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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

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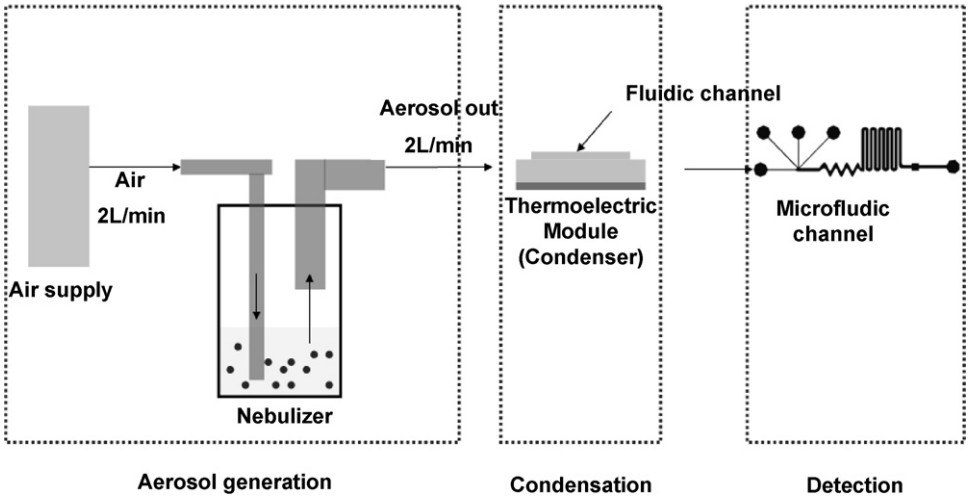

Fig. 1. Schematic diagram of our experimental set-up. The system consists of PAGS (Pneumatic Aerosol Generation System), a high-efficient condenser and a microfluidic chip for ATP extraction and detection of bioluminescence.

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 1.33×10^8 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 hydrosolized 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 pnuematic pump lacking a syringe or peristaltic pump. The system was only controlled by air pressure

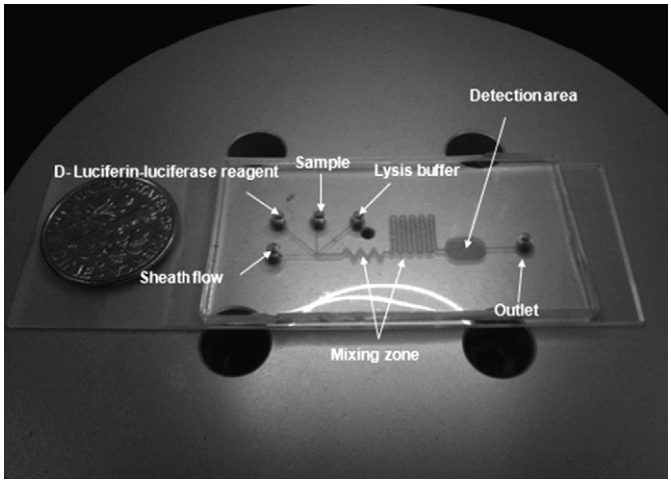

Fig. 2. Photograph of the microfluidic chip fabricated by soft lithography techniques. The chip is composed of four inlets, a mixing channel, a detection area, and one outlet. Dimensions are as follows: width of inlet channel, 100 μm; width of outlet channel, 400 μm; thickness of whole channel, 100 μm; length of diffusion area, 6 cm; detection area, 1 mm × 1 mm.