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# Integrating Carbon Nanotubes into Microfluidic Chip for Separating Biochemical Compounds

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## ABSTRACT

We present a new type of device to separate biochemical compounds wherein carbon nanotubes (CNTs) are integrated as chromatographic stationary phase. The CNTs were directly grown on the bottom of microfluidic channels on $Si/SiO_2$ substrates by chemical vapor deposition (CVD). Acetylene was used as carbon source and Ni was employed as catalyst. For electrokinetic separations, higher electrical field strength is usually required; therefore, the CNTs were constructed in pillar-array-form by patterning the catalyst layer. Electrical field strength of 2.0 kV/cm has been realized, which is more than one order of magnitude higher than the one reported so far. The systems were successfully used to separate a compound containing two Coumarin dyes, 240 mM C460 and 270 mM C480.

## INTRODUCTION

Over the past decade, the integration and miniaturization of chip-based analytical systems have been enormously developed [1]. Microchip electrophoresis combining with liquid chromatography is emerging as a promising separation technique to analyze biochemical compounds [2]. The mechanism of the separation technique is to employ a stationary phase to interact with solutes with different degrees for different molecules. In the research, main challenge presently is to discover more robust and powerful stationary phases which can be technologically integrated into microfluidic channels.

CNTs are very promising nanomaterial as a new stationary phase owing to their high hydrophobicity, surface-to-volume ratio, chemical stability and outstanding mechanical strength. Additionally, their growth processes are compatible with micro fabrication techniques. Carbon-based materials have been employed as stationary phase in capillary electrophoretic and microfluidic devices for separation [3]. In these studies, the CNTs were typically incorporated in a porous rod [4], immobilized on the inner channel wall [5] or deposited on the surface of beads that subsequently were packed [6]. Recently, a few research works on growing CNTs in microfluidic channels for electrokinetic separations have been reported [7,8]. To date the main

challenge to employ CNTs as stationary phase is the limitation of the electrical field strength (E) before bubble formation from electrolysis due to the metallic characterization of CNTs. The maximum E reported so far is around 150 V/cm, which puts a severe limitation on the fluid velocity that can be used. The low velocities results in plate heights larger than optimal due to peak broadening from diffusion. In our work presented here, E in microfluidic separation channel can be applied up to 2.0 kV/cm, which is more than one order of magnitude higher than the one reported so far.

**EXPERIMENT**

Figure 1 illustrates the process steps for fabricating the microfluidic chips on 4-inch Si/SiO2 wafers. An 8-μm thick $SiO_2$ layer was thermally oxidized on undoped 4-inch Si wafer. For anodic bonding purpose, a thin undoped amorphous Si (30 - 50 nm) was deposited on the $SiO_2$ surface by LPCVD (low pressure chemical vapor deposition). Microfluidic channels were patterned by conventional photolithography technique; the channels were made by wet etching. The separation channel had a 100-μm width and a 5-μm depth. A 10-nm $Al_2O_3$ layer was then deposited as catalyst supporting material, followed by a 5-nm Ni deposition as catalyst. The two

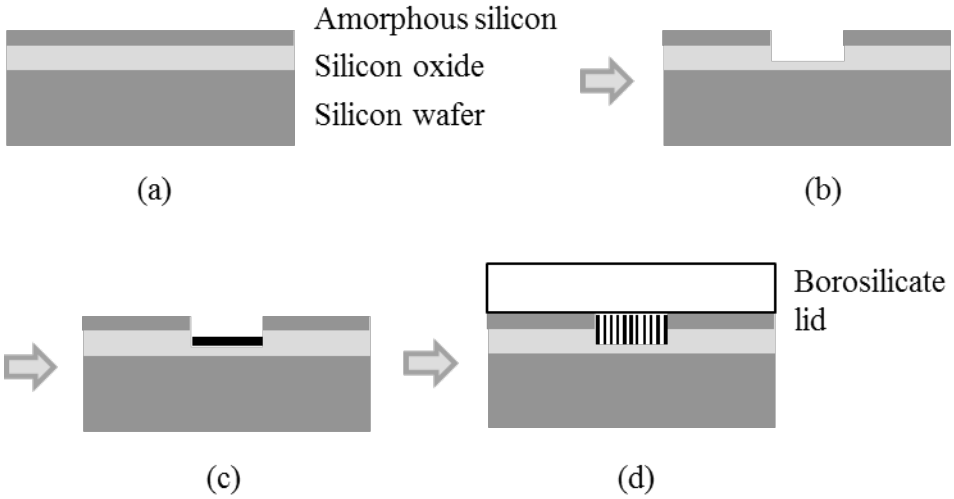
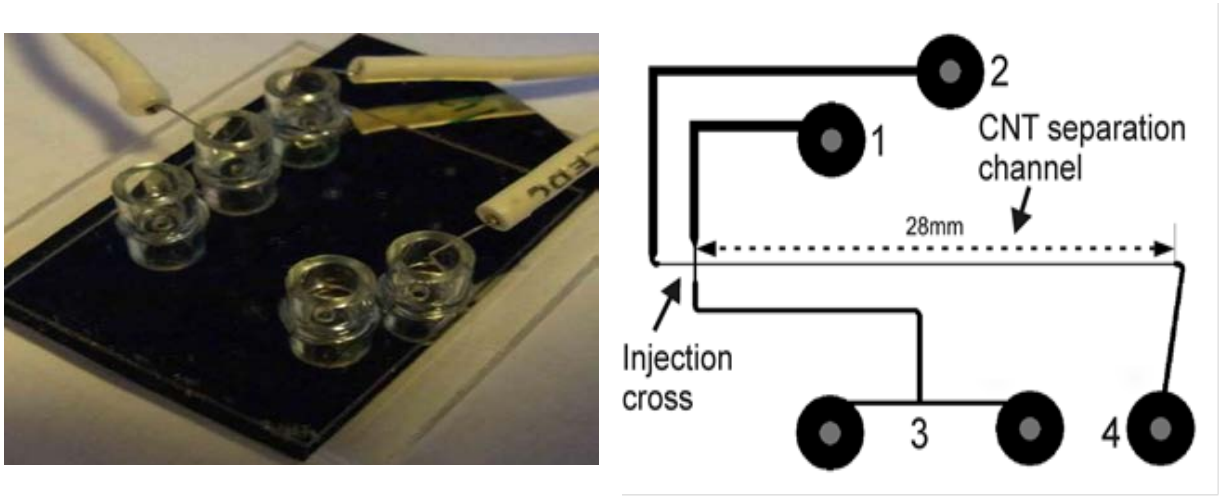



