GEL ELECTROPHORESIS IN POLYDIMETHYLSILOXANE FOR DNA PURIFICATION

矽膠材質 DNA 純化晶片

林 建 明[*]　胡 文 聰[**]　張 裕 釧[†]　林 畢 修 平[†]

Cheng Ming Lin　Andrew Wo　Yuh-Chuahn Jang　Hsiu-Ping P. Lin

[*]碩士研究員　[**]教授

[*,**] 國立台灣大學應用力學研究所

[†] 財團法人生物技術開發中心

[*]Research Fellow　[**] Professor

[*,**] Institute of Applied Mechanics, National Taiwan University, Taipei, Taiwan 10617, R.O.C.

[†] Development center for Biotechnology, Taipei, Taiwan 10617, R.O.C.

摘　要

本文目標朝向微小 DNA 純化系統進行，利用微機電系統和生物技術，可將流體元件微小化，可以達到實驗室晶片(lab-on-a-chip)的理想目標，我們所使用的軟性材料為 polydimethylsiloxane (PDMS) 彈性材料，可以快速製作微流道、微混和器和微過濾器等元件製成微流體晶片的初步原形。在微流道內注入洋蔡膠體當作分子篩子。為提供高壓電源加速分離 DNA 和雜質，利用電晶體 (bipolar junction transistor) 電路產生一高壓電源；實驗結果證明，在微膠體電泳實驗裡，較小的分子能通過膠體分子篩；螢光球因本身有較大的尺寸(0.9μm)而被膠體阻擋，而單股的螢光 DNA 分子可以從混合液中分離出來，本實驗設計的微流體晶片具有分離且有純化 DNA 的能力。

關鍵詞：DNA 純化、PDMS、電泳、洋菜膠。

Abstract

This paper reports progress towards DNA purification in a microfabricated device. In order to achieve the lab-on-a-chip, components – microchannel, micromixer, microfilter, and electrophoresis module are fabricated using polymeric material polydimethylsiloxane (PDMS) for fast prototyping. The microchannel is injected with agarose gel, which acts as a sieve for bio-samples. To expedite the electrophoretic process during differentiating DNA and impurities as they pass through the gel, a bipolar junction transistor high-voltage device is fabricated. Results show that fluorescent micro-spheres (0.9μm in diameter) are successfully separated from single strand DNA, which are collected in the micro-channel outlet.

Keywords: DNA purification, PDMS, electrophoresis, agarose gel.

1. INTRODUCTION

The purpose of nucleic acid assay is to detect predetermined nucleic acid sequences in biological fluid samples. Nucleic acid probe assays have been applied in biotechnology and medicine, *e.g.* detection of pathogens in foods and genetic diagnostics on human subjects [1]. The nucleic acid probe assays typically include polymerase chain reaction (PCR) to amplify copies of DNA to a detectable level. PCR requires a relatively pure DNA sample in aqueous solution, which is free of inhibitor during the process. For this reason, the extraction and purification of nucleic acid from biological samples are the critical steps. However, these complex chemical processes without miniaturization are most difficult and time consuming. MEMS technology, based on semiconductor processes, provides a platform to integrate nucleic acid assays within a microfluidic device.

A DNA extraction and purification (E&P) device has been designed [2]. The prototype is composed of three components: micromixer, microfilter, DNA E&P module. The goal of this work is to prepare DNA, extracted from living cells, for PCR amplification.

2. DESIGN AND FABRICATION

Layout of the microfluidic chip is shown in Fig. 1. Micromixer is designed for mixing *E-Coli* samples with lysis buffer, which can disrupt the cell wall in preparation for extracting chromosome DNA. After the microfilter, most debris of cell would be blocked, which ensure extraction of purest DNA molecules possible.

The fabrication process for the micro-device is as follows [2]. The designed patterns were printed on high resolution (4,000dpi) transparencies to be used as masks in photolithography. Soft lithographic technique is used for printing and molding using elastomeric stamps [3]. This has become a method of choice for fabricating microstructures for biological and microfluidics applications [4]. The whole design cycle, once established, is about 24 hours, and costs very minimal.

