Published in final edited form as:

IEEE Trans Nanobioscience. 2015 December ; 14(8): 811–817. doi:10.1109/TNB.2015.2478481.

A UAV-Mounted Whole Cell Biosensor System for Environmental Monitoring Applications

Yi Lu[†],

Department of Aerospace and Mechanical Engineering, University of Arizona, Tucson, AZ 85721 USA

Department of Biomedical Engineering, Pennsylvania State University, University Park, PA 16802

Dominique Macias[†],

BASIS Tucson North High Schools, Tucson, AZ 85716 USA

Zachary S. Dean,

Department of Biomedical Engineering, University of Arizona, Tucson, AZ 85721 USA

Nicole R. Kreger, and

Department of Aerospace and Mechanical Engineering, University of Arizona, Tucson, AZ 85721 USA

Pak Kin Wong* [Member, IEEE]

Department of Aerospace and Mechanical Engineering, University of Arizona, Tucson, AZ 85721 USA

Department of Biomedical Engineering and Department of Mechanical and Nuclear Engineering, Pennsylvania State University, University Park, PA 16802

Yi Lu: luyi@email.arizona.edu; Dominique Macias: dominiquenmacias@gmail.com; Zachary S. Dean: deanz@email.arizona.edu; Nicole R. Kreger: nicolekreger@email.arizona.edu

Abstract

This study reports the development of a portable whole cell biosensor system for environmental monitoring applications, such as air quality control, water pollution monitoring and radiation leakage detection. The system consists of a lightweight mechanical housing, a temperature regulating system, and a microfluidic bacterial inoculation channel. The overall system, which is less than 200 g, serves as a portable incubator for cell inoculation and can be mounted on an unmanned aerial vehicle for monitoring remote and unreachable locations. The feedback control system maintains the inoculation temperature within 0.05 degree Celsius. The large surface-to-volume ratio of the polydimethylsiloxane microchannel facilitates effective gas exchange for rapid bacterial growth. Molecular dynamic simulation shows effective diffusion of major gas pollutants in PDMS toward gas sensing applications. By optimizing the design, we demonstrate the operation of the system in ambient temperatures from 5°C to 32°C and rapid bacterial growth in microchannels compared to standard bacterial culture techniques.

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*Pak Kin Wong: ; Email: pak@email.arizona.edu

[†]These authors contribute equally to the work

Index Terms

Microfluidics; whole-cell biosensor; PDMS; gas diffusivity; environmental monitoring; portable incubator; PID control

I. Introduction

The increasing amount of harmful pollutants and volatile chemical compounds in the environment has drawn widespread public attention to demand effective monitoring techniques. For conventional “off-site” analysis, samples are collected and sent to an environmental testing laboratory to detect pollutants using physical and chemical gas sensing technologies. However, these analytical techniques are often expensive, time-consuming and labor-intensive. Some gas sensors have a low sensitivity and poor selectivity for analyzing gas mixture [2, 3]. More importantly, most conventional environmental sensing techniques can only sample specific time points.

The demand for continuous, on-site monitoring of environment pollutants has driven the development of field deployable chemical sensors that are fast, reliable, sensitive and cost-effective [4, 5]. Toward this goal, whole cell biosensors, which are highly sensitive and versatile, represent a promising strategy for environmental monitoring [6–8]. The cells may naturally, or be genetically maneuvered to, respond to different environmental insults including air pollution, water quality, biochemical toxin, and radiation leakage [2]. The amount of pollutants can be reflected by the proliferation, gene expression, and metabolic activities of the cell. For instance, the growth kinetics of cells could be an indicator of environmental stress and potential threats to human health. Several whole-cell biosensors have been developed to monitor the gas toxicity [9], air quality [10], and water pollution [11]. For monitoring remote or potentially dangerous locations, the whole-cell biosensor should be delivered and maintained in a proper culture conditions with a portable power source [12, 13]. The cells should also be properly interfaced with the environment, such as gas pollutants and radiation.

