Metastatic diagnosis of colon cancer by vertically aligned carbon nanotube based electromechanical biosensor

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ABSTRACT

A novel vertically aligned carbon nanotube based electrical cell impedance sensing biosensor (CNTECIS) was demonstrated for the first time as a rapid, sensitive and specific device for metastatic diagnosis of cancer cells. This biosensor is based on different mechanical entrapment fraction of metastatic cancer cells on vertically aligned carbon nanotube arrays in comparison with primary grades. After cell entrapment, electrical interactions between CNT tips and entrapped cell membranes, change the impedance of the biosensor depend on the concentration of entrapped cells. The device was fabricated through a photolithography process on Ni/SiO_2/Si layers.. CNT arrays act as both adhesive and conductive agents and impedance changes occurred as fast as 30 s (for whole entrapment and signaling processes). CNT-ECIS diagnosed the metastasis grade difference between SW48 and HT29 colon cancer cells (primsry and progressive grades of colon cancer) with the concentration as low as 4000 cells cm^{-2} on its surface.

Keywords: carbon nanotube, cancer metastasis, impedance biosensor

1 INTRODUCION

There is a great demand to develop a simple and rapid technique for the investigation of the metastatic grades of cancerous cells, as well as their interactions with drugs [1]. Electrical cell–substrate impedance sensing (ECIS) based techniques is one of the bioelectrical methods for monitoring the cell vital behavior such as cancerous transformation. Some structural constraints such as time consuming cell attachment process, requirement to biomarkers for selective detection and poor electrical sensitivity for low analyte concentration cause certain limitations in the ECIS applications in label free and rapid detection of specific cells, such as cancerous cells. Here our novel vertically aligned carbon nanotube based electrical cell impedance sensing biosensor (CNT-ECIS [2]) was demonstrated as a rapid, sensitive and specific device for metastatic diagnosis of colon cancer cells based on their mechanical deformability and the differences in their electrical properties without a need for any additional adhesive layer such as fibronectin, collagen, gelatin18 etc. which generate some unwanted electronic signals for both capacitive and resistive devices [2]. Such adhesive layers intervene with the response of the sensors and reduce the electrical interaction between cells and conductive electrodes. In addition, using CNT-ECIS devices, one does not need a time consuming cell attachment process in which, a minimum period of 20 min is needed before a reliable electrical signal can be acquired.

Our biosensor is based on the selective entrapment of metastatic cancer cells (which have more deformable cytoskeletal structures) on vertically aligned carbon nanotube arrays and leads to mechanical and electrical interactions between CNT tips and entrapped cell membranes, changing the impedance of the biosensor. Different mechanical properties of higher metastatic colon cancer cells from lower ones are employed to observe the significant differences in the entrapment fraction of such cancerous cells onto vertically aligned carbon nanotubes. Colon cancer cells were prepared in a cell culture. For higher metastatic live colon cancer cells, we have observed an entrapment fraction more than 75% whereas for lower metastatic grades, this entrapment fraction has been less than 30%. Cell viability studues were experimented to chek the live state of entrapped cells during signal extraction.

The impedance of the device after the entrapment of different grades of colon cancers (HT29 and SW48, the primary and progressive grades of colon cancer, respectively) were measured and compared for metastasis diagnosis. The detection of epithelial cells such as colon cells at higher metastatic cancerous transformation by means of their different entrapment on CNT arrays as well as electrical signal extraction from entrapped cells from CNT covered ECIS micro electrodes can be of great application in laboratory studies for cancer cell diagnosis.

2 EXPERIMENTS

The CNT-ECIS fabrication process starts by coating the Si surface with a thermally grown SiO_2 layer, followed by depositing a thin film of Ni as a catalyst for CNT growth and subsequent patterning of Ni using standard photolithography. One of the SEM images of such interdigital sensors is depicted in Figure 1.

