Integration of carbon nanotube arrays in lab-on-a-chip system for blood analyses separation and detection

Carbon nanotubes as filters in microfluidic device for bio-sensing applications

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ABSTRACT

Blood-cell-free serum is required for most clinical chemistry tests. At present bend micro channel and polymeric pillars are used in polymer based microfluidic devices (such as PMMA) for the blood filtration. In this study, we have fabricated carbon nanotube (CNT) pillars on silicon from 20-50 µm in diameter with ~10 µm spacing and integrate them inside the microfluidic channel with a view of using these for blood plasma filtration from whole blood, with passive capillary flow. Our main objective is to design a novel sensor, comprising CNT arrays, to filter/control whole blood flow, with an integrated micro patterned gold electrode which will be sealed by bonding into microfluidics structures. We have characterized the microfluidic channel by measuring the meniscus movement profiles. Also gold inter-digitated electrodes (IDEs) were fabricated on glass and immobilized with an antibody. These IDEs were used as an impedance-based biosensor using label-free antigen – antibody interaction. At a fixed frequency, the IDEs gave a linear response across the range of concentrations of secondary antibodies investigated (0 to 500 µg/mL).

Keywords: Hot embossing, Carbon nanotubes (CNTs), Poly Methyl Meth Acralate (PMMA), bio-sensing.

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INTRODUCTION

Impedance-based biosensors, based on an electrochemical impedance spectroscopy (EIS), measure the electrical impedance of an interface in AC steady state, with variable frequency but constant low amplitude DC bias conditions to avoid disturbing the probe layer [1]. A major advantage of impedance-based biosensors over most other types is their ability to perform label free detection, whereas biomolecule labelling can drastically change its binding properties [2]. Also, the yield of the target-label coupling reaction is highly variable; which is especially problematic for protein targets. Label-free operation offers additional advantages over endpoint detection in that it enables detection of target-probe binding in real time which can improve measurement accuracy and allows determination of affinity constants [3].

The remarkable properties of carbon nanotubes have motivated much research on their processing, integration, and applications. The incorporation of CNTs onto polymeric substrates provides a wide range of applications in various areas such as microfluidics, flexible microdevices, field emission and micro/nano electronics etc. Due to the low glass transition temperatures of most of the polymers it is extremely difficult to synthesize high quality CNTs directly on them. Various techniques like solution-based processing, soft lithography, casting into PDMS and hot embossing have been developed in recent years for incorporating CNTs onto

polymers [4–9]. Among these techniques, hot embossing has demonstrated the ability to transfer carbon nanotubes onto polymers with critical dimensions down to 10 μm [9, 10].

In this paper, we report a simple method for transferring vertically aligned carbon nanotubes onto PMMA substrates using hot embossing (HE), with a view of using them as filters for filtering blood cells from whole blood in impedance based bio-sensor. Also, using interdigited electrodes, AC impedance spectroscopic studies were performed with respect to various antibody concentrations.

EXPERIMENTAL DETAILS

The carbon nanotubes were produced using a Microwave Plasma Enhanced Chemical Vapour Deposition (MPECVD) system. The maskless UV lithographic technique was employed for the production of the microstructures on silicon surface. These micro-structures were fabricated using metal lift-off method on silicon wafer. A thin layer of cobalt (≈ 2nm) was used as a catalyst for CNT growth. These substrates were then transferred to the MPCVD chamber for nanotube growth [11]. Micro Raman studies was also performed on the samples before and after hot embossing. The microfluidic channels with the depth of ~30-50 µm were also fabricated on PMMA using hot embossing method [12]. Finally, the microfluidic channel with vertically aligned CNTs (VACNTs) on PMMA were bonded with PMMA lid with appropriate inlet and outlets using EVG system and tested with10 µm PMMA particles in DI water with red dye (to simulate blood conditions) for flow characterizations.

(A) Hot embossing of CNTs on PMMA

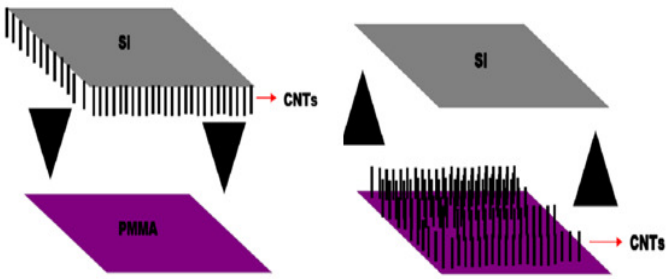


Figure 1. Schematic of hot embossing method using CNTs.

