DNA Extraction Chip Using Key-type Planar Electrodes

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ABSTRACT

This paper presents a novel method of DNA extraction from whole blood using time varying magnetic field. The novelty of this chip is that both mixing and separation steps are performed in a single chamber in less than a minute with no need for extra microfluidic channels. In order to extract DNA from white blood cells, whole blood is mixed with lysis buffer containing superparamagnetic beads. The mixing chamber is sandwiched between two key-type planar coils. Time varying magnetic field is generated within the mixing chamber to create efficient mixing. This process distributes the magnetic beads both temporally and spatially to achieve the desired mixing effect. Once the white blood cells are lysed, the exposed DNA molecules attach themselves onto the functionalized surface of the magnetic beads. Finally, DNA-attached magnetic beads are attracted to the bottom of the chamber by activating the bottom electrode. DNA molecules are extracted from magnetic beads by washing and re-suspension processes. The extracted DNA output was verified using bench-top PCR and gel electrophoresis.

Keywords: DNA chip, DNA extractor, mixer, magnetic bead

1 INTRODUCTION

Over the past decade, the advent of Micro-Electro-Mechanical Systems (MEMS) has created the potential to fabricate various structures and devices on the order of micrometers. This technology takes advantage of almost the same fabrication techniques, equipment and materials that were developed by semi-conductor industries. The range of MEMS applications is growing significantly and is mainly in the area of micro-sensors and micro-actuators. In recent years, miniaturization and integration of bio-chemical analysis systems to MEMS devices has been of great interest which has led to invention of Micro Total Analysis Systems (μ-TAS) or Lab-on-a-Chip (LOC) systems.

However, whilst there has been a great deal of work in core areas, for example, miniaturizing PCR for expedited amplification of DNA in the microchip format, less effort has been directed towards miniaturizing DNA purification methods. In fact, 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 bench-top 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. A problem with the currently known microfluidic devices is performing efficient chaotic mixing in these platforms, this usually needs existence of moving parts, obstacles, grooves, and twisted or three dimensional serpentine channels. The structures of these components tend to be complex, however, requiring complicated fabrication processes such as multi-layer stacking or multi-step photolithography.

Magnetic beads have been of great interest in both research and diagnostic applications. Functionalized surface of magnetic beads offer a large specific surface for chemical binding and may be advantageously used as a mobile substrate for bioassays and in vivo applications [1]. They may have various sizes ranging from a few nanometres up to tens of micrometres. Due to the presence of magnetite (Fe3O4) or its oxidized form maghemite (c-Fe2O3), magnetic particles are magnetized in the presence of an external magnetic field. Such external field, generated by a permanent magnet or an electromagnet, may be used to manipulate these particles through magnetophoretic forces and therefore, result in migration of particles in liquids. Due to the size and distribution of the small embedded iron-oxide grains, particles lose their magnetic properties when the external magnetic field is removed, exhibiting superparamagnetic characteristics. This additional advantage has been exploited for separation of desired biological entities, e.g. cell, DNA, RNA and protein, out of their native environment for subsequent analysis, where particles are used as a label for actuation.

This paper introduces a novel DNA extractor process using magnetic beads and time varying magnetic field. Extractions of DNA from white blood cells and Lymphoblast GM 607D have been tested using two different schemes.

Whilst dynamic mixing has been used for separation of DNA from whole blood using Y shape micromixer (chip 2), static mixing have been used for separation of DNA from Lymphoblast GM 607D using lysis chamber (chip 1). In both methods, magnetic field has been used for efficient mixing of the lysis buffer and cells. Once the cells are lysed, the exposed DNA molecules attach themselves onto the functionalized surface of the magnetic beads. Finally, DNA-attached magnetic beads are harvested by activating the holding magnet. DNA molecules are extracted from magnetic beads by washing and re-suspension processes. The extracted DNA output was verified using bench-top PCR and gel electrophoresis.

2 MICROFABRICATION

Both chips were built in-house by ChargeLabs, a consultant to the Project.

2.1 Chip 1: Lysis-mixing Chamber

Figure 1 shows a photograph of the assembled chip 1. The device has essentially a sandwich construction, consisting of three basic elements. These are i) an outer substrate layer of PMMA, ii) a central layer of PDMS containing the mixing/lysis chamber, and iii) a second outer PMMA substrate layer, broadly similar to the first.

