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Biosensors and Bioelectronics 20 (2005) 2079–2088
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Membrane-based on-line optical analysis system for rapid detection of bacteria and spores

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Received 4 June 2004; received in revised form 6 August 2004; accepted 24 August 2004

Abstract

We report here the adaptation of our electronic microchip technology towards the development of a new method for detecting and enumerating bacterial cells and spores. This new approach is based on the immuno-localization of bacterial spores captured on a membrane filter microchip placed within a flow cell. A combination of microfluidic, optical, and software components enables the integration of staining of the bacterial species with fully automated assays. The quantitation of the analyte signal is achieved through the measurement of a collective response or alternatively through the identification and counting of individual spores and particles. This new instrument displays outstanding analytical characteristics, and presents a limit of detection of ~500 spores when tested with *Bacillus globigii* (*Bg*), a commonly used simulant for *Bacillus anthracis* (*Ba*), with a total analysis time of only 5 min. Additionally, the system performed well when tested with real postal dust samples spiked with *Bg* in the presence of other common contaminants. This new approach is highly customizable towards a large number of relevant toxic chemicals, environmental factors, and analytes of relevance to clinical chemistry applications.

Keywords: Bacteria; *B. globigii*; Microchip; Epifluorescence; Immunoassay; *B. anthracis*

1. Introduction

Prompt detection and identification of potentially harmful bacteria is essential in the medical, environmental, and food industries. Recent events have emphasized the need for appropriate techniques to detect such micro-organisms when used in acts of bioterrorism. As bacteria require very little to grow, a great number of them can easily be manufactured for use as biological warfare agents. *Bacillus anthracis* (*Ba*), or anthrax, initially a threat to humans only through infected herbivores, has become such an agent. As evidenced by the anthrax attacks of October 2001 on US soil and the extensive list of potential biological warfare agents (CDCA), there is an urgent need for a detection system capable of detecting very low numbers of spores, with great selectivity, and in a timely fashion. Strategic locations for the detection of spores and bacteria include all mail-sorting facilities (Dull et al., 2002). For this particular application, continuous monitoring of the mailroom atmosphere is crucial in order to detect small amounts of contaminants while they are still contained within the facilities. As the postal office environment contains extreme amounts of paper and textile fiber, dust, and fluorescent brighteners, it represents a very complex matrix in which the detection of small quantities of spores is rendered very complicated.

Traditional methods of detection require the growth of single bacteria into bacterial colonies in different types of media, followed by a lengthy identification process involving morphological and biochemical tests (Francis et al., 2001; Paton and Jones, 1975; Kaprelyants and Kell, 1992; Phillips et al., 1983, 1985; Davey et al., 1999; Davey and Kell, 1997;

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doi:10.1016/j.bios.2004.08.046

Mason et al., 1995). These procedures, although often providing very accurate results, suffer from poor specificity, and repose on the expertise of highly trained personnel. Additionally, they often require complicated interpretation with complete analysis time typically approaching 72 h, which makes them unsuitable for online rapid analysis. Recent efforts have been directed towards developing approaches suitable for the entrapment or capture of bacteria, based on a combination of physical characteristics of the capturing medium and the affinity of the bacteria for a variety of chemical functionalities (Lee et al., 1997; Szczesna-Antczak and Galas, 2001; Chapman et al., 2001). While rapid, these methods are nonspecific, requiring completion of multi-step analysis for identification and quantification.