In this project, we developed a portable whole cell biosensor system that allows continuous environmental monitoring in remote locations (Fig. 1a–b). The design of the system includes a microfluidic channel for bacterial inoculation, a temperature regulating system, and a mechanical enclosure to house all components (Fig. 1c). Microchannels, which have a large surface-to-volume ratio, provide effective gas exchange for rapid bacterial growth without bulky support equipment [14]. The polydimethylsiloxane (PDMS) microchannel is fabricated using soft lithography (Fig. 1d). The chemical inertness, transparency, and gas permeability render PDMS an ideal material for whole cell biosensing applications. Toward gas pollutant detection applications, molecular dynamic simulation is performed to study the diffusivity of several major pollutant gases in PDMS. Furthermore, the system is designed such that it can be mounted on a quadcopter or other unmanned aerial vehicles (UAV) while providing an appropriate environment for bacterial growth. To evaluate the performance of the system, the viability of *E. coli* bacteria in the whole cell biosensor system is measured

and compared with standard bacterial culture techniques. This design may open new opportunities in developing novel strategies for environmental monitoring.

II. Materials and Methods

A. Portable Incubator Design

The design of the whole cell biosensor system consists of a mechanical housing, a temperature regulating system, and a microfluidic bacterial inoculation channel.

1) Mechanical Housing—The mechanical design of this project involves creating an enclosure to house the temperature regulating system (heater, temperature sensor and microcontroller) and the bacterial inoculation microchannel. Fig. 2a shows the housing has two layers which can hold the control board and bacterial inoculation microchannel separately. The design of the mechanical housing was first created in solid modeling CAD software (Solidworks, Dassault Systèmes, France) (Fig. 2a). Then the prototypes were printed using a 3-D printer (Makerbot Replicator 2, Makerbot, Brooklyn, NY) (Fig. 2b). The structural material was polylactic acid (PLA), a light, biodegradable thermoplastic aliphatic polyester. The housing was designed as lightweight as possible and had a clamping system for attaching to the quadcopter. The entire system weighed less than 200 g.

2) Temperature Regulating System—The electrical configuration of the temperature regulating system is shown in Fig. 2c. The device consists of four major components: a microcontroller Arduino Uno (ARDUINO, Italy), two temperature sensors LM35CH/NOPB (Texas Instruments, Dallas, TX) for measuring the interior and exterior temperature respectively, and a polyimide low voltage thermofoil heating element (KHLV-202, Omega Engineering, INC, Stamford, CT). The thermofoil heater is light, energy efficient, and fast in regulating the temperature (Fig. 2d). Three heaters with different power ratings (2.5 W/in^2 , 5 W/in^2 , and 10 W/in^2) were tested in this study. A Darlington transistor array (ULN2003A, STMicroelectronics, Geneva, Switzerland) was also applied in the device to drive the heating element.

3) Microchannel Fabrication—PDMS was chosen as the channel material for bacterial inoculation in this study. Among various elastomers, PDMS has a high gas diffusivity and is chemically inert. The microchannels for bacterial inoculation were fabricated by molding PDMS (Sylgard 184, Dow Corning Inc, Midland, MI) at 65°C for 5 hours. The PDMS was thoroughly mixed with the base and crosslinking agent at a 10:1 ratio. The mixed sample was degassed in a vacuum chamber for 5 minutes and poured onto an aluminum mold to a thickness of about 1.3 mm. The aluminum mold was machined using a computer numerical control system with micrometer precision (HAAS automation Inc., Oxnard, CA). The PDMS sample was cured on a hot plate at 65°C for 5 hours. In this study, microchannels with a length of 5 cm, width of 5 mm, and depth of $300\text{ }\mu\text{m}$ were used for bacterial inoculation. The PDMS channel layer and a glass substrate were sterilized and bonded using an atmospheric (air) plasma system. In order to minimize bacterial adhesion on the channel surface, the microchannel was incubated with 1% (w/v) bovine serum albumin (BSA) for 10 min and rinsed with phosphate buffered saline (PBS).