Figure 1. Schematic of the fabrication processes of microfluidic chips integrated with CNTs. (a) An undoped amorphous Si layer (30 - 50 nm) is deposited by LPCVD on $SiO_2$ (8 μm)/Si wafer. (b) Fluidic channels are patterned with photolithography technique. The separation channel has 100-μm width and 5-μm depth. (c) 10-nm $Al_2O_3$ is first deposited as catalyst support material and then 5-nm Ni is deposited as metal catalyst. These two films are patterned with photolithography process, resulting in the catalyst on the bottom of the separation channel. (d) CNTs are grown with CVD at 670 °C in an $N_2/H_2$ environment, and acetylene is used as carbon source. Finally, a borosilicate glass is anodically bonded onto the fabricated device to form fluidic channels.

layers were patterned by lift-off process on the bottom of the separation channel. CNTs were directly grown on the bottom of the separation channel by cooled-wall CVD (chemical vapor deposition). Inside the CVD reactor, the device was first annealed at 610 °C for 10 min in $H_2$ in order to make the Ni film into nanoparticles. In the growth process, acetylene ($C_2H_2$) was used as

carbon source; the pyrolysis of $C_2H_2$ and catalyst was performed in a $N_2/H_2$ environment at 670 °C. The height of the CNTs was adjusted to equal the depth of separation channel in order to obtain a uniform distribution of the CNTs throughout the channel. In addition, the CNTs grown here were multi-walled CNTs with diameter ranges of 30 – 80 nm, which are more rigid and suitable to be used in fluidic conditions. As a final step, a borosilicate glass lid was anodically bonded onto the amorphous silicon surface to form fluidic channels.

The optical image of a fabricated microfluidic chip and its layout are shown in figure 2 (a) and (b), respectively. In each chip, five holes aligned to the fluidic channels were made in the borosilicate glass lid, and five glass tubes were glued to the holes with epoxy as biochemical liquid reservoirs. In the experiment, Pt wires were employed as electrodes. CNTs were integrated with the separation channel (figure 2 (b).




(a) (b)

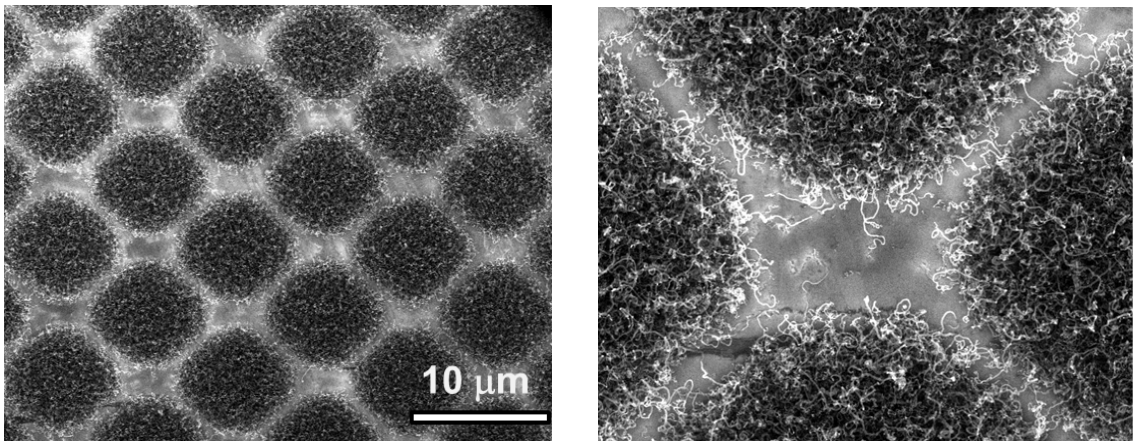
Figure 2. (a) Optical image of a microfluidic chip. In the chip, glass tubes are employed as biochemical liquid reservoirs and Pt wires are used as electrodes. (b) Layout of the chip. The reservoir 1 and 2 are for sample waste and injection, whereas the reservoir 3 and 4 are for buffer injection and ground contact. The CNTs are integrated in the separation channel, and its length is 28 mm.

