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An electrical bio-chip to transfer and detect electromagnetic stimulation on the cells based on vertically aligned carbon nanotubes

Saeed Rafizadeh-Tafti [a,b,1], Mohammad Hossein Haqiqatkhah [c], Mehrdad Saviz [d], Mohsen Janmaleki [e], Reza Faraji Dana [c], Somayeh Zanganeh [a,b], Mohammad Abdolahad [a,b,*,1]

[a] Nanoelectronic Center of Excellence, Thin Film and Nanoelectronic Lab, School of Electrical and Computer Engineering, University of Tehran, P.O. Box 14395/515, Tehran, Iran
[b] Nano Bio Electronic Devices Lab, School of Electrical and Computer Engineering, University of Tehran, P.O. Box 14395/515, Tehran, Iran
[c] Center of Excellence on Applied Electromagnetic Systems, School of Electrical & Computer Engineering, University of Tehran, P.O. Box 14395-515, North Kargar Avenue, Tehran, Iran
[d] Antenna Laboratory, School of Electrical and Computer Engineering, University of Tehran, P.O. Box 14395-515, North Kargar Avenue, Tehran, Iran
[e] Medical Nanotechnology and Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, P.O. Box 1985717443, Tehran, Iran

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ABSTRACT

A highly sensitive impedimetric bio-chip based on vertically aligned multiwall carbon nanotubes (VAMWCNTs), was applied in direct interaction with lung cancer cells. Our tool provided both inducing and monitoring the bio-electrical changes in the cells initiated by electromagnetic (EM) wave stimulation. EM wave of 940 MHz frequency with different intensities was used. Here, wave ablation might accumulate electrical charge on the tips of nanotubes penetrated into cell's membrane. The charge might induce ionic exchanges into the cell and cause alterations in electrical states of the membrane. Transmembrane electrostatic/dynamic states would be strongly affected due to such exchanges. Our novel modality was that, the cells' vitality changes caused by charge inductions were electrically detected with the same nanotubes in the architecture of electrodes for impedance measurement. The responses of the sensor were confirmed by electron and florescent microscopy images as well as biological assays. In summation, our method provided an effective biochip for enhancing and detecting external EM stimulation on the cells useful for future diagnostic and therapeutic applications, such as wave-guided drug-resistance breakage.

1. Introduction

Membrane voltage and charge states play crucial role in the regulation of living cells' functionality. As an example, a cell exposed to a strong external electric field, which experiences a build-up of opposing ion charges across its membrane, may fail to maintain cellular equilibrium and enter to apoptotic phase [1–5]. The impact of transmembrane charge accumulation on cellular vitality strongly depends on the intensity and duration of the stimulations. Controlling the intensity of such phenomena to maintain the cell in living state or transforming it to apoptosis, is a challenge which requires many biological assays such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) [6,7], flowcytometry [8] and etc.

Producing localized charge accumulation on cell membrane would cast new lights in investigating the charge-cell interactions applicable in diagnostic and therapeutic purposes. Vertically aligned carbon nanotubes (VACNTs) as nano-scale field enhancers [9] are good candidates in producing localized charge accumulation under the exposure of electromagnetic (EM) wave [10–12]. Cell's transmembrane electrical environment would be highly affected in direct interaction with wave-stimulated CNTs. In addition, nanotubes could penetrate into the cell inner parts without damaging its membrane as previously reported by our group [13,14]. Moreover, CNT based bioelectronics has been considerably developed in recent years since it exhibited anomalous physical and electrical properties [15,16].

There are many kinds of CNT-based biochips. Some of them are based on interacting with chemical agents in solutions as CNT's provide larger interacting area than smooth surfaces. Hence, they can be incorporated in many biomedical ligand-based applications [17]. Some others, have been used widely in DNA-based biochips due to nano-geometry and deflection properties of CNTs [18]. In another group of bio sensors, vertically aligned CNT's have been applied as both entrapping sites for cancer cells and signal extracting probes from the grasped cells [14,19,20]. Based on the extracted signals, several diagnostic approaches were proposed [21].

