Polymer microfluidic chip with embedded CNTs for DNA sample preparation

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ABSTRACT

A polymer-based microfluidic chip which has deposited carbon nanotubes (CNTs) onto the microchannel has been developed. Embedded CNTs were coated and used as functional area to immobilized DNA from bio sample. The advantage of having a large large surface area to volume ratio from deposited CNTs in micro channel introduces high binding capacity of DNA. The chip is capable of performing DNA preparation process like restriction enzyme digestion and ligation of DNA. The chip was associated with automated control system for liquid and temperature control.

Keywords: DNA sample preparation, carbon nanotubes, polymer microfluidic, lab-on-a-chip

1 INTRODUCTION

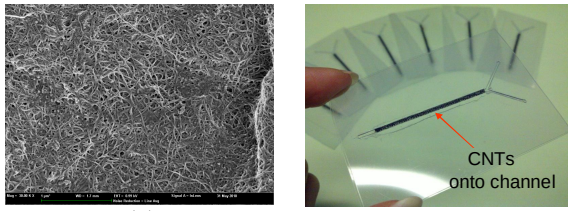
Rapid development of microfluidic device using nucleic acids for biomedical assay, environment monitoring, and life science research have been present in past decade [1-3]. Polymer microfluidic device is the main drive in this area since low cost of disposable devices and large kingdom of material type. As part of this movement, there is a challenge of fully integrated analysis system onto a single polymer device which advantage to reduce laboratory time, human interaction, and reagent and equipment costs. The pervious development from our group has been shown successfully from DNA extraction on polymer chip by using embedded carbon nano tubes (CNTs) [4].

In this study, we further developed a fabrication technique to deposit CNTs and coat with copolymer. The deposition of CNTs on polymer film has been developed by using ultrasonic embossing technique. Very large surface area to volume ratio from deposited CNTs into polymer channel enhances interaction area between bio-molecule to micro channel. Thus, accelerate reaction by having shorter time of diffusion is achieved.

2 FABRICATION

The polymer chip was fabricated from Polypropylene (PP) and Cyclo Olefin Polymer (OP) films. CNTs were deposited on COP film which was used as a carrier layer. Localized embossing of COP film together with CNTs onto PP substrate was performed by ultrasonic technique. Inspection by Raman spectroscope presented no Raman shift on CNTs after the embossing process (see Fig.2). SEM photo shows deposited CNTs on a polymer channel after ultrasonic embossing (Fig. 1(a)).

Figure 1(b) shows embedded CNTs onto polymer chips. The chip has designed as Y-shape by having two inlets and one outlet. CNTs were deposited in the channel area after combined inlet channels. High density of deposited CNTs layers shows in black color on the polymer channel.



(a) *(b)*

Figure 1. (a) SEM photo of embedded CNTs on polymer channel, (b) Embedded CNTs on Polymeric chip. The chip has two inlets and one outlet.

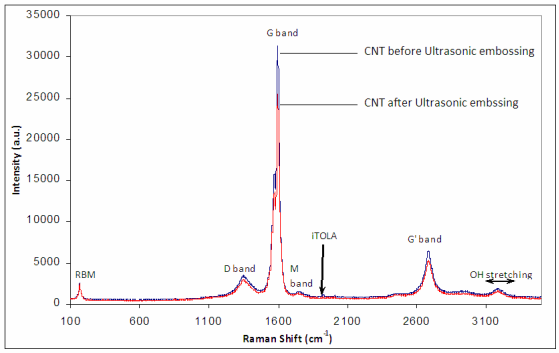


Figure 2. Comparison of Raman shift between before and after embossing process of CNTs on polymer.

3 METHODE

Preparation DNA libraries for genomic analysis has several processes such as purified genomic DNA, repair ends, ligation adapters, restriction digestion, and PCR [5-6]. In this work we demonstrate DNA conjugation and restriction digestion in polymer microfluidic chip. pUC 19

was used in this work. DNA will be immobilized in microfluidic channel during the processing time.