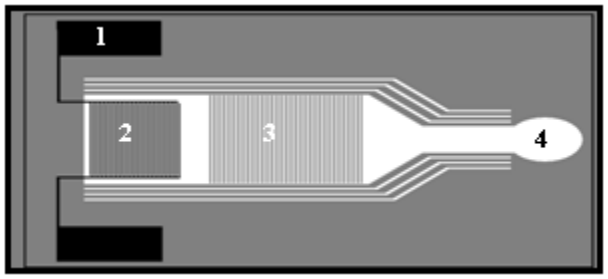
As shown in figure 1, the Si acts as a stamp on which the vertically aligned CNTs were grown as explained in previous section. Transparent PMMA discs of 4 inch diameter and 0.5mm thickness was utilized as molding substrate of which the glass transition temperature was about 115°C. A hot embossing system (EVG-520HE) as described above was used for the experiments. The hot embossing bonding parameters were 1kN-2kN applied force, temperature from 100-115^{o}C, and 2min embossing time. After embossing, the polymer substrate was retained in the chamber and cooled down to room temperature, then it was separated from the mold (CNTs grown on Si). The total cycle time was between 20-30min. After de-molding, the CNTs were transferred onto the polymer substrates.

(B) Impedance spectroscopy

In this work we also focus into the fabrication of interdigitated electrodes (IDEs) with various dimensions The IDEs were fabricated using conventional photolithography techniques [13, 14] on a gold coated glass slides purchased from Platypus technologies. AC impedance spectra for IDE were performed using a Solartron 1260 impedance gain-phase analyzer with a Solartron 1286 electrochemical interface from Solartron Analytical – UK. The impedance spectra was analysed and fitted using ZPlot and ZView software from Scribner Associates, Inc., Southern Pines, NC. The impedance measurements were performed using in 5mM of Fe $(CN_6)^{-3/-4.}$ The generator provided an output signal of known amplitude (10 mV) and the frequency range was typically swept between 100 KHz and 0.1 Hz. Then a formation of self-assembled monolayer (SAM) comprises a mixture of 11 mercapto-1-undecanoic acid (MUA) and 11-mercaptoundecanoal (MU) (from Sigma-Aldrich – UK). At a concentration of 4mM of MUA and 1 mM of MU are prepared in ethanol respectively and stored at room temperature. Then equal volumes of solution are mixed together and then added immediately to the gold surface which is exposed to the solution for about 12 hours (overnight). After adsorption electrode should be washed with ethanol to remove unbound thiols. SAM has been activated by EDC/NHS for 1hr then primary antibody (Anti-Rabbit IgG from Sigma-Aldrich) , with a concentration of (500 µg/ml) is incubated onto the electrode surface for 1 hour, followed by PBS rinse. The unbound sites of the IDE are blocked by using 1-Ethanolamince : HCl for 10 min. The secondary antibody (Rabbit IgG) from Sigma-aldrich, is prepared in different concentrations (1, 10 , 20 , 50 , 100 , 300 , 500 µg/ml) in PBS + 50 µl Tween20.

RESULTS AND DISCUSSION

Schematic of the overall device is shown in figure 2. The CNT pillars were hot embossed into the PMMA based microfluidic channel and the gold electrodes fabricated on glass were used as a lid. Here, the CNT pillars in certain geometry will act as a filter for filtering blood cells from the real blood and the plasma will pass through the CNTs and the proteins in the plasma will be detected by antibodies immobilized onto IDE's surface.



1. **Gold contact pads**
2. **Inter-digitated electrodes**
3. **Carbon nanotubes**
4. **Microfluidic channel**

Figure 2. Showing the schematic of microfluidic channel with CNT pillars for filtration and gold IDEs for impedance measurements.

This work has a two fold interest a) integration of CNT into microfluidic channels as a filter b) bio-sensing using gold IDEs. Here we will discuss both of them individually.