The presented research investigates a novel method to electrically monitor the effect of EM wave stimulation on CNT-penetrated lung cancer cells. Here, the CNT arrays have been grown in the architecture of helical electrodes as an impedimetric biosensor to detect the vitality of the cells exposed by EM wave. A homogenous electrode surface has

* Corresponding author.
E-mail address: m.abdolahad@ut.ac.ir (M. Abdolahad).
[1] Authors with same contributions.

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been achieved for the cells to spread on the CNTs. Then, the whole package has been stimulated by EM wave in the frequency of 940 MHz (standard frequency introduced by International Commission on Non-Ionizing Radiation Protection (ICNIRP) in 1998).

Overall, the CNT arrays have been applied as both charge based stimulators of cellular membrane by external EM wave and impedance based sensors to monitor any variations in cells' vitality. The excess charges induced on lung cancer cells attached to stimulated CNTs, would affect cellular functionality. The intensity of the EM wave has a key role in the amount of charge perturbations on CNT-cell composites which determine the destructive strength of ionic exchanges in cells' vitality. This has been investigated by impedance signals and approved by florescent and field emission scanning electron microscopies (FE-SEM) as well as biological assays such as MTT. This lab-on-chip provides an effective tool for controlling membrane permeability, transmembrane potential and levels of apoptosis caused by EM wave in future therapeutic applications. Also, the vitality assessment by impedance spectroscopy can be used as a diagnostic bio-chip.

2. Materials and methods

2.1. Sensor fabrication process

At first, Si wafer (100-Atecom Technology Co.) was cleaned by the standard Radio Corporation of America (RCA) method (RCA#1 method with $NH_4OH:H_2O_2:H_2O$ solution and volume ratio of 1:1:5), rinsed in deionized (DI) water and blow-dried by air. Thermal oxide was then grown on the wafers in wet oxide furnace at 1050 °C with the assistance of H_2O (g) for 2.5 h. Then, a Ni thin film with the thickness of about 9 nm was deposited on the Si substrate using e-beam evaporation system (Veeco Co.) at the temperature of 120 °C and with depositing rate of 0.1 Å/s. After that, the wafer was cut in to pieces desired for the sensors (25 mm^2). Then, the Ni layer was patterned in the shape of circular electrodes using standard photo-lithography process (Fig. 1-b) in which a thin layer (400 nm) of positive photoresist (Microchem-S1813) was spin coated on the surface. After illumination with Mask-Aligner System (Karl Suss Co.), the samples were chemically developed. Then, the Ni layer in undesired region was etched using Ni-etch solution ($HNO_3:H_3PO_4:CH_3COOH$, 3:3:1) and after that the photoresist was washed using acetone. Acetone, etchant and RCA components were procured from Merck Co. Finally, the samples were held in a direct-current plasma enhanced chemical vapor deposition reactor (DC-PECVD SensIran Co. Iran) to grow vertically aligned multi-walled carbon nanotubes (VAMWCNT) on desired places (Fig. 1-c). The specifics of the growth process were discussed in the next section. The width of the CNT covered micro-electrodes were 70 μm with 50 μm distance between (Fig. 1). Also, the length and diameter of nanotubes were about 2.5 μm and 55 nm respectively (Fig. 1-d, d1, and d2).

2.2. CNT growth

Using DC-PECVD the samples were annealed at 650 °C in a dynamic H_2 environment with a flow rate of 35 standard cubic centimeters per minute (SCCM) for 10–15 min. Thermally treated Ni layer was hydrogenated by plasma with a power density of 5.5 W cm^{-2} for about 5 min to obtain Ni nano-grains. The CNTs were grown on the Ni seeds in the same chamber containing a mixture of H_2 and C_2H_2 gases with flow rates of 35 and 5 SCCM at a temperature and pressure of 650 °C and 0.28 kPa, respectively. The size and geometry of nanotubes are presented in Fig. 1-d, d1 and d2. Also, the physical and electrical characteristics of CNTs were experimented by X-ray photoelectron spectroscopy (XPS), Raman spectroscopy, lifetime measurement and I-V characterization