To achieve deep channel structure, negative photoresist SU-8 100 (MicroChem Corporation, Newton, MA) is adopted to fabricate the master.

Cured PDMS is bounded on glass after oxygen plasma treatment about 20 ~ 60 second. For instant bonding, it is found that low RF power and short treatment time are best.

To disturb the laminar-like flow thus enhancing mixing, a simple passive micromixer, Fig. 2, is used. Results are found to be adequate, although in further effort optimization is being studied.

Agarose gel, concentration at 1% (w/v) was used to serve as molecular sieve, which blocks huge molecules (≥ 200nm) from passing through. The gel was injected into the middle channel between two reservoirs. The inlet reservoir received mixture of DNA, proteins, and other organelles. Only smaller negative charged molecules (≤150 ~ 200nm) could pass through the gel under an electric field. Therefore, the downstream reservoir would contain DNA and smallest molecules. In general gels with a pore size from 150nm at 1% (w/v) to 500nm at 0.16% are used, and pore diameter up to 800 nm is achievable at 0.075%. The surface of PDMS is essentially hydrophobic which prevents agarose gel solution into the microchannel. Figure 4(a), agarose solution was of course carefully introduced into the injection port between inlet and outlet to fill the microchannel with gel. TAE buffer is also injected into the inlet and outlet ports.

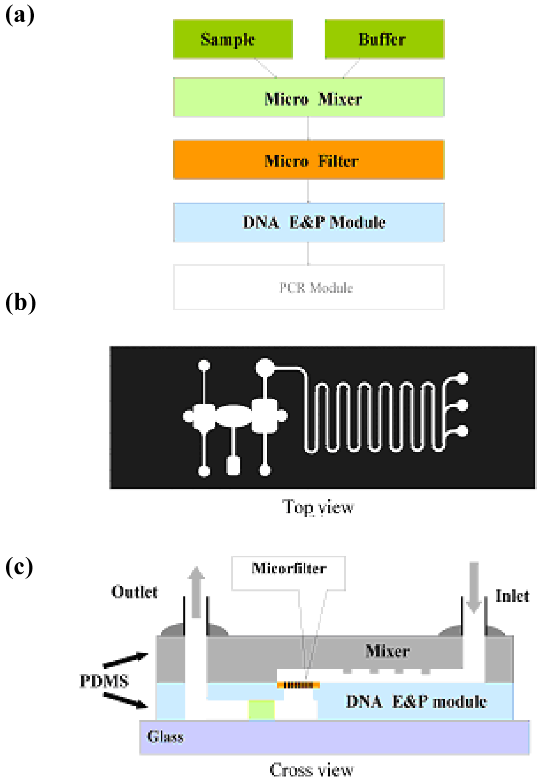

Fig. 1 (a) Flow chart of the microfluidic system for DNA E&P chip. (b) The diagram presents the concept that is "samples in and DNA out". As shown in (c), the proposed microfluidic system consists of two layers of microstructure

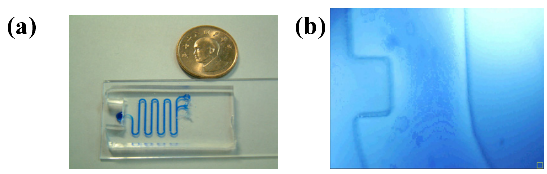

Fig. 2 (a) Micro mixer used in the preliminary design. (b) The scale of the microchannels is 500μ*m* in width and 100μ*m* in height

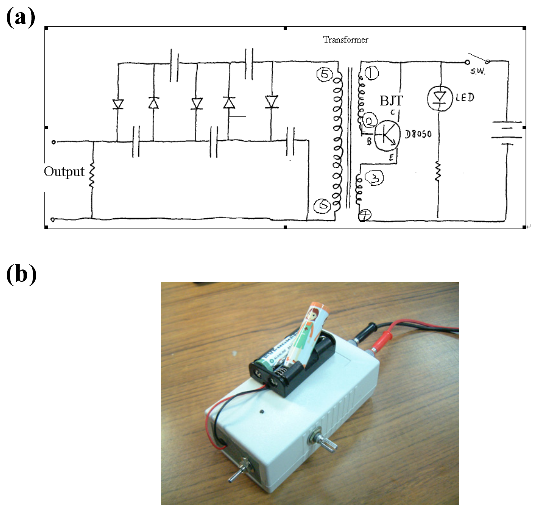

Fig. 3 (a) The electric circuit of the high DC voltage. (b) Photograph of the portable high DC voltage, low-power power supply

Thus, before injection of gel, oxygen plasma is used to treat the surface being hydrophilic. A high voltage power supply with a transformer and a BJT (Bipolar Junction Transistor), Fig. 3, was built to shorter the times for the electrophoresis process [6].

