A microfluidic ATP-bioluminescence sensor for the detection of airborne microbes

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Abstract

Airborne pathogenic microorganisms are hazardous bioaerosols which often cause serious respiratory diseases. To prevent airborne infectious disease, real-time detection and monitoring systems of airborne pathogens are needed. Since ATP (adenosine triphosphate) is a major biological energy source, the detection of ATP from aerosol reflects the existence of living microbes. Therefore, we developed a new biosensor to detect ATP from aerosols in real-time using an aerosol condensation system, a microfluidic channel, and an ATP-bioluminescence transducer. The condensation system enabled aerosol microbes (4 L) to be hydrosolized (0.2 ml) in 2 min. The bacterial intracellular ATP was then extracted in the passage through the microfluidic channel. The concentration of ATP could be determined by a bioluminescence sensor integrated in the channel. In this study, we used *B. subtilis* and *E. coli JM110* as model airborne microbes. Our system can determine the existence of airborne microbes within 10 min. In the future, the application of our device will extend to the detection of fungi and consequently contribute to improving indoor air quality.

Keywords: Airborne microbes; Aerosol condensation; Microfluidic channel; ATP-bioluminescence sensor

1. Introduction

The real-time detection of microorganisms such as virus, bacteria, and fungi is an emerging and rapidly evolving field of research. The spread of airborne pathogens like measles, anthrax, *Legionella*, influenza, smallpox, and rhinovirus is often regarded as major threats of public health since they cause severe airborne infectious diseases with high mortality rates [1]. Furthermore, most bacterial and viral pathogens can be used for biological weapons capable of immense destruction [2]. These airborne diseases can spread rapidly by means of airborne transmission from person to person via the respiration of pathogenic bioaerosols. In order to prevent the transmission of such airborne infectious diseases and control dangerous biological particles in public places and dwellings, efficient real-time detection systems are required.

Conventionally, the detection of airborne bacteria has been achieved by collecting and culturing, a method which is very effective but requires a long incubation time (at least 24 h). In general, the collection of living organisms is achieved by commonly used sampling methods in aerobiology, e.g. filtration, air washing, impingment, and impaction [3].

In recent years, new techniques have been developed to replace the standard sampling method in order to reduce the duration of testing. Mainelis et al. [4] developed a new bioaerosol sampler, called as electrostatic precipitator, which utilized an electric field to deposit charges on bacterial samples and a solid agar as a bacterial growth media. After the development of the sedimentation method, Vadrot et al. [5] adapted the polymerase chain reaction (PCR) for direct detection of *Mycobacterium tuberculosis*. Deloge-Abarkan et al. [6] tested and compared the main principles for bioaerosol collection methods, i.e. solid impaction, liquid impingement and filtration, and they performed fluorescent in situ hybridization (FISH) for airborne *Legionella* bacteria detection. Most recently, Sengupta et al. [7] reported a detection method based on Raman spectroscopy which relies on inelastic scattering, or Raman scattering of monochromatic light, usually from a laser in the visible range. They utilized a silver coated bioanalyte suspension to obtain an enhanced Raman spectrum, and then could detect and characterize airborne bacteria rapidly by injecting the suspension through a light scattering chamber.

ATP (adenosine triphosphate) is the most important biological fuel in living organisms. Detecting ATP originating in air...
could thus be an important method for detecting living organisms like airborne pathogens, although the critical biohazard concentration is yet unknown. Quantitative measurements of ATP have been applied to biological and environmental systems for years. For example, the growth of bacteria was monitored in real time by the measurement of bioluminescence [8,9]. However, most of the applications in the area of ATP detection were limited to food and hygienic systems, for instance, determining the surface cleanliness of kitchen using traditional hygiene swabbing method plus ATP-bioluminescence [10] and evaluating the microbial load on hands or domestic surfaces by ATP-bioluminescence monitoring as a surrogate marker [11].

In this paper, we demonstrate a new real-time detection system to measure ATP extracted from airborne microbes using (1) a condensation system to concentrate aerosol, (2) a microfluidic channel to extract bacterial ATP, and (3) a bioluminescence sensor to measure ATP content. Our technique will help improve environmental monitoring methods to prevent airborne infectious diseases.

2. Materials and methods

2.1. Microbes and reagents

Bacteria were obtained from Korean Culture Center of Microorganisms (KCCM). *E. coli* JM110 (ATCC 47013) and *B. subtilis* (ATCC 6633) were grown in nutrient culture media at a temperature of 37 °C for 24 h. Bacterial growth was monitored using a spectrophotometer and bacteria were harvested in midlog phase (optical density of 0.53), corresponding to about 1.33 × 10⁹ cells/ml. In this study, bacteria are destroyed by a lysis buffer which was purchased from Bioneer Co. (South Korea). D-luciferin-luciferase reagent (Lucipac W kit, Kikkoman International, Japan) was used to induce bioluminescence at 560 nm by a reaction with ATP.

