PNNL-13951

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Development of an Automated Microfluidic System for DNA-based Detection of Pathogens

BS Hagan
CJ Bruckner-Lea

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Prepared for the U.S. Department of Energy
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Energy Research Undergraduate Laboratory Fellowship Program

Washington State University

Pacific NW National Laboratory

Richland, WA

July 15, 2002

Prepared in partial fulfillment of the requirements of the Office of Science, DOE Energy Research Undergraduate Laboratory Fellowship under the direction of C. Bruckner-Lea in Chemical Biological Non-proliferation Program (CBNP) at Pacific Northwest National Laboratory. [**are you supposed to give reference to the funding program? If not, omit reference to CBNP program]

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Abstract

Development of an Automated Microfluidic System for DNA Collection, Amplification, and Detection of Pathogens. SERENA HAGAN (Washington State University, Pullman, WA 99163) C. BRUCKNER-LEA (Pacific Northwest National Laboratory, Richland, Washington 99355).

This project was focused on developing and testing automated routines for a microfluidic Pathogen Detection System. The basic pathogen detection routine has three primary components; cell concentration, DNA amplification, and pathogen detection. Cell concentration is achieved by using magnetic beads that held in a flow cell by an electromagnet. Sample liquid is passed through the flow cell and bacterial cells attach to the beads. These beads are then released into a small volume of fluid and delivered to the peltier device for cell lysis and DNA amplification. The cells are lysed during initial heating in the peltier device, and the released DNA is amplified using polymerase chain reaction (PCR). Once amplified, the DNA is then delivered to a laser induced fluorescence detection unit in which the sample is detected. These components create a flexible platform that can be used for pathogen detection in liquids derived from environmental samples such as water, sediments or aerosols. Future developments of the system will include on-line DNA detection during DNA amplification and improved protocols to minimize the analysis time and minimize the use of reagents.

Research Category Chemistry and Engineering

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Is this being submitted for publication: No
DOE Program: Chemical Biological Non-Proliferation Program (CBNP)

Introduction

Recently the bio-terrorist attacks against the United States have raised concerns regarding pathogen detection. Pathogens can be found in many places; in the food we eat, the water we drink and even the dirt we walk on. Several methods for pathogen detection are already available, but the detection processes are lengthy and call for highly skilled technicians. This research project at Pacific Northwest National Laboratory (PNNL) has focused on developing a *biosensor system* that completely automates both sample preparation and detection for unattended operation. This system could then be applied to pathogen detection in environmental samples, food processing and water monitoring and could also be applied to the medical field in disease diagnostics and drug discovery.

Development of a completely automated pathogen detection system was first addressed in the development of renewable surfaces for separation and detection. These surfaces would need selective chemistry for bioseparation and/or detection by use of microbeads. The process of pathogen detection starts with aliquoting a small solution of derivatized microbeads, which are automatically packed from a slurry into a microcolumn, perfusing them with samples and wash solutions, and detecting the bound analyte molecules either directly on the beads or after elution from the microbeads. After each analysis, the derivatized microbeads are flushed from the system and new microbeads are used for the next analysis. Automation of the routine above is achieved by using a sequential injection fluidic system, which can handle volumes ranging from many milliliters to microliters. This renewable surface-sequential injection approach is sometimes referred to as “BEADS”, for Biodetection Enabling Analyte Delivery System. The central feature

of a BEADS system is the use of microparticles for separation and sensing. Scientists at PNNL have developed several different renewable separation columns for handling many different particle types, but the general characteristic of all of the flow cells is the ability to capture particles, perfuse particles, and release particles after use.

DNA sample processing is required to purify and concentrate DNA and therefore improve detection limits, and also remove components of the sample that interfere with DNA amplification or detection. A person with a pipette traditionally conducts the steps of purification and concentration, however the PNNL automated biosensor system completes the sample preparation and detection of the cells unassisted. Some commonly used sample preparation steps prior to DNA analysis are: concentration of the cells of interest, lysis of the cells to release DNA, purification of DNA, DNA amplification, and finally detection. Over the past several years scientists at PNNL have developed microfluidic modules to conduct each of these unit operations.

DNA purification

Sample purification is important to allow cell amplification and detection. Without purification, some of the components in environmental samples often inhibit amplification and detection. The DNA sample preparation work at PNNL began with the purification of DNA from soil extracts. Soil is a very challenging sample medium given that organic soil components, such as humic acids, will inhibit DNA amplification (e.g. PCR), and the presence of other soil components, such as proteins, can interfere with detection. As a result, several different types of microbeads were evaluated to

assess their ability to purify DNA from soil when used within automated renewable microcolumn systems. A PNNL magnetic flow cell was used in combination with superparmagnetic particles that included a 16S rDNA capture oligonucleoted to purify DNA from a sediment sample. A rotating rod renewable separation column was also used for purifying DNA from sediment, but in this case, a total DNA binding reagent that consists of irregular particles was used [6]. In both cases, the DNA eluted from the columns was suitable for PCR. In addition, purification results were similar to batch experiments with the same reagents [2, 6]. These results indicated that the BEADS automated approach could be used to automate DNA purification using many different bead types. The fluidic system design for purification simply depends upon the bead type and bead chemistry of choice.

