Abstract

Molecular microbiologists have been developing assays to achieve rapid detection of harmful microorganisms in coastal waters and have developed a need for miniaturized, portable sensors. To meet these needs, the developments here show a thermocycling PCR chip and a reconfigurable fluidic processor. Data shows the ability of the chip to cycle through and maintain common nucleic acid amplification temperatures. The chip has an embedded resistive heater. This heater was patterned on copper-laminated Liquid Crystal Polymer (LCP) using maskless photolithography techniques. The flow cell was etched into the polycarbonate with a CO2 laser. These layers were bonded together with SU8 under constant heat and pressure. The fluidic processor is a simple 24 volt solenoid valve and peristaltic pump configuration. The reconfigurability of the processor is used for prototype testing during fluidic management development and will allow for rapid integration of the PCR chip with a commercially available electrochemical device into a portable, semi-automated, microbial sensor.

Introduction

USF’s Center for Ocean Technology has teamed up with NOAA’s Atlantic Oceanographic and Meteorological Laboratories to design a portable PCR-based electrochemical sensor for the rapid detection and improved monitoring of microbial contaminants in coastal waters (Figure 1). This poster illustrates progress that has been made towards combining a new thermocycling flow cell with rapid, molecular assay technologies (Reference 1), to a commercially available, portable, electrochemical sensor developed by Alderon Biosciences, Inc., Durham, N.C. (Figures 1 and 2).

Thermocycling Flow Cell

This thermocycling flow cell will essentially act as both the PCR chip and the electrochemical sensor. Comprised of the bonded layers shown in Figure 1 and 3, the temperatures of the fluids inside the cell are regulated via a 4-wire resistive measurement circuit and a 5 volt power supply (Figure 4).

Configurable Fluidic System

The basis of the configurable fluidic processor that will be integrated into the biosensor was previously developed at USF (Reference 2). A customized version of this fluidic system will automatically deliver the PCR sample and the electrochemical reagents necessary for amplification and detection through the thermocycling flow cell. Microfluidic manipulations will occur through the use of air displacement in a 3-way valve/manifold pump system (Figure 7). By adjusting pump speeds and valve timing the experiments in Figure 8 show fluidic control on the microliter scale.

Summary

We have successfully demonstrated the ability of the flow cell to cycle PCR temperatures. Additional work is being performed to optimize the cell for a successful PCR reaction. Once that work is completed, electrochemical probes will be integrated into the flow cell and tested with the use of the fluidic management system.

References