B. Heat Transfer Analysis

Finite element analysis was performed to estimate the temperature uniformity and study the heat transfer characteristics of the system. The results were applied for optimizing the design and select the appropriate heating element for operating in a large range of ambient temperature. Modeling and numerical simulations were performed by Comsol Multiphysics software (v4.4, COMSOL Inc., Los Angeles, CA). In the simulation, the heat transfer in solid module was applied for calculating the time-dependent temperature distribution in the model. The temperature field was estimated based on the energy equation:

$$\rho_m c \left(\frac{\partial T}{\partial t} + u \cdot \nabla T \right) = k \nabla^2 T + Q \quad (1)$$

where k , ρ_m and c are the thermal conductivity, density, and specific heat of the fluid, respectively. Q is the heat generated from the heating element. A 2-D finite element model, which modeled the 3D printed PLA culture chamber, the bacterial inoculation microchannel, and the heating element, was developed to evaluate the uniformity of the temperature distribution in the entire culture chamber and the heating time constant for each heating elements at three different boundary conditions, specifically ambient temperatures at 5 °C, 20 °C, and 35 °C.

C. Molecular Dynamic Simulation of Diffusivity

For gas sensing applications, the diffusion of the gas pollutant in PDMS should be considered. The diffusion coefficient describes the diffusive flux due to a chemical gradient of the substance, as defined in Fick's first law:

$$J = -D \nabla \phi \quad (2)$$

where D is the diffusion coefficient of the substance and J is the diffusion flux, which is proportional to the concentration gradient of the substance, ϕ .

Diffusion coefficients of CO, O₂, O₃, CO₂, SO₂, and NO₂ in an isothermal-isobaric (NPT) ensemble (i.e., constant number of moles, pressure and temperature) at 300K and 1 atm were determined by molecular dynamic (MD) simulations using the Materials Studio (Accelrys, San Diego, CA). Simulations of penetrant molecules diffusing in a PDMS microstructure were computed as a function of time by solving Newton's equations of motion. For each gas, a total of 100 penetrants were used as the diffusing species. Penetrants were introduced into the system at the "random walk" trajectory in the PDMS microstructure without overlap. To compute the diffusion coefficients, the mean-square displacement (MSD) of the penetrant molecules was tracked and the diffusivity was estimated by means of the kinetic theory:

$$D = \frac{1}{6N_a} \lim_{n \rightarrow \infty} \sum_{i=1}^{N_a} \langle [r_i(t) - r_i(0)]^2 \rangle \quad (3)$$

where N_a is the number of diffusive atoms in the system and $r(0)$ and $r(t)$ are the position coordinate of the penetrant molecules at initial and time t during the simulation. $|r(t) - r(0)|$ represents the displacement of the penetrant molecule. A time step of 1 fs (1×10^{-15} s) was applied in this study. Equation 3 assumes that the diffusion coefficient is a constant and is

proportional to the slope of the MSD with respect to time. In this study, the MSD of CO, O₂, O₃, CO₂, SO₂, and NO₂ were calculated from 120 ps trajectories to estimate the average diffusive motion of each gas molecules in PDMS.

D. Pathogens and physiological samples

E. coli clinical isolate (EC137) was applied in this study. The bacterium was isolated as part of a research protocol approved by the Stanford University Institutional Review Board. Before the experiment, bacteria were first inoculated in Mueller-Hinton broth (BBL, Becton Dickinson, East Rutherford, NJ) to early exponential phase and were diluted to appropriate concentrations for the experiment.

E. Assembled System and Flight Test

To demonstrate the ability of the system for environmental monitoring in remote areas, the whole cell biosensor system was mounted and carried by a UAV (Phantom 2, DJI, Shenzhen, China). The maximum lifting weight of the quadcopter was approximately 300 grams. The whole cell biosensor system weighed about 200 grams and could be carried by the quadcopter (Fig. 3). The quadcopter also carried a 5200 mAh smart battery (DJI, Shenzhen, China), which was used to drive the cell-based biosensor systems. The biosensor was mounted on the quadcopter a 3D printed clamping system. The flight test showed the flight time was over 20 minutes with the whole cell biosensor system (Supplementary Video 1).