Immobilization of DNA onto CNTs in the chip was achieved by conjugation between streptavidin and biotin. The surfaces of CNTs were first coated with copolymer (PEG-PEI). Mixture of PEI, 10 wt% and PEG, 10 wt% in water, with a volume ratio of 1:9, was flowed in the chip and incubated overnight at room temperature. The next day, the chip was rinsed with DI water at 50 µl/ min for 10 minutes. The chip was dried with nitrogen gas. Biotin, together with 20.5 mg of ester, was added in 10 ml of 15 mM DMF. The mixture was injected in the chip. Overnight incubation at room temperature was carried out to allow binding of biotin onto CNTs (Fig. 3(a)). In the following day, the chip was rinsed with 15 mM DMF and DI water. Lastly the CNTs which have biotin on the surface were incubated with streptavidin, 1.5 mg in PBS, 0.01 M for 15 minutes at room temperature (Fig. 3(b)). After the chip was rinsed with DI water and dried with nitrogen gas, it was ready for use.

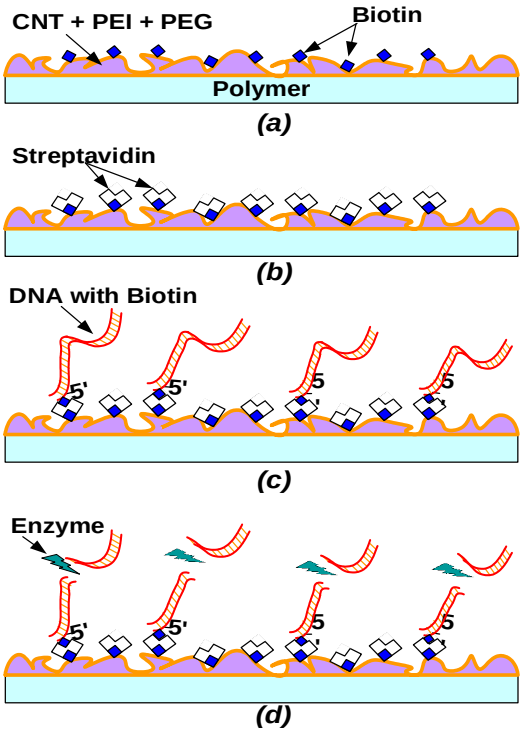


Figure 2. Diagram of surface treatment (a), coated with biotin and streptavidin (b), DNA bound on chip (c), and digestion of DNA (d).

Figure 3 (a) and (b) show AFM photo of CNTs chip before and after deposition of Biotin-Streptavidin respectively. Surface of CNTs after preparation process (Fig. 3(b)) has formed small roughness on the CNTs due to deposition of PEG-PEI. . In-house automation control which can handle multi sample inputs and temperature control was used as an interface for the CNT chips in our experiment.

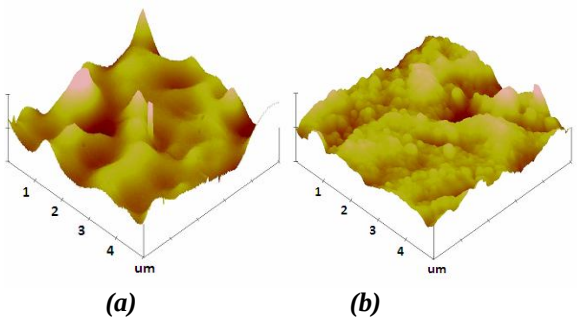


Figure 3. AFM images of surface CNTs before (a), and after PEI-PEG coating with streptavidin-biotin (b).