(A) Integration of CNT into microfluidic channels

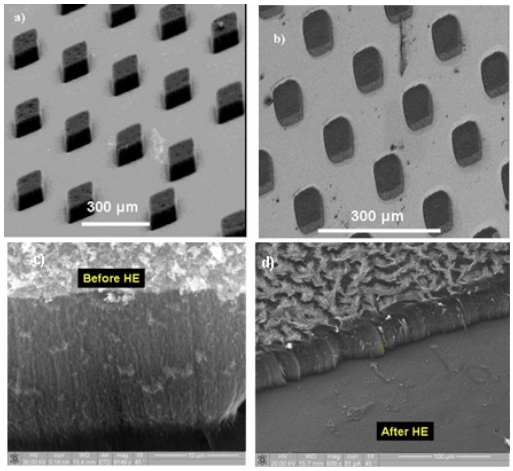
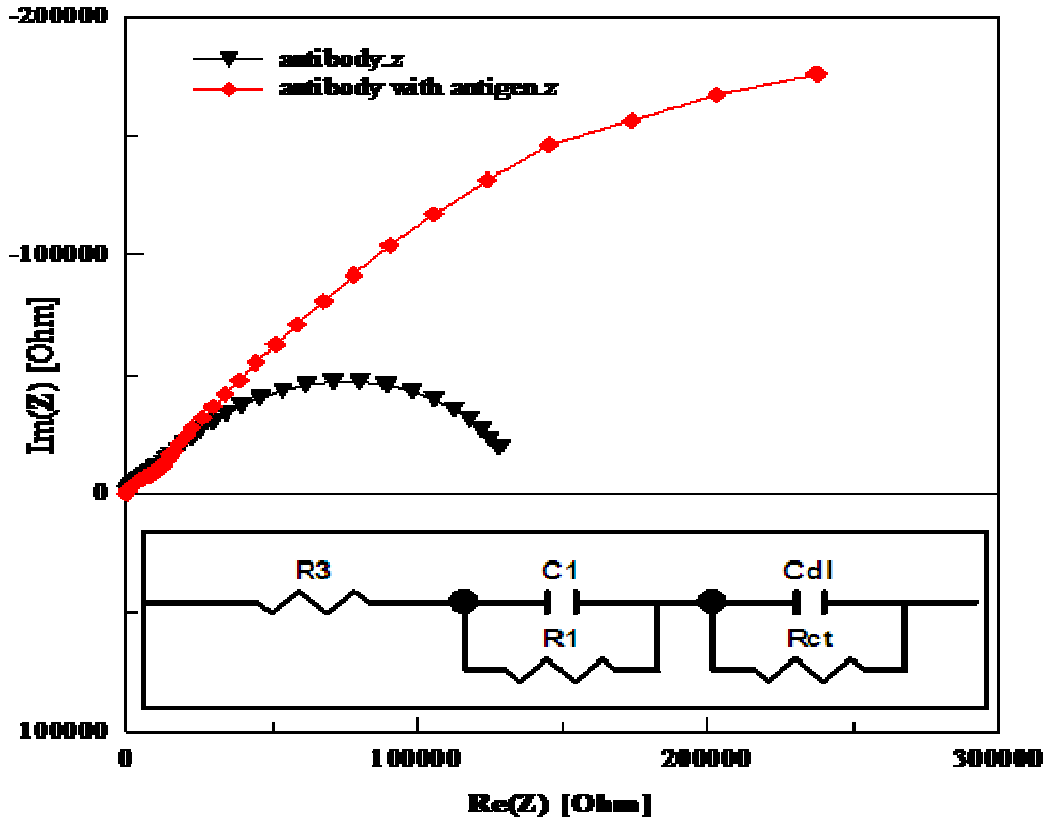


Figure 3(a, b, c, d). Represents SEM images of CNTs before and after HE.

