190 s
						~3000 s

F1: PHEMA matrix; F2: PS matrix; F3: silica gel matrix. I_0 : Fluorescence intensity under deoxygenated condition; I_{21} : Fluorescence intensity under air condition; I_{100} : Fluorescence intensity under oxygenated condition.