Integrated Microfabricated Devices for Genetic Assays

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ABSTRACT

Genetic assays are powerful technology drivers and excellent candidate applications for miniaturization technologies because the demand for inexpensive genetic information is essentially unlimited, and the cost and time for the assay decrease with sample volume. Genetic information is stored in long DNA molecules in solution. Processing of DNA molecules in the microscale hence requires the implementation of microfluidic devices capable of handling, mixing, thermal cycling, separating, and detecting nano and pico liter liquid samples. This paper discusses the implementation of integrated microfluidic devices for DNA processing.

INTRODUCTION

Genetic tests (or assays) have an enormous scope of applications in biotechnology and medicine, ranging from agriculture and farming [1] to the detection of pathogens in foods [2] to genetic diagnostics on human subjects [3]. Currently about 400 diseases are diagnosable by molecular analysis of nucleic acids, and this number is increasing daily. Many more assays will follow as more genetic information is discovered by major research undertakings such as the Human Genome Project. Humans have approximately 100,000 genes that could be potentially tested for defects or the propensity for diseases with essentially the same procedure.

Recently, there has been much interest in the implementation of microfluidic devices for genetic assays. These devices are excellent candidates for miniaturization because of their high demand as well as the fact that the same microfabricated part can be used for many different assays by changing the nature of its reagents, not the device construction.

DNA PROCESSING TECHNIQUES

Conventional genetic assay protocols use five fundamental chemical procedures for the analysis of DNA [4].

Chemical Amplification: is required when the sample is present at concentration levels that are too low for any direct test. The amplification basically consists of a set of reactions that allow a DNA molecule or fragment to duplicate. Molecular replication takes place when a double stranded fragment is first thermally separated (or denatured) into two strands followed by the polymerase enzyme action at a reduced temperature. Further amplification is hence obtained by repeating the procedure. This scheme is known as the polymerase chain reaction (or PCR). Amplification factors as large as 2^30 are possible permitting detection based on single DNA molecules.

Fluorescent Tagging techniques are commonly used for detecting both single and double stranded DNA fragments by introducing in the mixture a suitable label molecule that binds to the fragment. Light-emitting tags are extremely sensitive permitting the detection of individual molecules [5] in femtoliter samples. Hence these dyes are almost universally used for the visualization of DNA fragments.

Restriction Digestion is used to fragment a DNA molecule. Restriction endonuclease enzymes split double stranded DNA at specific locations. Restriction enzymes are essential for analyzing chromosome structure and sequencing very long DNA molecules into fragments that are more readily analyzed. The size distribution of such fragments can serve as a fingerprint for a DNA molecule.

Electrophoresis is a technique used for separating DNA fragments of different sizes from a mixture DNA fragments in solution are negatively charged; therefore they drift under the presence of an applied field. The fragment mobility depends on the type of mobile phase and the fragment size. Therefore if the mixture is introduced as a single band at a starting point in a mobile phase, the fragments are separated into bands composed of different sizes of DNA as they drift in a “race track” fashion. Separations provide important information about the fragment distribution in a sample, and when combined with Sanger’s randomly truncated replication scheme [6], it leads to the fragment sequence.

Hybridization Techniques use the highly selective hydrogen bonding of two complementary single strands of DNA forming a double strand. In hybridization-based DNA analyses one of the strands is known (an immobilized DNA probe) and the other unknown. The presence of a double strand in the mixture (detected by fluorescence) is indicative of a match; hence hybridization serves is a sequence detection mechanism.

A typical DNA assay may involve a combination of the above techniques. For example the process may include the extraction of nucleic material from a cell followed by amplification, staining, restriction digestion, and electrophoretic separation or hybridization.

MICROFABRICATED DEVICES

Over the last decade much there has been much activity in the miniaturization of assays. Scaling down results in increased throughput, reagent cost reductions due to miniscule reaction volumes, and lower system costs due to introduction of batch fabrication techniques to produce chip-scale devices. The first amplification microdevice was developed by M. A. Northrup [7] at LLNL. This device consists of a 1 mm² microwell cavity structure with polysilicon heaters formed in a silicon substrate by anisotropic etching. This low-mass structure can be heated at rates of 15 °C/s and cycle times of about 1 min, roughly four times faster than in a conventionalycler. Electrophoresis microdevices, constructed using 3-7 cm long glass planar capillaries 5-10 μm in diameter have been demonstrated to yield very rapid sequencing-grade separations [8]. About 450 fragments were distinguishable in about
10 minutes separation time using fields of 200 V/cm. The chip separation is about 3-5 times faster than conventional CE and 50 times faster than slab gels. Micromachined hybridization array devices can be micromachined using lithographic techniques [9] producing a very large number (> 10⁶) of distinct micrometer-sized DNA probe "pixels". This technology is now commercially mature.

Most of these devices use an expensive fluorescent microscope to read out of the signal; hence they lack miniature detectors. On-chip detection [10] has recently been accomplished permitting now the realization of completely integrated devices.

INTEGRATED ASSAY MICRODEVICES

The devices discussed above are good demonstration vehicles for feasibility of molecular assays in microscale environments, but their practical implementation for low-cost applications presents more problems.

A solution to these problems is an inexpensive integrated fabrication technology that accommodates all the necessary fluid, thermal, and detection functions under a common substrate. We have fabricated an example integrated device shown in Figure 1 incorporating simple injectors, mixer, heating chamber, and separation channel with detectors. Diode detectors were constructed in a silicon substrate with a thick optical filter deposited on the surface that blocks UV excitation. Heaters and electrodes are next defined on the surface and passivated with silicon dioxide and p-xylene. A network of capillaries and chambers is next formed above the surface by bonding an etched glass wafer on top of the silicon substrate. In this device, sample and reagents can be driven through the system pneumatically or by heated gas trapped in pockets behind the sample, and the sample spread is controlled through a series of hydrophobic stops. This device demonstrated the motion, amplification, separation, and detection of DNA samples integrated in a single part.

![Figure 1: Example integrated glass device with injectors, mixers, amplification chamber, separation and detection [10].](image1)

We have successfully run genotyping assays involving the amplification, separation and detection of Mycobacterium tuberculosis DNA. Figures 2-3 show examples of migration and on-chip detection of DNA fragments. The presence of individual bands has been observed with S/N of about 100.

![Figure 2: Injection and separation of DNA fragments on integrated device. The channel is 500x50 μm². (50 bp ladder, 0.13 μg/μL, SYBR Green, 8 V/cm, 10%T:2.6%C polyacrylamide) [10].](image2)

![Figure 3: Low-resolution separation of DNA fragments recorded with on-chip detector diodes thus eliminating the need for expensive readout optics [10].](image3)

Integrated DNA assay microdevices have an enormous number of commercial applications. We believe these will continue to fuel very rapid technological advances which are needed to improve the resolution of these assays to the same level available in laboratory instruments.

References