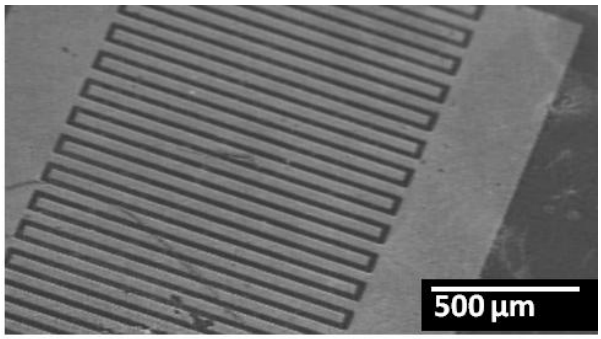

Figure 1.SEM image from CNT-ECIS microelectrodes.

Finally, the sample is placed in a direct-current plasma enhanced chemical vapor deposition (DC-PECVD) reactor to grow vertically aligned multi-walled carbon nanotubes (MWCNT) on desired places. Details about this growth are reported elsewhere [3]. The CNT beam length and diameter range from 2 to 12 µm and 20 to 75 nm, respectively (please refer to Fig. 1 for the SEM image of a typical CNT-ECIS device). Highly ordered CNTs have been achieved with desired patterns and geometries (figure 2) .

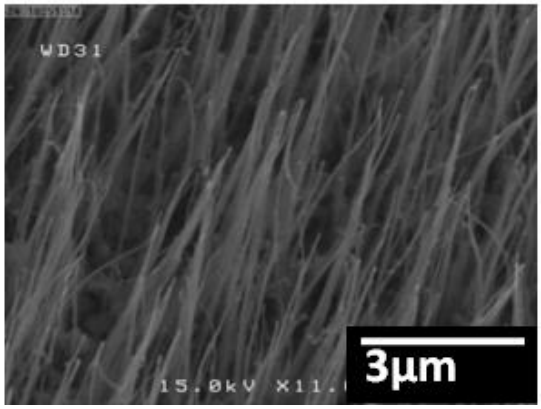

Figure 2. Elecron micrograph from vertically aligned carbon nanotube arrays.

SW48 and HT29 cell lines, which were isolated from grades IV and I of human colon tumors have been used in this investigation. These cells were obtained from the standard cell banks and maintained at 37^{o}C (5%CO2, 95% air) in RPMI-1640 medium (Sigma 8758) supplemented with 5% fetal bovine serum (Gibco), and 1% penicillin/ streptomycin (Gibco). The fresh medium was replaced every other day. The biocompatibility of CNT structures during their interaction with cells were experimented by green florescent protein (GFP) expression method as shown in Figure 3. HT29 and SW48 colon cancer cells (primary and progressive grades of colon cancer respectively) were flown over the surface of the sensor with the same primary concentration (4000 cells/cc) with the flow rate of 2.5 cc/min. The whole entrapment and signaling processes were carried out in less than 1 minute.

3 RESULTS AND DISCUSSION

The florescent microscope images from SW48 cancer cells which were tagged by green florescent protein (GFP) before seeding on CNT surface have been shown in figure 3. The expression of GFP (green image of the cells) is a confirmation of cell viability after entrapment on CNT structures.

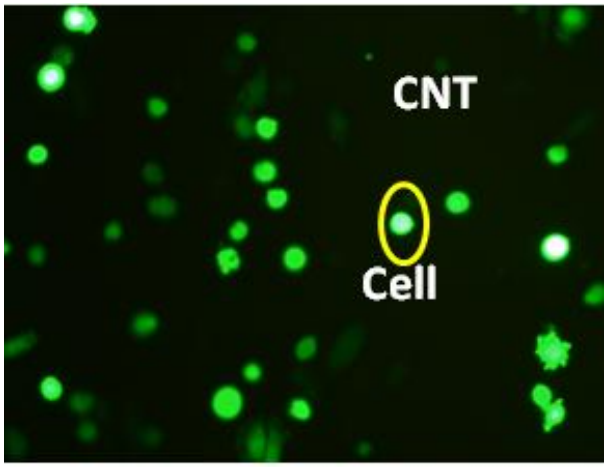

Figure 3. Florescent microscopy image from GFP tagged cancer cells entrapped on CNT arrays.