Biotinylated pUC19 at 15µl with concentration of 100ng/µl was used. At first, the CNTs chips were washed with 1xB&W buffer for 200µl at 50µl/min flow rate. DNA was first flow in the chip and incubated at room temperature. Four chips were tested in this experiment. Chip 1 and 2 were incubated for 10 minutes and Chip 3 and 4 were performed at 20 minutes of incubation time. During incubation time, biotinylated DNAs were conjugated with streptavidin on CNTs (Fig. 2(c)). Covalent binding of DNAs has strong bonding which can withstand chemicals and temperature. The amount of DNA conjugates onto the chips was quantified by a Picogreen test after collecting the supernatant.

DNA which was immobilized on chip was further digested with Hind III Enzyme, 0.5µl in 10x NE buffer, 10µl and Nuclease free water, 39.5µl (Fig. 3(d)). The chip was incubated at 37° C for 15 minutes before loading the enzyme into the chip. Restriction digestion process was performed at 37°C for 2 hours.

4 RESULTS

The supernatants from four chips after DNA immobilization on chips were collected and quantified. The quantification of non-binding DNA on chip was done by a Picogreen technique. Calculation of binding DNA on chips from four chips shows in Figure 4. Three sets of sample with the same chip were measured. The results preset binding of DNA on chip achieve up to 190ng DNA or 6.6x1019 copies from Chip 4 (Fig. 5). Result of higher DNA binding believes that due to longer incubation time. However, the incubation period is needs to optimize.

Restriction digestion on chip was performed. Supernatants from Chip 3 and 4 were collected and run on gel electrophoresis to qualify digested DNA. Figure 6 shows the gel electrophoresis as the correct DNA band size of 297bp was observed on the gel, when compared to the ladder and control sample.

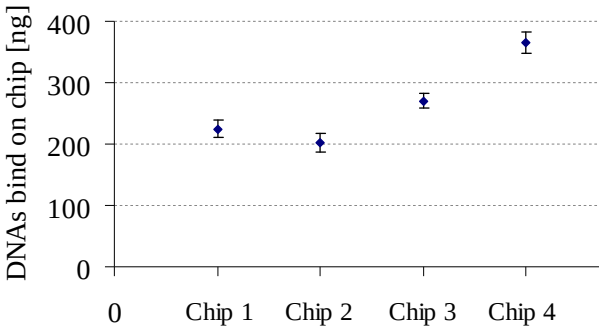


Figure 4. Amount of DNA binds on CNTs chips, Four chips were tested with two inclubation time. Chip 1 and 2 were inclbated at room temperature for 10 mintues and 20 minutes in chip 3 and 4 (n=3).

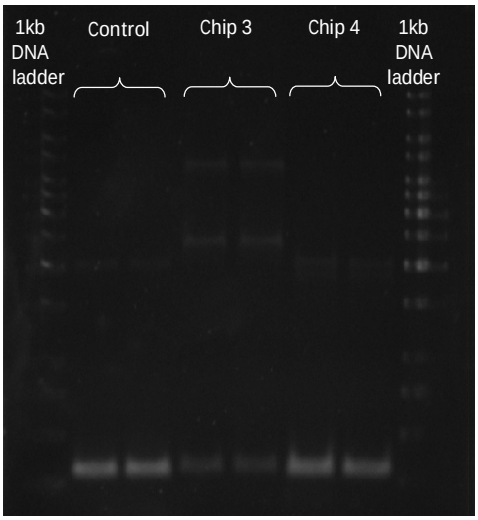


Figure 5. Gel electrophoresis of DNA after restriction digestion. Digested DNAs, 297bp, from chip were collected from chip 3 and 4 to verify and compare with control sample.

CONCLUSIONS

We have fabricated embedded CNTs on polymer chip that functions DNA sample preparation. DNA conjugation and restriction digestion on chip were tested and quantified. Deposition of CNTs on polymer enhances a binding capacity due to large surface per volume ratio. Polymer chip is cooperated with off-chip liquid control system. The system is able to program liquid flow and temperature on chip. The established platform can be integrated with other DNA sample preparation process such as purification and PCR.

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