To characterize the microfluidic chips integrated with CNTs, experiments of separating biochemical compounds were carried out. The buffer used was 50 mM ammonium acetate (pH = 7.0) with 90% acetonitrile. The chips were preconditioned by flushing with a 0.1 mM NaOH solution for 10 min to stabilize electroosmotic flow, followed by flushing with the buffer solution for 10 min. The chips were then loaded in a confocal microscope equipped with a 100 W halogen lamp (Leica DMLB, Leica Microsystems, Denmark). In the measurements, biochemical compounds were excited at wavelength of 375 nm with UV diode laser module (8 mW, Shanghai Dream Lasers, China) through an optical fiber. An objective lens with a 10x magnification was used for collecting emission light; the lens was connected to a photomultipler tube (H5784-02, Hamamatsu, Denmark) to detect fluorescence and data. The filtercube had a band pass filter at 450-490 nm for excitation and a long pass emission filter at 515 nm for fluorescence collection (Filtercube I3, Leica Microsystems, Denmark). A control system

connected with high voltage power supply was used to generate electrokinetic fluidic flow (Microfluidic Tool Kit, Alberta Microelectronic Corporation, Canada).

## RESULTS AND DISCUSSION

For an electroosmotic flow generated in a microfluidic channel, exerted external E needs to be in the ranges of 0.5 – 1 KV/cm in order to eliminate a band broadening from diffusion due to low mobile phase velocities. On the other hand, for the channel integrated with metallic multi-walled CNTs, it is infeasible to apply so high potentials because of electrolysis of oxygen and hydrogen resulted from large potential differences between the carbon nanotubes and the surrounding electrolyte. Therefore, in order to reduce these potential differences, the CNTs need to be patterned into pillar arrays, see figure 3. If treating each CNTs pillar as an electrode and considering onset electrolysis potential is 1 V, at E = 1 kV/cm, the potential drop over a 10 μm length pillar is just 1 V. Based on above considering, we fabricated chips with different pillar lengths and measured their I-V characteristics. In the measurements, the microfluidic channels were filled with a 50 % 10 mM Tertraborate buffer (pH = 9.2) / 50 % acetonitrile solution. For pillar length of 8.0 μm, E can be up to 2 kV/cm without bubble formation, which was same as the one measured from the channel without CNTs [9].




(a) (b)

Figure 3. Top views of SEM images of the patterned CNTs pillar arrays inside the separation channel. (a) The pillar length along the channel is 8 μm. (b) the gap between pillars is 2 μm.

The measurement results of separating two Coumarin dyes, 240 mM C460 and 270 mM C480, are shown in figure 4. The microfluidic chip measured has a CNTs pillar length of 8 μm. The separation experiments were performed at E = 400 V/cm, 2 s gated injection at separation length of 2.6 cm. The three curves shown in the figure were from different chips with same experimental conditions, reflecting a good reproducibility.

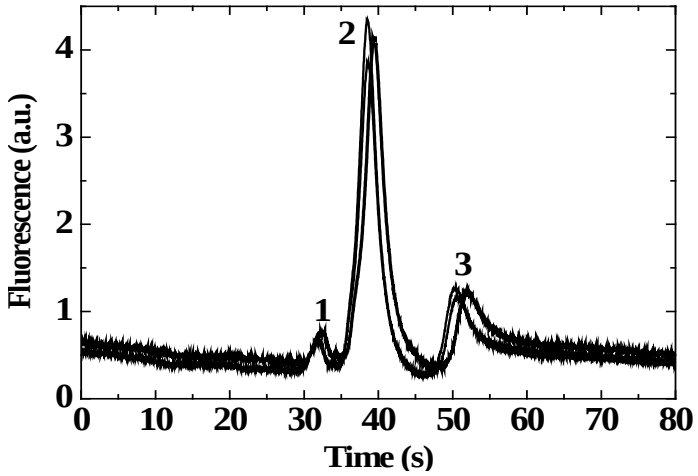



Figure 4. Electrochromatographic separation of Coumarin dyes. The three emission peaks represent (1) C460 impurity, (2) 240 μM C460 and (3) 270 μM C480.

## CONCLUSIONS

A microfluidic chip integrated with CNTs for electrochromatographic separation is presented. The results of proof-of-concept separation experiments show CNTs can be used as robust and powerful stationary phase in chip-based microfluidic system. In the future, different types of CNTs will be employed as stationary phases in order to optimize the systems for various biochemical analysis applications.

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