Figures 4 and 5 present the SEM images of SW48 and HT29 cancer cells entrapped on CNT covered ECIS elecrodes, respectively. CNT arrays act as both adhesive and conductive agents.

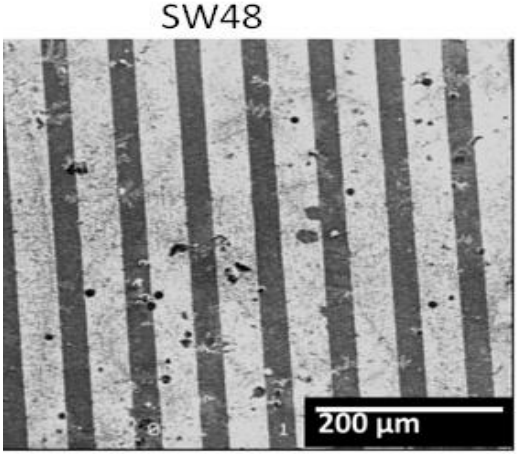

Figure 4. SEM image of SW48 metastatic cancer cells entrapped on CNT-ECIS surface.

About 40% increase in the level of entrapment of SW48 cells has been observed. The number of entrapped HT29 cells was 30% of the primary cell solution whereas this number was more than 75% for SW-48 cells. All other experimental conditions, including cell solution, speed of cell pouring and substrate temperature have remained the same. We propose that the above phenomenon occurs because of the stiffness difference of HT-29 and SW-48 cells.

As stated before, cancer cells with higher metastatic grades have more deformable structures and are generally softer than lower metastatic ones. So during interaction with partially flexible CNTs (due to their high aspect ratios), they can be trapped more significantly. Attention must be paid to the fact that higher metastatic cancer cells are less adhesive than other ones, so the surface proteins and integrins of SW48 cells might have less contribution in their higher fraction of entrapment on CNTarrays in comparison to HT29 ones. The SW48 cells which have highly deformable cytoskeletal structures [3,4] are entrapped on the CNT beams with observably higher fraction.

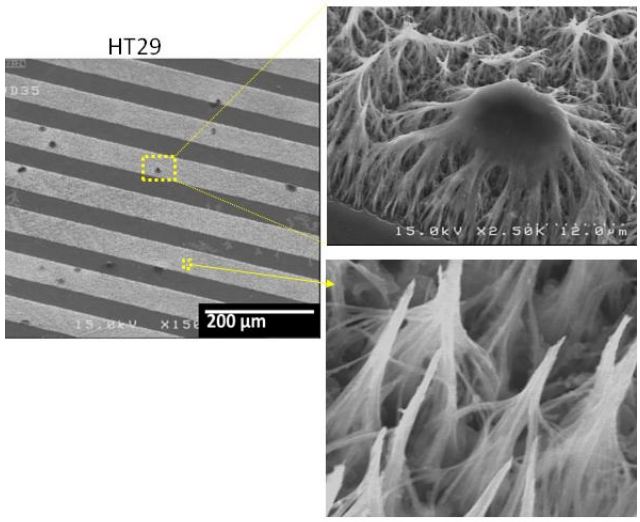

Figure 5. SEM micrograph of primary cancerous HT29 cells entrapped on CNT-ECIS. The fraction of cells entrapment are noticably lower than metastatic ones (SW48) as seen in Figure 4. The geometry of entrapped single cell (top right) as well as the agglomeration of nanotubes after flowing of cell solution (bottom right) are observable.

In addition for more elaboration about the immedeate entrapment of cancer cells on CNT arrays we take optical images from CNT surface just 5 seconds after cell entrapment process. As shown in figure 6, one can see the entrapped SW48 cells on nanotubes just after flowing process. The dark regions in this figure correspond to the places coated with nanotubes.