2.2. Aerosol generation and condensation system

As shown in Fig. 1, our whole experimental system consists of three major parts: aerosol generation, condensation, and ATP detection. An air supply system filters out airborne dust to introduce clean air into the nebulizing equipment via a mass flow controller. Bacteria-liquid droplets were generated at a flow rate of 2 L/min by using a 1-jet nebulizer (MRECN24). The size of the droplets was analyzed using an aerodynamic particle sizer (APS) (TSI 3321).

The aerosolized bacteria were hydrodolized by a thermoelectric module mounted on the condensation system. The aerosol from the nebulizer was condensed on a cold plate (about 4 °C) in the thermoelectric module. All aerosol generation systems are controlled by a pneumatic pump lacking a syringe or peristaltic pump. The system was only controlled by air pressure

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**Fig. 1.** Schematic diagram of our experimental set-up. The system consists of PAGS (Pneumatic Aerosol Generation System), a high-efficient condenser and a microfluidic chip for ATP extraction and detection of bioluminescence.

**Fig. 2.** Photograph of the microfluidic chip fabricated by soft lithography techniques. The chip is composed of four inlets, a mixing channel, a detection area, and one outlet. Dimensions are as follows: width of inlet channel, 100 μm; width of outlet channel, 400 μm; thickness of whole channel, 100 μm; length of diffusion area, 6 cm; detection area, 1 mm × 1 mm.
from the clean air supply system. We call this system PAGS (Pneumatic Aerosol Generation System). We could find the optimal PAGS flow rate that generates the best mixing efficiency of the microfluidic system by calculating a volumetric flow rate with cross-sectional area of tube and average flow rate.

2.3. Microfluidic system

The ATP analysis system is made up of a microfluidic channel and an electric circuit. In the microfluidic channel, the captured airborne microbes are dissolved by lysis buffer and their ATP can be extracted in a continuous flow. The electric circuit is for transducing the electrical signal after the detection of ATP-driven bioluminescence. Fig. 2 shows the micro-channel network fabricated by a soft-lithography technique using a silicon wafer, a spin coater, UV aligner, PDMS, a photoresist (SU-8 2050), and a few chemicals. The micro-channel is composed of four inlets, a mixing channel, a detection area, and one outlet. The four inlets are separately connected to four tubes from the pneumatic system and condenser, and transfer bioaerosol sample, D-luciferin-luciferase reagent, lysis buffer, and DI water. The width of the inlet channel is 100 μm, and the outlet channel is 400 μm wide. The thickness of the entire channel is 100 μm. The mixing area of this channel network is constructed in a serpentine pattern to enhance mixing efficiency, and the length of the diffusion area is around 6 cm. The detection area is 1 mm × 1 mm which is the same size of the detector photodiode which detects the ATP bioluminescence, to which is attached an electric circuit.

2.4. Detection of ATP bioluminescence

The electric circuit was designed to measure the intensity of bioluminescence caused by the reaction between ATP and D-luciferin-luciferase reagent. Fig. 3 shows the design and a photograph of the electric circuit, which consists of four parts: regulator, detector, low pass filter, and amplifier.

To detect the bioluminescence, we used a photodiode (Model: BS520, Sharp, USA) which has a peak absorption at 560 nm. The photodiode transferred the bioluminescence at a current as low as 10^−8 A. To increase the sensitivity of the electric circuit, a 500 Ω resistance (sensitivity control resistance element) was used to produce an order of voltage output. To remove the noise which is caused by high frequency and the 60 Hz power supply, a butterworth filter was integrated into the circuit. For digitizing the analog signal, an MP150 (Biopac, USA) was used. Acknowledge 3.8.1 software was used to monitor the digital signal, and Matlab 6.5 was used for signal processing and calibration.
3. Results and discussion

3.1. Analysis of bioaerosols

In order to detect airborne microbes, bacteria-containing aerosol is generated in the nebulizing system as shown in Fig. 1. The droplet size (av. ∼3 μm) and the concentration of nebulized microbes were determined by using APS and an Anderson sampler. Various flow rates (1, 2, 3, and 4 L/min) were introduced into the nebulizer with microbe samples having different cell concentrations (10^6–10^8 cells/ml). After sampling the aerosol microbes using the 6-stage Anderson impactor that contains nutrient agar, we incubated the collected microbes for 24 h. Then, the concentration of bioaerosol was obtained by counting the number of colonies of the sampled microbe (data not shown). Based on the size distribution data of nebulized microbe, we determined that the optimal flow rate for aerosol generation was 2 L/min.

3.2. Optimization of flow rate in the microfluidic channel

In the microfluidic channel, we needed to find out optimal flow conditions to maximize the mixing efficiency of each reagent (bacteria sample, lysis buffer, D-luciferin-luciferase reagent and sheath flow); this procedure maximized the output signal of the ATP/D-luciferin reaction. First, we varied the flow rates from 1 to 10 L/min using a micro-syringe pump, and then examined the mixing efficiency in the mixing zone and the detection area of the microfluidic channel using an optical microscope. In order to observe the mixing efficiency microscopically and easily, we used a mixture of two transparent chemicals, phenolphtalane and sodium hydroxide, which create a deep purple color when they react. From the flow test we determined the optimal mixing flow rate to be around 1 L/min. Reynolds number applied in this experiment was about 0.18. In our microfluidic system with Re ≪ 1, slower flow rates can lead to faster mixing rates due to the serpentine feature of microchannel and molecular diffusion [12].