3. RESULTS AND DISCUSSIONS

To prove that the DNA E&P module could extract DNA and filter out other big molecules, fluorescent microspheres (Duke Scientific Corp.) and single strand DNA (ssDNA), a primer used in genomic sequences analysis, are both placed in the inlet reservoir prior to the gel entrance. The microspheres are activated at 542nm with maximum emission near 612nm (red). The nominal diameter of these spheres is 0.9µm, which is bigger than the gel pore size of 0.15µm. During the electrophoresis, they migrate toward anode, the same direction as DNA.

The Fluorescence of the ssDNA, is activated at 494nm and maximum emission is near 530nm. These DNA molecules are small (20bp ssDNA is about 6nm in length), which makes differentiation between noise and fluorescence difficult. To increase the intensity of fluorescent DNA, a 100µm wide (40µm depth and 40mm length) was fabricated.

Figure 4(b) shows the results of electrophoresis in the 100µm wide channel. The 0.9µm microspheres are adsorbed on the interface between buffer and gel, while the fluorescent ssDNA, emitting green fluorescence, pass through the 1% (w/v) gel at a field strength of 250V/cm. Figure 4(c) and 4(d) show the change of intensity of fluorescent DNA more clearly in the microchannels — as more DNA molecules gather in outlet the fluorescent intensity increases.

4. CONCLUDING REMARKS

This work has designed, fabricated, and tasted the sieve of a DNA purification module via 1% (w/v) agarose gel.

Augmented by an in-house high voltage device, we demonstrated the separation of single strand DNA from the mixture of single strand DNA and fluorescent microspheres using agarose gel electrophoresis. The success suggests progress toward a potentially low-cost device that can extract DNA from more mixture of bio-solution.

Progress is underway to separate red blood cells, white blood cells, and blood plasma for rapid blood analysis.