Figure 7 shows the electrical impedance changing diagram of two CNT-ECIS sensors exposed to HT29 and SW48 cell solutions with frequencies ranging from 100 Hz to 120 kHz. The impedance of the device shows a marked increase for the case of SW48 cells in comparison with HT29 ones. By referring to figure 4 and 5, we speculate that the variations in the fraction of cell entrapment for tese two cases could be the reason behind such a difference.

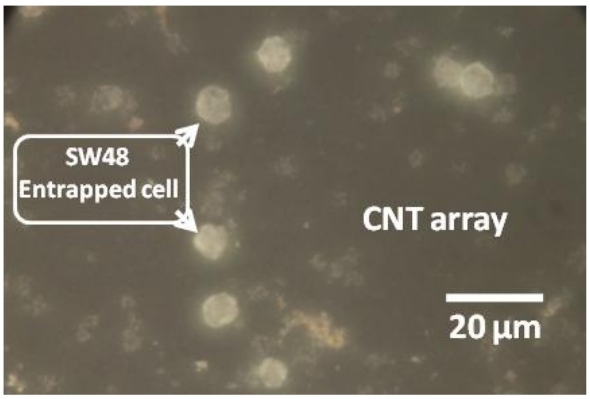

Figure 6. Optical microscopy image from entrapped SW48 cells on CNT arrays immedeatly after flowing process.

A higher fraction in the cell entrapment leads to more coverage of the sensor effective area which in turn leads to a rise in the measured impedance. The observed change in the impedance (300 Ω) has been recorded just 30 s after flowing the cells across the sensor surface. The whole entrapment and signaling processes were done in less than 1 minute. CNT-ECIS detected the metastatic cancer cells with the concentration as low as 4000 cells cm^{-2} on its surface.

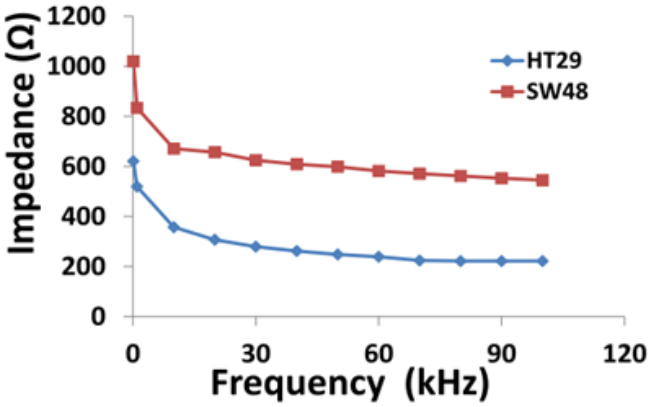

Figure 7. The impedance spectrum of a CNT-ECIS sensor measured at different probe frequencies. The sensing frequency window in this experiment has been plotted within which the difference between SW48 (metastatic grade) and HT29 (primary grade) colon cancer cells is quite observable.

The measured impedance differences between the SW48 and HT29 cells covered electrodes in such a short time is

the result of fast and selective grade dependent entrapment of cancer cells on CNT tips, as well as good electrical interaction between the cancer cell membranes and CNT conductive beams.

4 CONCLUSION

A CNT-ECIS biosensor by means of patterned vertically aligned MWCNT arrays on SiO_2/Si substrates have been fabricated for rapid and high resolution colon cancer metastasis diagnosis. Deformability differences of various metastatic grades of cancer cells resulted in their different fraction of entrapment on CNT arrays. Vertically aligned CNT arrays act as both entrapping and conductive agents in this device. It has been observed from our investigations that higher metastatic cancer cells have more fraction of entrapment and the impedance of the device was further increased after their entrapment in comparison with primary cancerous grade ones. The response time was just 30 s after cancer cell suspension flow across the ECIS electrodes surface. We speculate that strong mechanical and electrical interactions between CNT tips and cell membranes are responsible for such a rapid and marked response. Further elaboration on the mechanism of the cell entrapment as well CNT penetration through the membrane of cells is being pursued.

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