Cell concentration

One main goal of this project was to detect whole bacteria, as opposed to bacterial DNA, from samples. This step is important because the detection of whole bacteria means that live bacteria are likely present, whereas bacterial DNA would be present even if the bacteria were lysed. In order to detect the whole bacteria, they first have to be concentrated and purified. The PNNL magnetic flow cell was used for concentrating *E. coli* 0157:H7 from poultry rinsate and water samples. These samples, ranging in size from 100ul to 100ml, were passed through the magnetic flow cell and the bacterial cells attached to the magnetic beads. These were then processed in about 15 minutes, and PCR and cell growth were used to estimate capture efficiency. Capture efficiencies were also estimated by absorbance using UV-Vis Spectrometer or by bead-counting using a

Luminex flow cytometer. Capture efficiencies ranged from 30-70%; and were comparable to batch reactions with the same reagents and samples. This research illustrated some of the attractive features of the magnetic flow cell. For instance, the magnetic flow cell can be used to capture and release particles during sample perfusion in order to enhance mixing, and it can rapidly process large samples to generate small, purified samples that are suitable for detection on a biochip [4].

DNA amplification

DNA amplification using PCR is one method that can be used to detect very small numbers of organisms, even down to individual cells. In order to include automated DNA amplification withing the BEADS sample preparation system, it is necessary to have a DNA amplification module that allows processing without manual or robotic transfer of samples and reagents to reaction chambers. Therefore, scientists at PNNL have designed, a temperature-controlled flow-through DNA amplification module. DNA reaction tubes, about 1 mm ID Teflon, are sandwiched between two Peltier blocks. The DNA sample and amplification reagents are delivered to the tube, and the temperature is cycled in order to double the amount of DNA product with each thermal cycle. The system provides a uniform, accurate temperature within the temperature-controlled region of a tube, and also allows the use of multiple reaction tubes. A PCR reaction volume of 20 microliters is used in this device, and reaction volumes ranging from 5 to 50 microliters have also been amplified using this format. This amplification module was used in series with the PNNL magnetic flow cell to purify and amplify *E. coli* 0157:H7 DNA from a sediment sample. In addition to efficient DNA amplification, the ability to automatically

clean the fluidics system between analyses is an important aspect of the flow-through format. Therefore, PNNL scientists have spent a lot of time and effort in developing cleaning protocols and analyzing "blank" samples to verify system cleanliness. The current cleaning protocols using DNAZap have allowed system reuse for more than 50 analyses without detectable sample carryover.

Detection

A flow-through fluorescence detection unit was made specifically for the BEADS DNA analysis system, and other techniques have also been used for detection. The fluorescence detection unit uses a DPSS Laser light, which excites Fluorescent probes found in the DNA sample. These probes produce light that can be detected by a PMT detector. Other techniques used for detection have included planar microarrays and also suspension arrays. Multiplexed detection using planar microarray is currently being used for detection of *E. coli* virulence markers. Also in progress is the use of suspension arrays for multiplexed pathogen detection. Suspension arrays consist of color-coded microbeads, where each microbead color has a different surface chemistry for biomolecule capture. BEADS devices have been used to automate the binding of samples onto the microbeads prior to bead analysis using a standard flow cytometer. The flow cytometer is used to analyze individual beads, to determine the bead color and whether fluorescently tagged molecules are bound to the bead surface.

Automated Biodetection

Recently, scientists at PNNL have assembled the components mentioned above to form an integrated DNA sample preparation and detection system that is designed for unattended monitoring in the field. The outline of the process begins with a liquid sample ranging from microliters to hundreds of milliliters in size. This sample is aspirated into the system and bacterial cells are concentrated onto magnetic beads using the PNNL magnetic flow cell. The magnetic beads are then moved to the peltier for cell lysis and DNA amplification using Polymerase Chain Reaction. Finally, the amplified DNA is fluorescently detected. The fluidics and electronics are separated into two portable boxes, with the fluidics box being about 25 cm on each side (Figure 2). The instrument is currently being tested for the detection of *E. coli* 0157:H7, a common food and water pathogen that can cause severe illness.

System Control Software

The key to integrating the electronics and the fluidics systems is the system control software. This software is written in "C" using LabWindows/CVI software from National Instruments. The user interface allows easy configuration to match system hardware(Pumps, Valves, Detector, etc..). It has a graphical user interface for manual control as well as flexibility to script experiments and run with one keystroke. The intuitive panel layout shows real-time data output in graphs and numbers. The instrument Interface has a multi-threaded design and independent process strings to prevent data loss and instrument interference. This interface is also adaptable for different types of instrumentation. The flexibility of the software allows for the integration of the fluidics and the electronics to form one automated sensor system.