III. Result and Discussion

A. Heating Performance

Finite element analysis was conducted to select the appropriate heating rate of the thermofoil. In the simulation, three common power ratings (heat flux), 2.5 W/in², 5 W/in², and 10 W/in², were chosen from the 28 Volts DC series (Omega engineering, Bridgeport, NJ). Practically, the heater is powered with 12 volts DC; the equivalent power rating should be 0.459 W/in², 0.92 W/in², and 1.837 W/in², respectively. In the simulation, each power rating was tested at three different boundary conditions (10°C, 20°C, and 30°C) to estimate the performance of the system in different ambient temperatures. Fig. 4a shows the representative simulation result of the temperature distribution in the cross-section (width × height plane) of the system (Supplementary Video 2). Fig. 4b shows the average temperature in the microchannel as a function of time with different power ratings and ambient temperatures. The thermofoil of 10 W/in² was capable of heating the microfluidic chips from a wide initial temperature (10–30°C) to the designated temperature (37 °C) in a few minutes.

The heating performance of the system was also estimated experimentally. Fig. 4c shows the transient heating profiles with a general lab incubator and the thermofoil heater with different controllers. The time required by the thermofoil to heat the system to 37°C was approximately 240 seconds, which was three times faster than that of the lab incubator. An Arduino Uno microcontroller was programmed to regulate the temperature inside the system. Different heating elements and temperature controllers were compared by monitoring the temperature of the sensor every second for a duration of 15 minutes. An

on/off controller was first tested. The controller was programmed to turn off a mechanical switch when the temperature reached 37.5°C and turn on the switch when the temperature reached 36.5°C. The on/off controller had a rapid temperature rise. However, the temperature had a large fluctuation (± 1 °C) and was sensitivity to the environmental temperature. To obtain a steady temperature for bacterial growth, a proportional-integral-derivative (PID) controlled system, which continuously calculates the error value between the measured temperature and the desired temperature, was demonstrated to maintain the temperature at 37 °C within 0.05 °C. The thermofoil with the PID controller allowed accurate temperature control and was applied for the rest of the study. The performance of the system can be improved by adjusting the PID parameters in different ambient conditions. We, therefore, developed a hybrid PID strategy to enhance the robustness of our system. In particular, the PID parameters are adjusted according to the ambient temperature (Table 1). We tested our system with the hybrid PID controller at three different ambient conditions, including the refrigerator (5° C), the laboratory (20°C), and the oven (32°C). The results demonstrate the whole cell biosensor system with the hybrid PID strategy can regulate temperature precisely and effectively (Fig. 4d).

B. Gas Supply and Consumption

Gas supply and consumption are important considerations for environmental monitoring using whole cell biosensors. PDMS is gas permeable, oxygen and gas pollutants can diffuse into the medium inside the microfluidic inoculation channel. The maximum gas flux F_{max} by diffusion through the PDMS layer can be approximated by the diffusion equation:

$$F_{max} = D_{PDMS} \left(\frac{\Delta C}{\Delta z} \right) \quad (4)$$

where D_{PDMS} , C and z denote the diffusivity of gas in PDMS, the difference of gas concentration across the PDMS layer and the thickness of the PDMS layer.

Molecular dynamic (MD) analysis was performed to study the diffusivity of several gases in PDMS (Supplementary Video 3). As shown in Fig. 5a, the model system consisting of 50 short PDMS chains ($n=10$) and 100 gas molecules was created in this study. Fig. 5b shows molecular dynamic simulation of mean-square displacements (MSD) of O₂, O₃, CO, CO₂, SO₂, and NO₂ molecules in PDMS as a function of time. As predicted in equation 3, the MSD increased linearly with time in the duration of the simulation (100 ps). The diffusivities of the gases were then determined from the slopes of data (Fig. 5c). Table 2 lists values of diffusivity for oxygen and several major gas pollutants produced by human activity. D_{est} represents the diffusion coefficient estimated by MD simulations and D_{exp} is values obtained experimentally in a previous study [1]. The diffusivities of O₃, CO, SO₂, and NO₂ in PDMS, however, have not been reported. The MD simulation results for O₂ and CO₂ are in good agreement with the experimentally values, suggesting the applicability of MD simulation for estimating the diffusivity. We observed a decreasing trend of the diffusion coefficient with the molecular size of the penetrant molecules (Fig. 5d). Our results suggest the diffusivity of these major gas pollutants in PDMS are on the same order of magnitude, which supports the usage of PDMS microchannel for environment monitoring applications.