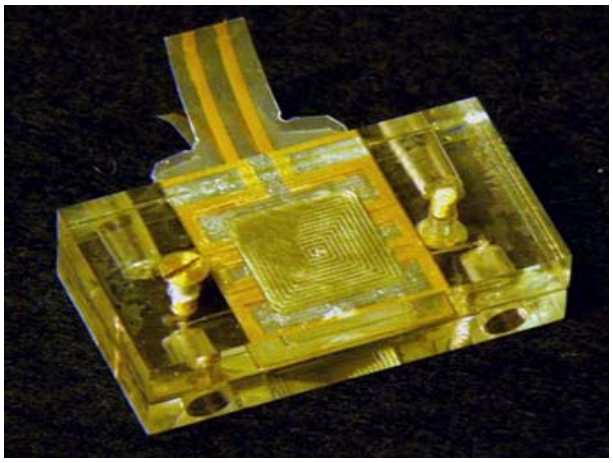

Figure 1: A photograph of assembled fluidic Chip 1.

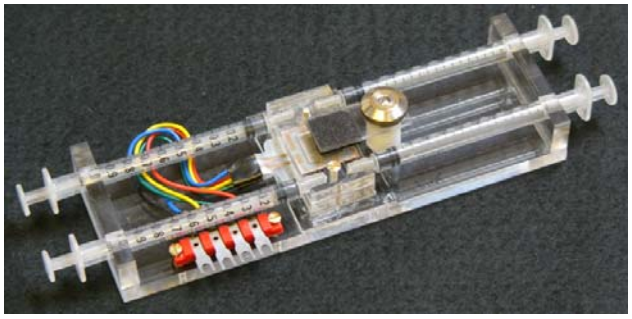

Figure 2: Completed device in docking station.

Both PMMA substrates contain a planar coil array to produce the magnetic fields for the mixing process, centred over the middle lysis chamber. Conventional ferric chloride etching and thermal mask have been applied to etch the coil arrays from Mylar-backed copper foil, a composite consisting of a 30µm layer of copper, held on a 70µm Mylar backing with an adhesive layer some 10µm thick.

Backing these coil arrays are square pieces of high-permeability material to act as magnetic amplifiers. Mu-metal sheet being available, this was employed as having superior permeability characteristics to the more conventional Permalloy. Two pieces approx 13mm square were cut, then flattened and surface lapped to just over 150µm in thickness. They were then finished to 12.50mm square, and their corners radiused to allow seating in a chamber milled with an 800µm cutter.

The substrates were cut from 5.8mm cast sheet of PMMA, machined to 40mm by 22mm overall dimensions, and edge-polished. The centre portions of each were then milled to provide the recess for the planar coil foils and the seating beneath for the mu-metal magnetic amplifiers.

This arrangement completes the lower substrate. The upper substrate additionally carries porting and drillways to allow liquid communication with the vias formed in the central PDMS component. Connexion to these ports is made via standard Luer female tapers formed into the substrate material.

The functional interfaces of both substrates are finally spin-coated with a thin (~6µm) layer of PDMS, resulting, on assembly, in a working chamber entirely faced in this material, whose long-term biocompatibility is well known.

The central PDMS layer consists of a sheet of cast PDMS, 150µm in thickness, with a rectangular cut-out, 12mm by 10mm forming the operational chamber. The shorter sides of this chamber are provided each with two cutout vias, ~250µm in width and some 8mm in length, each terminating in a 1.5mm diameter punching, to connect with the porting on the top substrate. On assembly, the device sections are secured together with two small screws, passing through one substrate and the central layer, and threaded into the second substrate. This allows adequate permanent clamping pressure between the layers of the device, whilst retaining the option of opening the device for cleaning or repair in extreme cases (Figure 1). Finally, the completed microfluidic chip is located in a docking station, which provides an electrical connector for interfacing with the drive electronics, and mechanical support and alignment for up to four standard 0.25” OD syringes (Figure 2).

2.2 Chip 2: Y-shape Micro-mixer

Figure 3 shows a picture of the y-shape micro-mixer. The device has a very simple structure consist of a capillary channel and external electromagnets. Capillary micro-channel was made using warming the glass up to the

melting point and subsequent sudden stretching. This resulted in a very uniform capillary channel with an internal diameter of 150 μm and the external diameter of 250 μm.

Electromagnets used in this assembly are external to the capillary micro-channel. The electromagnets were constructed using winding of 60 turns of copper wire of diameter 60 μm onto a Mu-metal core which has been castellated in one end to produce sharp magnetic gradient tips. Castellated tip has been milled down to 200 micron and with the same 250 micron width and length. The teeth of the magnetic core are positioned on both sides wherein they are horizontally offset such that the teeth of one core are positioned between the teeth of the other core. An additional electromagnet has been used down the capillary micro-channel for collecting beads after mixing process. Finally, the completed chip is located on a PMMA substrate with electrical connectors.

