CHAPTER 4

Self-Contained, Fully Integrated Biochips for Sample Preparation, PCR Amplification and DNA Microarray Analysis
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Abstract

Rapid developments in back-end detection platforms (such as DNA microarrays, capillary electrophoresis, real-time polymerase chain reaction and mass spectrometry) for genetic analysis have shifted the bottleneck to front-end sample preparation where the 'real' samples are used. In this chapter, we present a fully integrated biochip device that can perform on-chip sample preparation (including magnetic bead-based cell capture, cell preconcentration and purification and cell lysis) of complex biological sample solutions (such as whole blood), polymerase chain reaction, DNA hybridization and electrochemical detection. This fully automated and miniature device consists of microfluidic mixers, valves, pumps, channels, chambers, heaters and DNA microarray sensors. Cavitation microstreaming was implemented to enhance target cell capture from whole blood samples using immunomagnetic beads and accelerate DNA hybridization reaction. Thermally actuated paraffin-based microvalves were developed to regulate flows. Electrochemical pumps and thermopneumatic pumps were integrated on the chip to provide pumping of liquid solutions. The device is completely self-contained: no external pressure sources, fluid storage, mechanical pumps, or valves are necessary for fluid manipulation, thus eliminating possible sample contamination and simplifying device operation. Pathogenic bacteria detection from ~ mL whole blood samples and single-nucleotide polymorphism analysis directly from diluted blood were demonstrated. The device provides a cost-effective solution to direct sample-to-answer genetic analysis and thus has a potential impact in the fields of point-of-care genetic analysis, environmental testing and biological warfare agent detection.

Introduction

Molecular approach to detect low abundance markers in biological tissue is becoming critical to assess genetic and environmental interactions. The miniaturization of biological assays to the chip level carries several advantages. On-chip assays use reduced volumes of reagents (2-3 orders of magnitude as compared to traditional bench approaches) and allow for reducing cost per reaction and improving reaction kinetics.13 On-chip reactions are performed in miniature channels or chambers that can be distributed on the device wafer at high density. This high population of identical reaction paths allows for the development of highly parallel analytical systems with high

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system throughput. Furthermore, integration of several assay functions on a single chip leads to assay automation and elimination of operator involvement as a variable. The microfluidic lab-chip device with capabilities of on-chip sample processing and detection provides a cost-effective solution to direct sample-to-answer biological analysis. Such devices will be increasingly important for rapid diagnostic applications in hospitals and in-field bio-threat detection.

A fully integrated biochip needs to perform all functions including sample preparation, mixing steps, chemical reactions and detection in a miniature fluidic device. Most of the currently demonstrated microfluidic or microarray devices pursue single functionality and use purified DNA or homogeneous sample as an input sample. On the other hand, practical applications in clinical and environmental analysis require processing of samples as complex and heterogeneous as whole blood or contaminated environmental fluids. Due to the complexity of the sample preparation, most available biochip systems still perform this initial step off-chip using traditional benchtop methods. As a result, rapid developments in back-end detection platforms have shifted the bottleneck, impeding further progress in rapid analysis devices, to front-end sample preparation where the ‘real’ samples are used.

Since the early work of Harrison and coworkers on chip-based capillary electrophoresis (CE), the advances of the microfluidic lab-on-a-chip technologies have been multi-directional and have addressed the following issues: high throughput of the analysis, functional complexity, level of integration, on-chip sample preparation and low-cost fabrication and manufacturing. Several researchers have developed devices allowing for performance of multi-step assays using complicated channel networks, while pumps, valves and detectors were left off-chip and were built into the desktop test station. Others argued for integrating all functional components into the chip and preferred portable solutions. The latter efforts led to ingenious demonstrations of on-chip valving and pumping schemes in an attempt to depart from traditional Microelectro-mechanical Systems (MEMS) approaches that are complicated in fabrication and therefore expensive. Various materials have been used in the fabrication of lab-chip devices. Lithographic techniques, adapted from semiconductor technology, have been used to build chips in glass and silicon. Unconventional lithography techniques such as soft lithography have been used to fabricate reproducible microstructures of biological materials offering a multitude of possibilities to explore as molecular diagnostic tools. Recently, with increasing emphasis on disposable devices, the use of plastics and plastic fabrication methods has become popular.

Most of the integrated microfluidic work has been directed towards the integration of DNA amplification with CE. Several research groups, including our group, have vigorously pursued integration of sample preparation, polymerase chain reaction (PCR) and DNA microarrays and miniaturization of the whole DNA microarray analysis into a single device. Anderson et al have reported an integrated system that performed RNA purification from a serum lysate, followed by PCR, serial enzymatic reactions and nucleic acid hybridization. Yuen et al reported a microchip module design for blood sample preparation (white blood cell isolation), PCR and DNA microarray analysis. Our group has focused on developing efficient and simple on-chip mixing, valving and pumping techniques and using electrochemical detection based microarray that makes the integrated biochip system desirable for applications valuing portable solutions such as point-of-care diagnostics, in-field environmental testing and on-site forensics. We developed an integrated biochip device that integrated sample preparation with PCR and DNA microarray for sample-to-answer DNA analysis. The on-chip analysis starts with the preparation process of a whole blood sample, which includes magnetic bead-based target cell capture, cell preconcentration and purification and cell lysis, followed by PCR amplification and electrochemical microarray-based detection.

This chapter begins with a description of the design and fabrication of the DNA analysis integrated device, followed by a discussion of the individual microfluidic components essential to this integrated device. Next, a demonstration of the performance of the device for integrated nucleic acid analysis, including pathogenic bacteria detection from ~ mL whole blood samples and single nucleotide polymorphism analysis directly from blood samples, is presented.