Figure 3 shows the transfer of carbon nanotube forest and patterns from Si (a, c) to PMMA (b, d). From the scanning electron microscopy image, it is clear that vertical alignment of the features is maintained from this process. A close up image shows that the tubes are embedded in the polymer layer with little distortion of the polymer around the nanotube patterns as a result of the heating of the polymer. Previously CNTs were transferred to polymers [9], however in our technique we can transfer CNTs patterns on large areas and can also be used in batch processing. This technique also has advantage as it can be used to fabricate microfluidic devices.
Using the above mentioned CNT pillars/forest on PMMA we have successfully fabricated microfluidic devices as mentioned in experimental section. The dyed water flow through the CNTs based microfluidic devices has been thoroughly studied and we observed that in our measurement geometries the time (filling time) of the fluid flow across the channel without CNT pillars (≈ 5 sec) was less than that of with CNT pillars (≈ 16 sec). We have successfully filtered the PMMA particles using CNT forest.

(B) Bio-sensing using impedance spectroscopy

We measured the impedance before and after adding the secondary antibodies to the electrode, as shown in figure 4a. The whole frequency spectra showed significant difference in both quantities of impedance (real and imaginary parts) and this increment in impedance was due to the change in double layer capacitance (C_{dl}) and charge transfer resistance (R_{ct}).


-200000
-100000
0
100000
Im(Z) [Ohm]
antibody_z
antibody with antigen.z
R3
C1
Cdl
R1
Rct
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100000
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Re(Z) [Ohm]

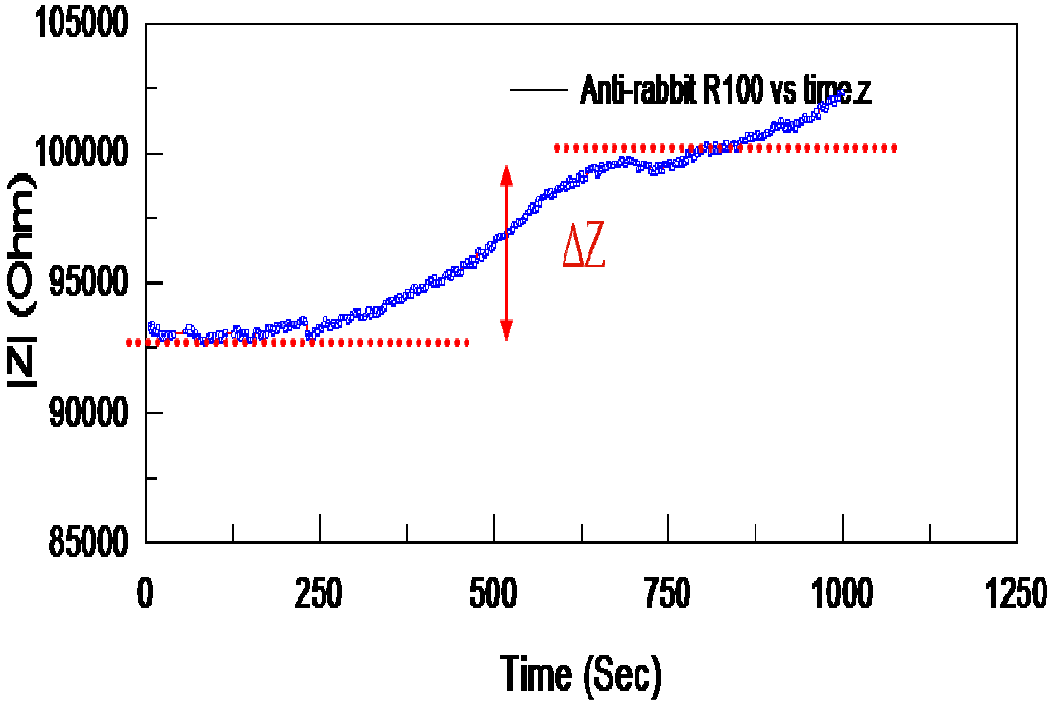
105000
100000
95000
90000
85000
|Z| (Ohm)
Anti-rabbit R100 vs time.z
ΔZ
0
250
500
750
1000
1250
Time (Sec)

Figure 4. (a) Showing the Nyquist plot before and after adding secondary antibodies, (b) Showing the magnitude of impedance |Z| vs. time at a fixed frequency.

To obtain the change in impedance, we fixed frequency at 113 Hz, and then secondary antibodies were injected to measure change in impedance |Z| vs. time as shown in figure 4b. At a fixed frequency, the biosensor gave a linear response across the range of concentrations of secondary antibodies investigated (0 to 500 µg/mL), as shown in figure 5. The limit of detection was comparable to that observed by Ko *et al* [15] and in addition the biosensor reported here is label free.