3 EXPERIMENTAL SECTION

3.1 Materials

The materials used for the experiments with chip 1 are listed here: Lymphoblast GM 607D 4x106 cell/ml in FCS. (Grown in RPMI 1640 with 2mM glutamine and 15% foetal calf serum). DNA purified from GM 607D lymphoblast cells using Wizard Genomic DNA Purification Kit (Promega). Dynabeads DNA Direct Universal Prod. No 630.06, Kit containing beads in lysis fluid, wash buffer and resuspension buffer. 1.1x mastermix AB-0575/A, Primer F&R D3S3717. 2% agarose gel with 5μl ethydium bromide/100mls, Hyperladder 1 Bioline cat. No. 33025, DNA coated beads.

3.2 Expriment Steps

The chip 1 was washed through with wash buffer and drained. Two Hamilton syringes were loaded to the 10μl marking, one with DNA coated beads the other with FCS and each was attached to an inlet. Two plastic syringes were attached to the outlets, the one diagonal to the serum-containing syringe having its plunger removed. The serum sample was injected into the chip. The outlet syringes were swapped. The bead sample was injected into the chip. The current was switched on for one minute at 3A and 4 Hz to mix the contents of the chamber. Top magnet was switched off, leaving the bottom magnet to attract the beads and hold them. After 2 minutes, 50μl of wash buffer was pushed through an inlet syringe at approximately 1μl/second. This was repeated at the other inlet making a total of 100μl of washings collected in a syringe. The chamber was sucked out gently. The magnet was switched off. The washing procedure was repeated to collect the washed contents of the chamber. The run was repeated with each Hamilton syringe loaded to 20μl. This was repeated with cells in serum and beads in lysis fluid injected into the chamber but the volume injected was 20μl.

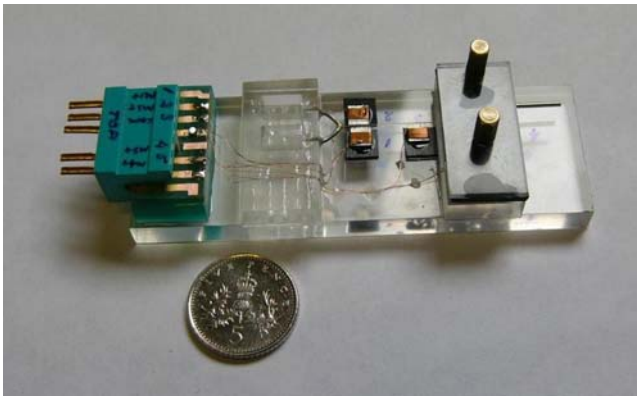

Figure 3: A photograph of chip 2, Y-shape Micro-mixer.

3.3 PCR

A 39 cycles of PCR was performed on the samples with 22μl mastermix, 1μl primers and 2μl samples. The presence of lysis fluid in the samples may act as an inhibitor to PCR therefore; samples with lysis buffer (approximately 100ul) were put on the magnet to remove the beads. The beads were then resuspended in 20μl of resuspension buffer.

4 RESULT AND DISCUSSION

4.1 Extracting DNA from Cells – Chip 1

Figure 4 shows a molecular weight marker of PCR products. Table 1 indicates detail of each column corresponding to Figure 1. Using DNA coated beads, DNA amplification in the harvested bead sample has been shown. This would indicate that the magnet held the beads in the chamber during the wash stage after which they were successfully harvested. This assumes that the washing stage was efficient over the whole area of the chamber. DNA amplification was seen in the wash sample. These beads could have been washed out of the chamber. DNA coated beads would have been washed from the dead space between the end of the inlet syringe and the chamber so DNA amplification was expected here. DNA amplification was present in all the harvested bead samples, both on the beads and free in solution therefore the system was overloaded with DNA and the washing was inefficient. As there are beads in the harvested samples, the magnet seems to have held the beads during washing. Amplified DNA from the wash sample bead may indicate some loss of beads from the chamber during washing.

4.2 Extracting DNA from Blood - Chip 2

Numerical simulation for the structure similar to this chip has clearly shown the chaotic mixing of the magnetic particles [2-3]. A preliminary experiment was carried out on chip 2 to extract DNA from whole blood. The stages are similar to previous section except the whole blood and the

lysis buffer containing the superparamagnetic beads were fed into the micro-channel via the two inlets of the Y-shape inlet ports. The switching magnetic field was applied as befor. DNA was amplified from the washed beads from the 20µl whole blood samples run through chip 2 with both heparin and EDTA as blood anticoagulants. DNA was amplified from the washed beads from the heparinised plasma sample obtained from chip 2.