With the diffusivity of the different gases in PDMS, we can apply these values to estimate the maximum diffusion flux F_{max} through the PDMS layer. For example, one of the prerequisites for the normal function of the whole cell biosensor is to maintain the viability of bacteria, which requires a sufficient supply of oxygen. In the current microchannel design, the PDMS thickness z is 1 mm. The oxygen concentration in the atmosphere is 0.2 mol/m^3 . The diffusivity of oxygen in PDMS is $4.1 \times 10^{-9} \text{ m}^2/\text{s}$ [1]. Using equation 4, the maximum oxygen flux is estimated to be $\sim 50 \text{ pmol/s}$, which is sufficient to support the growth of *E. coli* to 10^9 cfu/ml [14, 15].

C. *E. coli* Viability Testing

The performance of the whole cell biosensor system was evaluated by growing bacteria under different ambient conditions. The bacterial growth inside the biosensor was also compared with other standard bacterial culture techniques. The *E. coli* were pre-cultured and diluted to a concentration of OD 0.01 ($\sim 10^7 \text{ cfu/ml}$). Fig. 6a shows an image of the channel at the beginning of the experiment. The bacteria were also incubated in a flask in a conventional incubator and a flask in an orbital shaker. At the beginning, the bacterial growth rates were similar in all conditions. The growth rates could be clearly distinguished after 2 hours. The viability of the bacteria was visually inspected by optical microscopy (Fig. 6b) and the growth of the bacteria was determined by measuring the absorbance with a spectrophotometer. Fig. 6c shows the concentration of EC137 after 2 hours of inoculation. The viability of the bacteria in the biosensor was significantly higher compared to culturing with a flask. Interestingly, the growth in the whole cell biosensor system was $\sim 20\%$ higher than the orbital shaker incubator. This is likely due to the small thermal inertia of the microchannel, which can be heated up rapidly compared to the flask. As shown in Fig. 4d, the microchannel only needed 5 minutes to heat up the microchannel with sample solution to 37°C . The sample solution in a flask in a conventional incubator took at least 15 minutes to reach 37°C (Fig. 4c). Therefore, the heating rates correlated with the bacteria growth rates and provided an explanation in the difference.

The growth of bacteria in the system under different ambient temperatures was also compared. The experiment was performed in three different ambient temperatures which represent three different field temperature, in the lab temperature ($\sim 21^\circ\text{C}$), in a refrigerator ($\sim 5^\circ\text{C}$) and in the outside field ($\sim 32^\circ\text{C}$), respectively. The growth rate of bacteria has a comparable result in the three different conditions (Fig. 6c), demonstrating the ability of the portable incubator system to operate in various environmental conditions.

IV. Discussion

In this study, we developed and optimized the mechanical, thermal, and electrical components of a microfluidic whole cell biosensing system. The possibility of being carried by a UAV opens up new opportunities for sensing in remote and unreachable areas. For instance, the system can be applied for testing air quality and pollution levels. To explore this possibility, the diffusivities of several gas pollutants, including CO , O_2 , O_3 , CO_2 , SO_2 , and NO_2 , were evaluated. Rapid cell growth was demonstrated, suggesting effective gas exchange of the system. Additional tests should be performed to evaluate of the ability of the

system for environmental monitoring. Other engineering interfaces should also be incorporated in order to detect other major air pollutants, such as lead and particulate matter, and environmental contaminations, such as radiation and water pollutant.

The system with a gas permeable microchannel and PID temperature control represents an effective platform for culturing cells under various environmental conditions. The ability to culture living cells in the system provides the foundation for a myriad of potential whole cell biosensing applications. In this study, we applied *E. coli*, which has a short doubling time, to demonstrate the performance of the system. In the future, other cell types, such as slow-growing bacteria, fungi and mammalian cells, with different growth characteristics and genetic modifications can be incorporated to enhance the functions of the system. Other optical and electrochemical readouts, such as gene expression and metabolic activities, can also be applied to evaluate the response of the cells [16]. The system may also have applications in phenotypic antimicrobial susceptibility testing in the future [17–19].