Most commonly available assays for the detection of spores or bacteria involve the use of enzyme-linked immunosorbent assays (ELISA) (Morais et al., 1997). While demonstrating high specificity, reproducibility, and capacity for multiplexing through the use of specific antibodies, these methods generally require lengthy analysis times, and are not compatible with real-time analysis. A large amount of efforts has been made recently to decrease analysis time and improve sensitivity and selectivity through the application, modification, or combination of various techniques. These include polymerase chain reaction (PCR) (Wu et al., 2002; Makino et al., 2001; Radosevich et al., 2002; Wilson et al., 2002; Iqbal et al., 2000; Belgrader et al., 1999, 2001; Cheun et al., 2001), electrochemical transduction (Dill et al., 2001, 1997; Yu, 1996, 1998; Brewster et al., 1996; Brewster and Mazenko, 1998; Mazenko et al., 1999; Gau et al., 2001), optical and microarray detection (Cheng et al., 1999; Chuang et al., 2001; Song et al., 2002; Rowe et al., 1999; King et al., 2000), flow-through immunofiltration (Morais et al., 1997; Abdel-Hamid et al., 1999a,b; Weimer et al., 2001), acoustic sensors (Ivnitski et al., 1999), capillary electrophoresis (Shintani et al., 2002), flow cytometry (Davey et al., 1999), and oligonucleotide probes and hybridization detection schemes (Steinert et al., 2002; MacGregor et al., 2001; Bockelmann et al., 2002; Gau et al., 2001; Yang et al., 2002; Liu et al., 2001) However, general sensor strategies rarely feature together the highly desired long list of attributes necessary for the creation of an "ideal sensor", as is demonstrated by the small number of commercially available sensing units (Ivnitski et al., 1999).

Methods based on PCR analysis have been chosen by the US Postal Service (USPS) as a preliminary technological response to an urgent need for a rapid detection method for *B. anthracis*. Despite the excellent specificity, sensitivity, and recent outstanding advances of this technology (McBride et al., 2003; Belgrader et al., 2003), some of the drawbacks include difficult sample preparation, long analysis time, the need for trained personnel, high reagent costs, potential contamination, false positives, and poor adaptability to multiplexing. In a similar manner to the Joint Biological Point Detection System (JBPDS) (NATIBO, 2001), which was designed to detect a biological agent within a minute upon release and identify the species in less than 15 min, our goal is to design and build a system that would use a two-step approach for the detection and identification of bacteria and spores. For document-handling applications, as required by USPS, it is desirable to develop rapid tests that can be used in conjunction with PCR confirmation tests to create a practical and cost-effective methodology suitable for the identification of bioterrorist threats (Fox et al., 2002). Further, the availability of such a rapid and specific spore and bacteria detection system would have a profound impact on food/water safety and important humanitarian efforts.

We have recently reported the use of a bead-based microchip technology (Lavigne et al., 1998; Goodey et al., 2001; Curey et al., 2001) to measure pH, detect metal cations, enzymes, proteins, DNA, and to assess cardiac risk through the monitoring of C-reactive protein in human serum (Christodoulides et al., 2002). These microchip-based applications repose on the use of polymer microspheres placed in the micro-etched wells of a silicon chip. By applying a mechanical entrapment strategy and the standard optical methodology of our system, we demonstrate here an efficient visualization of bacteria and spores. This novel approach is exploited and demonstrated here in the context of online detection of bacillus spores in mail-handling facilities.

2. Experimental

2.1. Flow cell

The flow cell assembly was created from a three-piece stainless steel cell holder consisting of a base, a support and a screw-on cap. Two circular polymethylmethacrylate (PMMA) inserts house the Nuclepore® (Millipore, USA) membrane. These two PMMA inserts have been drilled along their edge and one side to allow for passage of the fluid to and from the chip through stainless steel tubing (#304-H-19.5, Microgroup, Medway, MA). The tubing, which was fixed with epoxy glue in the drilled PMMA inserts, had an outer diameter of 0.039″ (19.5 gauge), and a 0.0255–0.0285″ inner-diameter. The bottom PMMA insert is modified in order to feature a drain and to contain a plastic screen disc (Pall Corporation, New York) that acts as a support for the filter. The top insert also features an additional outlet, which is used for regeneration of the filter. Silicone tubing is fitted on the stainless steel tubing, and as such is readily compatible with a wide range of fluidic accessories (i.e. pumps, valves, etc.) and solvents. The flow cell was shown to be resistant to leaks and pressures generated by flow rates as high as 20 mL/min.

2.2. Fluid delivery, optical instrumentation and software

The complete analysis system consists of a fluidics system composed of four P625/275 Instech peristaltic pumps (Instech, Plymouth, PA), dedicated to the delivery of the analyte,