The result obtained is shown in Figure 5 and Table 2. This clearly demonstrates that DNA can be successfully extracted from blood using this chip in a few minutes.

Research is continuing to optimize the chip 1 operating parameters and to quantify the amount of DNA that can be extracted from whole blood in a few minutes.

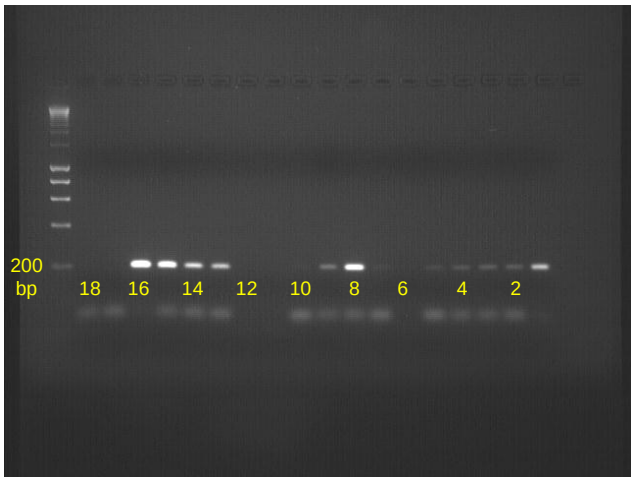

Figure 4: Molecular weight marker of PCR product (chip 1)

Lane	Sample	ProP[1]	PriP[2]
1	DNA/bead wash	++	+/-
2	DNA/bead sample	+	+
3	DNA/bead wash	+	+
4	DNA/bead sample	+	+
5	Cells+beads/ wash/ beads	+/-	+
6	Cells+beads/wash/supernatant	-	-
7	Cells+beads/ sample/ beads	+/-	+
8	Cells+beads/sample/supernatant	+++	+
9	Cells+beads/ wash/ beads	+/+	+
10	Cells+beads/ wash/ beads	+/--	+
11	Cells+beads/wash/supernatant	-	-
12	Cells+beads/wash/supernatant	-	-
13	Cells+beads/ sample/ beads	++	+
14	Cells+beads/ sample/ beads	++	+
15	Cells+beads/sample/supernatant	+++	+
16	Cells+beads/sample/supernatant	+++/+	-
17	dwater	-	+
18	dwater	-	+

[1]ProP: Product Present, [2]PriP: Primers Present

Table 1: Column information for Figure 4.

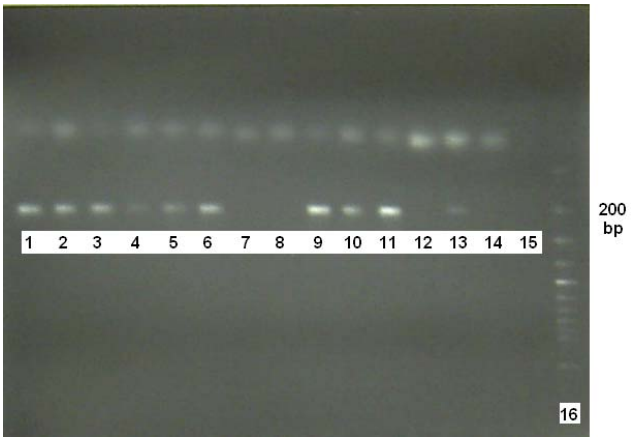

Figure 5: Molecular weight marker of PCR product (chip 2)

Lane	Sample	DNA Amplified
1	EDTA / Blood / Beads	0.5 µL
2	EDTA / Blood / Beads	1.0 µL
3	EDTA / Blood / Beads	2.0 µL
4	EDTA / Blood / Beads	0.5 µL
5	EDTA / Blood / Beads	1.0 µL
6	EDTA / Blood / Beads	2.0 µL
7	EDTA / Serum / Beads	1.0 µL
8	EDTA / Serum / Beads	2.0 µL
9	Heparin / Blood / Beads	0.5 µL
10	Heparin / Blood / Beads	1.0 µL
11	Heparin / Blood / Beads	2.0 µL
12	Heparin / Serum / Beads	1.0 µL
13	Heparin / Serum / Beads	2.0 µL
14	Negative Control	
15	Negative Control + Beads	

Table 2: Column information for Figure 5.

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