3.3. Condensation system

As presented in Fig. 1, the condensation process is needed to concentrate and hydrosolize the aerosol samples followed by nebulizing. First, the condensation system should produce high concentrations of microbe hydrosol to obtain a sufficient amount of ATP. ATP should be extracted from the microbes by lysis buffer and subsequently react with D-luciferin-luciferase reagent in the channel. As shown in Fig. 4A, the volume of bacterial hydrosol was proportional to the time course, and the average sampling rate was about 0.12 ml/min. In our experiment, the condensation time was adjusted to 2 min because a volume of 200 μl was enough to operate the ATP measurement. Second, we evaluated the condensation efficiency at various concentrations of airborne bacteria. Condensation efficiency was evaluated by measuring how much of the original samples were recovered by the condenser. After collecting bacterial samples, optical densities were measured by a spectrophotometer and compared with the original concentrations of airborne bacteria. As shown in Fig. 4B, the recovery yield is nominally 95% at all ranges of concentration, which is a ratio between concentrations of hydrosolized microbes after condensation and those of the original aerosolized microbes prior to condensation. Consequently, it is believed that the condenser can generate the hydrosol microbes in an accurate and stable manner.

3.4. Calibration of electric circuit and measurement of pure ATP

Prior to the optical measurement of ATP induced bioluminescence, we had to determine the reference value of the output signal produced by the electric circuit. Calibration was conducted in the state of no reaction between D-luciferin and ATP reagents in the microfluidic channel. For the calibration, we injected D-luciferin-luciferase reagent and deionized water into microfluidic channel, which will not emit detectable luminescence. The output signal of the circuit was 0.85 V in the absence of ATP, which is regarded as a reference value. Every experimental ATP bioluminescence datum was normalized to this reference value.

In order to determine the relation between changes in output voltage and ATP concentration, the bioluminescence was measured by photodiode and the results are presented in Fig. 5. The
3.5. ATP-bioluminescence vs. concentrations of airborne microbes

The intensity of bioluminescence emitted from bacterial ATP is a very important factor to quantitatively evaluate the amount of bacteria in real-time. After optimizing the nebulization, the aerosol condensation, and the optical detection of ATP bioluminescence, real bacterial samples were applied to our system. An excess of D-luciferin-luciferase reagent was introduced in the microfluidic reaction chamber and subsequently mixed with ATP extracted from the captured bacteria to enhance ATP-bioluminescence in a short period. We observed that the bioluminescence intensity rapidly increased within a few minutes after injecting sample as shown in Fig. 6. Note that the experimental data have been acquired immediately after the injection of sample. Thus, the rapid increase of the output signal indicates that the response time caused by the ATP/D-luciferin reaction is very short (around a few seconds). As expected, higher concentrations of bacteria produced a stronger intensity of bioluminescence. In Fig. 6, the normalized intensities of *E. coli JM110* are 0.15 at a concentration of $10^6$ cells/ml and 0.4 at $10^7$ cells/ml. In case of *B. subtilis*, the signal intensities are 0.04 at $10^6$ cells/ml and 0.1 at $10^7$ cells/ml. Concerning the detection limit of our device, we here present the raw value of light intensity for airborne microbes which was 1 V ($10^6$ cells/ml), 1.68 V ($10^7$ cells/ml) for *E. coli JM110* and 0.9 V ($10^6$ cells/ml), 1 V ($10^7$ cells/ml) for *B. subtilis*. These values correspond to around $10^{-12}$ M of pure ATP concentration in our experiment (see Fig. 5). A molecular weight of ATP is 573.1 g/mol, and total mass of ATP extracted from a bacterial cell is $5.0 \times 10^{-17}$ g (equivalent to $8.7 \times 10^{-20}$ mol) [13]. Thus, the concentration ($10^{-12}$ M) of ATP we measured will correspond to the bacterial concentration of $1.2 \times 10^7$ cells/ml.

It is now proven that airborne microbes can be rapidly detected in real-time by the sequential treatment of bioaerosols, including condensation, ATP extraction and detection. In the future, we will use our system to examine other airborne microbes (i.e. fungi). It is believed that our proposed system
can be used for military purposes, in or out-door air quality monitoring systems, air pollution monitoring systems in public transportation systems, and other uses.

4. Conclusions

The real-time detection of airborne microbes is of great importance as they often cause severe respiratory diseases. However, the technologies aiming at analysis of living organisms in the air are still lacking since the concentration of aerosol is very sparse by itself, and it takes a relatively long time to incubate and examine airborne microbes by conventional methods. In this paper, we demonstrated a real-time detection method for airborne microbes by ATP-bioluminescence sensor; this method will shed light on the research of environmental monitoring system to prevent the spread of infectious microbes.

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References


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