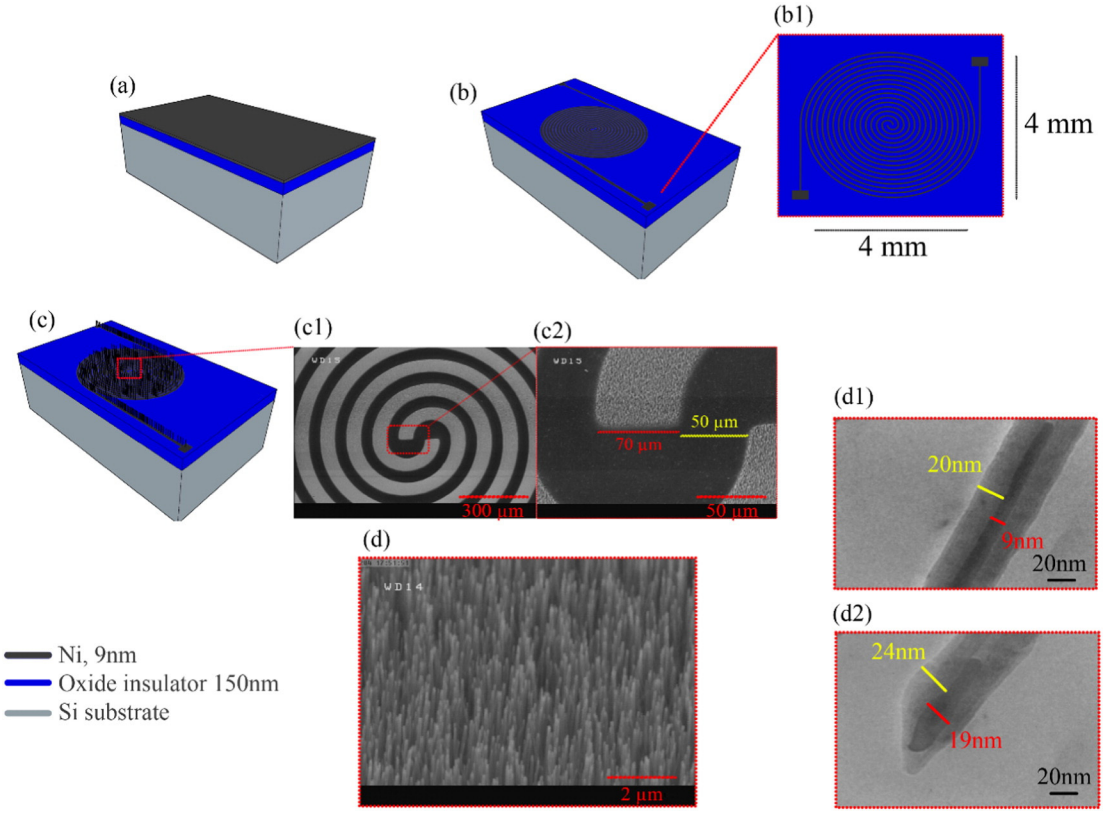

Fig. 1. Schematics from the fabrication process of VAMWCNT based biosensor. The process included: (a) Oxide growth on Si wafer followed by deposition of Ni layer (9 nm) on oxide. (b, b1) Patterning the Ni catalyst layer and formation of the helical blades. (c) CNT arrays were formed by locating the sample in DC-PECVD in C_2H_2 and H_2 ambient in 680 °C enhanced by plasma (I = 32 mA and V = 580 V). (c1, c2) Dimensions of the electrodes are shown. (d) FESEM of VAMWCNT array. (d1 & d2) TEM images of the tip and wall of a MWCNT. The thickness of the wall and trace of Ni catalyst in the nanotube are observable.