V. Conclusion

In summary, a portable whole cell biosensor system was successfully developed. The system is lightweight and can be transported by a UAV. The temperature regulating system can precisely maintain the inoculation channel 37 ± 0.05 °C in a wide ambient temperature range. This ability is essential for maintaining the long-term cell viability for continuous environmental monitoring. We have demonstrated that the system has superior performance over traditional culture techniques. The diffusivity of common gas pollutants in PDMS suggests effective gas diffusion of these gases for air pollution monitoring. The system is anticipated to be applied in various whole biosensing applications in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by the National Institutes of Health (U01AI082457, R44AI088756, DP2OD007161 and ES06694).

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Fig. 1.

(a) Schematic of a UAV-mounted whole cell biosensor systems for remote sensing applications. (b) Photograph of the whole cell biosensor system mounted on a quadcopter during operation. (Insert) The whole cell biosensor system is attached to the bottom of the quadcopter. (c) Mechanical housing of the portable incubator system with the temperature regulating system. (d) Image of a 300 μm height PDMS microfluidic channel for bacterial inoculation.

Fig. 2.

(a) A 3D drawing of the mechanical housing of the whole cell biosensor system created by SolidWorks. (b) A 3D printer system for manufacturing o the mechanical housing. (c) Electrical design consisting of a customized electrical circuit for PID control. (d) A thermofoil for regulating the temperature of the system.

Fig. 3.

Flying test. The system is attached to the bottom of quadcopter. Maximum flight time is approximately 20 minutes.

Fig. 4.

(a) Simulation result of temperature distribution in a cross-section of the portable incubator with the microfluidic chips. (b) Simulation result of the average temperature on the microchannel as a function of time with three different power rating (2.5 W/in², 5 W/in², and 10 W/in²) and three ambient temperature boundary condition (10 °C, 20 °C, and 30 °C). (c) Comparison between a general lab incubator and a thermofoil heater with different control strategy. (d) Portable incubator with hybrid PID system at three different ambient temperature (5 °C, 22 °C, and 32 °C).

Fig. 5.

(a) The model system consisting of 50 short PDMS chains ($n=10$) and 100 gas molecules. (b) Average mean-square displacements of O₂, O₃, CO, CO₂, SO₂, and NO₂ molecules in PDMS. (c) Diffusivity of O₂, O₃, CO, CO₂, SO₂, and NO₂ molecules in PDMS. (d) Diffusivity of gases as a function of the molecular size.

Fig. 6.

Microscope images for *E. coli* (a) initially and (b) after 2 hours of incubation in the biosensor. Scale bar: 20 μ m. (c) Comparison of bacterial growth in a 300 μ m microchannel with the portable incubator (SBL Incubator) at three ambient temperature, 5 °C, 22 °C and 32 °C,. An Erlenmeyer flask inside a shaking incubator (Shaker Incubator), and a static Erlenmeyer flask (Classic Incubator) (**, $P < 0.01$)

Table 1

Hybrid PID strategy: PID parameters will self-adapt to the different ambient temperatures

	P	I	D
5 °C	<i>120</i>	<i>1</i>	<i>2500</i>
22 °C	<i>50</i>	<i>0.3</i>	<i>1500</i>
32 °C	<i>10</i>	<i>0.03</i>	<i>8</i>

Table 2

Diffusivities of oxygen and major gas pollutants. The unit of diffusivity is (m^2/s). The values of the estimated diffusivity (D_{est}) are for the temperature of 300 K; the experimental values (D_{exp}) are obtained from ref [1].

PDMS (T=300K)		
Gas	D_{est}	D_{exp}
O ₂	3.717×10^{-9}	4.1×10^{-9}
O ₃	3.597×10^{-9}	NA
CO	3.847×10^{-9}	NA
CO ₂	2.207×10^{-9}	2.6×10^{-9}
SO ₂	1.877×10^{-9}	NA
NO ₂	1.555×10^{-9}	NA