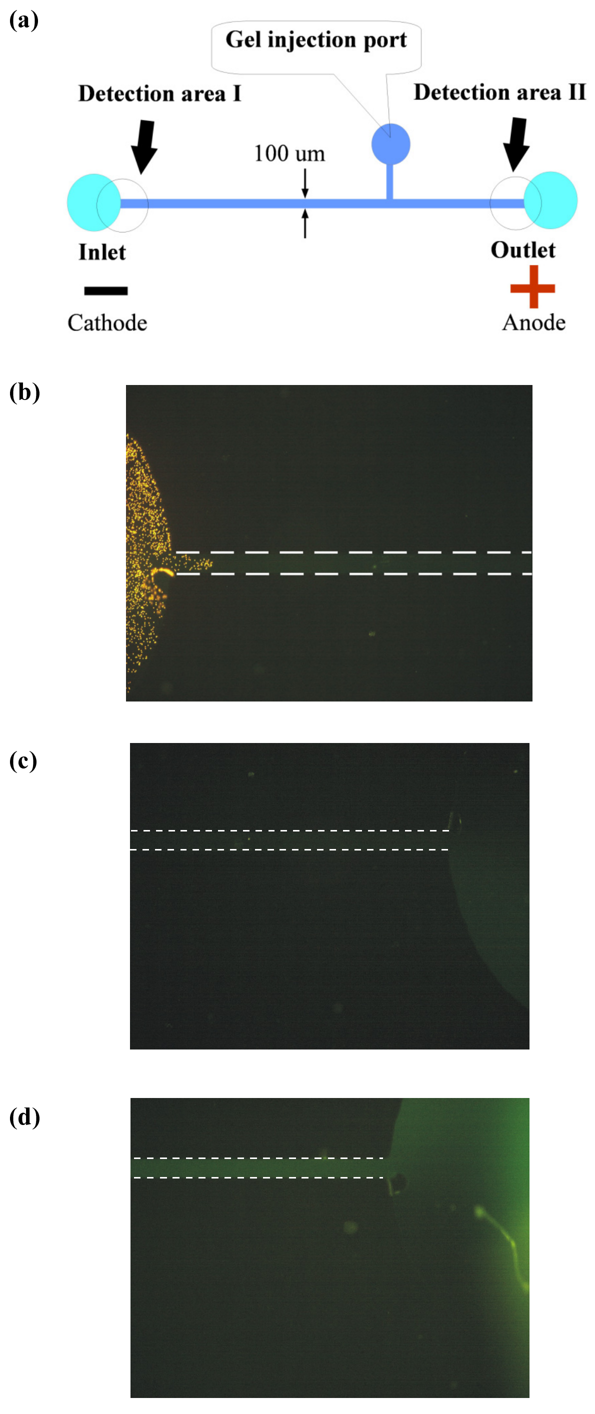

Fig. 4 (a) A 100µm wide microchannel was fabricated by PDMS molding method. The channel length is 40mm long, 100µm width, and 40µm depth. (b) Photograph shows fluorescent of ssDNA and microspheres mixture. The microspheres were adsorbed by agarose gel after electrophoresis. As more DNA molecules gather in outlet, the fluorescent intensity increases near detection area II. Photographs (c) and (d) show the increase of fluorescent at the outlet

ACKNOWLEDGEMENT

Partial financial support from the Development Center for Biotechnology is gratefully acknowledged.

REFERENCES

[1] J. Buitkamp and J. P., Epplen, "Modern genome research and DNA diagnostics in domestic aninals in light of classical breeding techniques," *Electrophoresis*, Vol. 17, 1996, pp. 1−11.

[2] C. M. Lin, "A low cost DNA extraction and purification chip for nucleic acid probe assay," Master thesis, Institute of Applied Mechanics, Department of Engineering, National Taiwan University, 2003.

[3] Y. Xia and G. M. Whitesides, "Soft lithography," *Angew. Chem. Int. Ed.,* 37, 1998, pp. 550−575.

[4] G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang and D. E. Ingber, "Soft lithography in biology and biochemistry," *Annual Review of Biomedical Engineering,* No. 3, 2001, pp. 335−73.

[5] R. H. Liu, M. A. Stremler, K. V. Sharp and M. G. Olsen, Members, "Passive mixing in a three-dimensional serpentine microchannel," *Journal of Microelectromechanical Systems*, Vol. 9, No. 2, June 2000.

[6] D. K. Cheng, *Field and Wave Electromagnetics*, 2nd ed., Addison Wesley, 1989.

Cheng Ming Lin (林 建 明) 1997年高雄中學畢業，2001年台灣大學造船及海洋工程學系學士，2003年台灣大學應用力學研究所碩士。

Andrew Wo (胡 文 聰) 1990 年美國麻省理工學院博士，1986 年美國麻省理工學院碩士，1982 年美國維真尼亞大學機械航空系學士，專長：微流體力學研究。

Yuh-Chuahn Jang (張 裕 釧) 1998 年國立陽明大學生物化學研究所博士，1989 年國立台灣大學農業化學系學士，專長：生物化學、分子生物學、鈣離子分析、RNA 表現的分析環境微生物篩選與改良用分子生物技術。

Hsiu-Ping P. Lin (林 畢 修 平) 1968~1977 年美國亞利桑那州立大學植物學／微生物學碩士、博士，重要成就：經濟部九十年度科技研究發展專案計畫「環境生物技術與產品研發第二期三年計畫」優良計畫主持人獎。經濟部八十九年度科技研究發展專案計畫「環境生物技術與產品開發四年計畫」全程優良計畫獎。

收稿日期 93 年 6 月 21 日、修訂日期 93 年 6 月 30 日、接受日期 93 年 7 月 7 日
Manuscript received June 21, 2004, revised June 30, 2004, accepted July 7, 2004