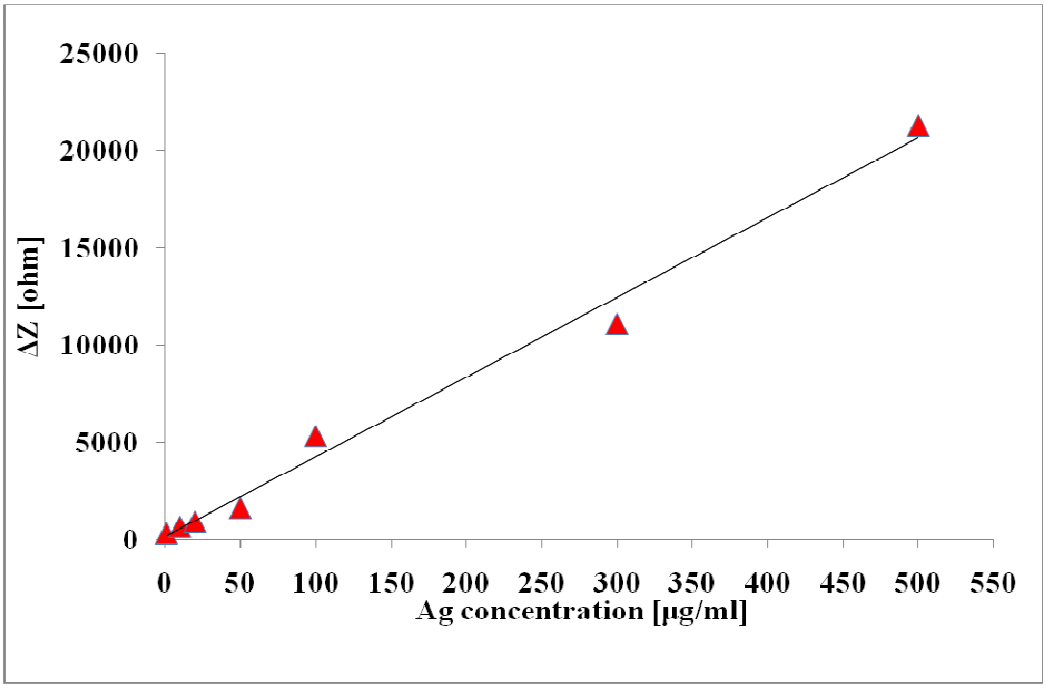

Figure 5. Change in magnitude of impedance as a function of increased secondary antibody concentration

Table 1. Equivalent circuit model components for different concentrations of secondary. ab. interacted with primary AB

Sec.AB [µgmL^{-1}]	R_s [KΩ]	R_{CT} [KΩ]	C_{DL} [µF]×100	ΔZ [KΩ]
0	2.157	1.363	4.06	0
1	2.571	1.661	2.13	0.335
10	2.469	1.992	1.78	0.677
20	2.396	2.066	1.83	0.925
50	3.058	15.431	1.33	1.627
100	3.266	22.616	1.06	5.338
300	3.597	65.304	0.660	11.08
500	3.543	66.466	0.806	21.30

Equivalent circuit modeling revealed significant changes in both R_{CT} and C_{DL} following secondary antibody binding (Table 1). Both components are influenced by the physical interaction of the primary and secondary antibody but also by the change in electric field, which results from the introduction of surface bound carboxyl anions and ammonium cations present on the surface of the proteins.

CONCLUSION

In this paper, we have reported a simple process protocol for making a microfluidic bio-sensor using CNTs as a filter to filter out blood cells from whole blood. The process of fabrication of the sensor is fast, simple and low cost in mass production, the results are sensitive and the operation is user-friendly. This novel approach included CNT transfer into microfluidic devices and the impedance experiments were carried out directly without any reagents, measuring the specific binding of a non-labelled antibody with a coated antigen. We have also observed a quite significant enhancement of the sensitivity of impedance-based biosensor by using of the interdigited electrodes. The achievement of this work will also provide an innovative direction for creating a high sensitive impedance-based microstructured biosensor; the anticipated device will detect analytes with high sensitivity, selectivity, rapidly and at low cost.

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