Discussion and Conclusions

Concentration and purification of DNA is necessary in order to achieve sensitive DNA-based detection of pathogens in environmental samples. Research at PNNL has lead to a reusable fluidics approach for completely automating DNA sample preparation. Over the last six years, scientists at PNNL have developed and demonstrated the use of automated fluidics modules for cell concentration, DNA purification, DNA amplification, and detection. They have also built an integrated system and are currently testing the system for the detection of organisms in environmental samples.

Future work at PNNL will focus on the development and testing of integrated systems that include automated sample preparation and detection, as well as simplified fluidics designs and multiplexed detection methods for analyzing many DNA sequences within one sample. Work is also needed to develop new bead chemistries for multiplexed analysis, and also cleaning reagents and protocols to minimize reagent volumes and analysis time.

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Acknowledgements

I thank the United States Department of Energy—Office of Science for giving me the opportunity to participate in ERULF summer program.

My thanks also go to my mentor Cindy Bruckner-Lea[1] and entire staff at the William R. Wiley Environmental Molecular Science Laboratory at Pacific National Laboratory in Richland, Washington. Special thanks go out to my lab partners Brian Dockendorff[1] and Shankari Gunaratnam[1]. Also, thanks goes to Michelle Nichols, Education Specialist.

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The research described in this paper was performed at the William R. Wiley Environmental Molecular Science Laboratory, a national scientific user facility sponsored by the United States Department of Energy's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory.

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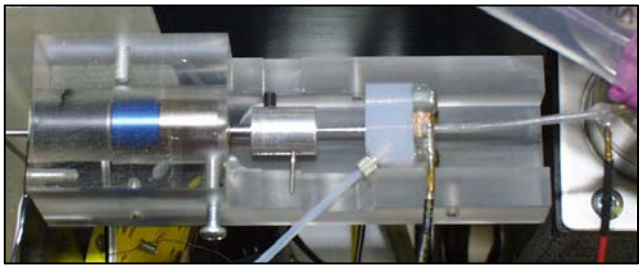

Figure 1: rotating rod used for concentration of DNA

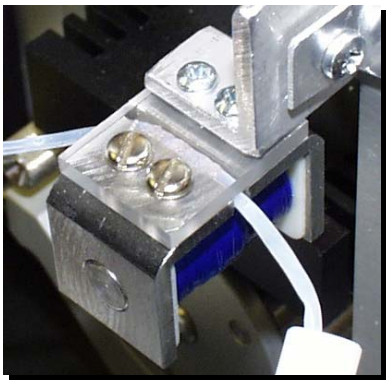

Figure 2: magnetic flow-through cell used for concentration of cells

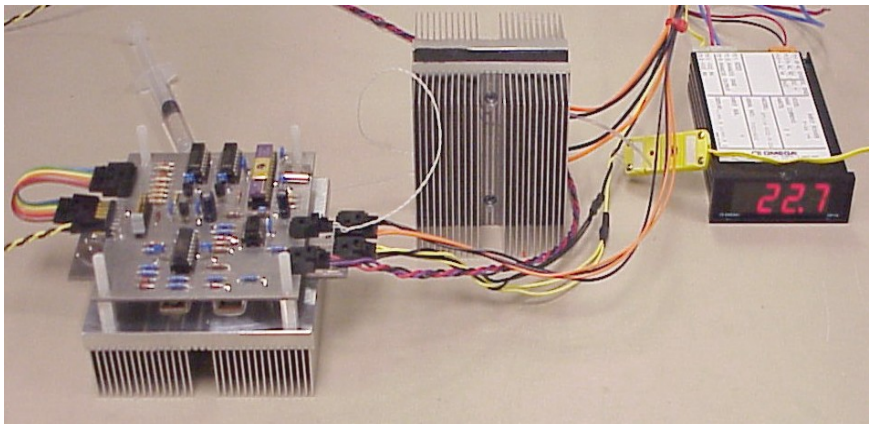

Figure 3: Peltier device used for amplification of DNA through thermal cycling

Figure 4: field portable device including both the electronics and the fluidics

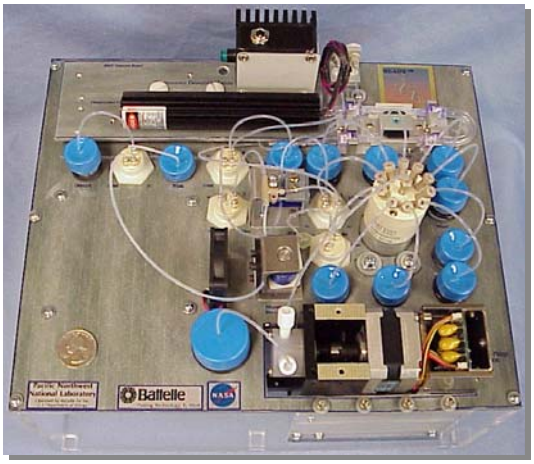

Figure 5: fluidics unit including the sequential injection device, the magnetic flow-through cell, and the fluorescence detector.