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# Carbon nanotubes based biosensor for detection of cancer antigens (CA-125) under shear flow condition

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ABSTRACT

The detection and determination of the cancer biomarkers is very crucial to diagnose at the early stages. We implement to design the biosensor which can improve the detection by reducing the process time, cost and space. The research approach mainly is focused on developing a surface modification protocol for better sensitivity under the shear flow rate conditions using carbon nanotubes (CNTs). The interdigitated electrode transducer was modified using functionalized CNTs for signal enhancement. The biosensor was integrated with PDMS microfluidic channels for controlled self-driven flow. In this article, the role and functionality of the CNTs for signal enhancement are summarized. The experimental results provide the evidence of disease-specific antigen (CA-125) detection from a micro volume of biofluid sample using the CNTs modified interdigitated electrodes under capillary flow condition.



## 1. Introduction

One of the leading gynecologic cancers is the ovarian cancer which is the cause of deaths of the women worldwide. Although ovarian cancer has a 92% 5-year survival rate at stage 1, only 15% of ovarian cancer cases are found at early stages. In most cases, they are found in the stages 3 and 4 having 5-year survival rates of 39 and 17%, which explains the high mortality rate for ovarian cancer. The potential ovarian cancer biomarker CA-125 suffers a low sensitivity at early stages and it shoots up at the later stages making it a top biomarker for the ovarian cancer. As a result, appropriate determination and quantification of the CA-125 levels is very vital for determination and monitoring the stages of cancer in patients [1–4]. There are various sensing techniques for quantification of the biomarkers like optical, piezoelectric, magnetic, thermometric and many more. These sensing techniques are both sensitive and selective but consist of expensive, bulky setup and time consuming mechanism [5,6]. Therefore, there is need for the development of a portable, inexpensive and in-vitro electrochemical based biosensor for on-site sample screening. The electrochemical technique is rapidly developing as this technique encounters all the challenges faced by other sensing methodologies. The electrochemical technique enables to directly provide information on the event of biorecognition that includes induced capacitance and resistance changes at the electrode or the substrate surfaces allowing for label-free biosensing. The electrochemical method is not only applicable to specific antigens and antibodies, but is also used for detection of enzymes, cells, microorganisms and DNA hybridization [7].

The interdigitated electrodes based electrochemical sensing techniques have drawn greater attention in recent years due to their high sensitivity within a smaller setup. Zhu et al. reported the enhancement of the sensitivity by 100 times using interdigitated nano biosensor compared to conventional biosensor for detection of reversible redox species [8]. The interdigitated electrodes based biosensor has advantages like miniaturized size, mass production, low power consumption and ease to operate [9–16]. The interdigitated electrodes based biosensor produces a capacitive signal which is used as the detection principle [17–21]. The biomarkers possess electrical charge distribution which produces unique signal under the electrical field generated by the interdigitated electrodes [8,9,23]. The biomarker interaction or the conjugation

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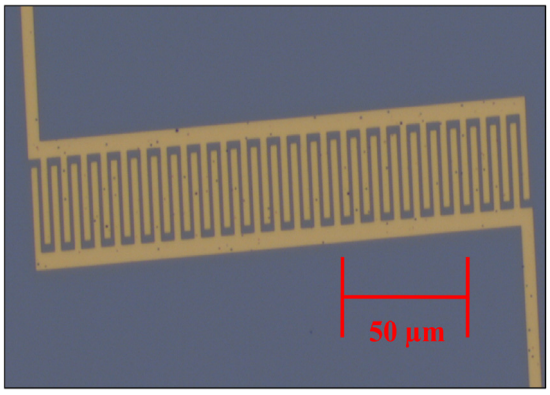


**Fig. 1.** The microscopic image of the interdigitated electrodes.

produces interference in the electric field which changes the dielectric values of the medium that gives certain capacitance values which is measured and analyzed [10,22].

The CNTs have attracted the attention in recent years due to their ultra-high specific surface area and outstanding electrical, mechanical and electrochemical properties. The CNTs in particular, due to their large length-to-diameter aspect ratios provides high surface to volume ratio which enables it to obtain high ultra fast detection of biological species even at low concentration [22,23]. The CNTs in the field of biosensing have advantages like better electron-transfer for sensing activity, higher stability and longer durability [24]. Additional to this, functionalized CNTs can be used to attach or bind any desired chemical species for enhancing the solubility and biocompatibility of the tubes [25]. Though the CNTs are very good for biosensing, but their research study and applications on the interdigitated electrodes based capacitive biosensor under shear flow rate conditions are limited. This research intends to study and understand the sensitivity of the CNTs based sensor under dynamic conditions of the analytes on top of the sensor surface. The scope of the research is to optimize and check the stability and functionality of the CNTs based sensor when the CA-125 cancer antigens passes through the integrated microfluidic channel.

## 2. Materials and methods

### 2.1. Chemicals and apparatus

Phosphate buffer saline (PBS), 1-ethyl-3-(-3- dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), Thiourea ($CH_4N_2S$) were purchased from Sigma Aldrich (USA). Carboxylic functionalized Carbon nanotubes (CNTs) were purchased from Cheap tubes. The CA-125 monoclonal antibodies and the CA-125 antigens were purchased from Meridian Life Science. The Polydimethylsiloxane (PDMS) base and curing agent were bought from Fisher Scientific. De-ionized water was used throughout the experiments.

### 2.2. Sensor fabrication

The Interdigitated electrodes are developed using photolithography technique. A thermal oxidized based silicon wafer is spin coated with a positive tone photoresist PMMA-6. The coated silicon wafer is patterned using JEOL JBX6300-FS Electron beam Lithography equipment. The patterned silicon wafer is developed with MIBK: IPA for 60 s and washed with IPA for another 60 s and then dried with Nitrogen gas. Following this step, an approximate of 15 nm thickness of Titanium is layered on top of the patterned silicon substrate as Titanium improves the adhesion of gold on Silicon. Around 95 nm thickness of Gold is deposited on top of the Titanium coated Silicon substrate by high vacuum evaporator (Kurt J. Lesker PVD-75 Evaporator). After the metal depositions, the lift-off process is performed by removing the positive tone photoresist by cleaning the substrate in acetone ultrasonic bath for 3 mins and then followed by rinsing with Isopropenol and de-ionized water in order to prevent redeposition. Fig. 1 shows the microscopic image of the patterned interdigitated electrodes.

The interdigitated electrodes were cleaned multiple times with ethanol and de-ionized water before addition of other chemical layers. The sensor surface was coated with Self Assembled Monolayer (SAM) by incubating the sensor in 50 mM solution of Thiourea overnight followed by rinsing with ethanol and de-ionized water. The formation of the SAM layer was confirmed using two point electrical probe station. The CNTs were placed on top of the modified sensor with SAM layer.

The surface of the CNTs having carboxylic functionalized group were activated using 50 mM concentration of EDC and NHS for approximately 4 h. The surface activation of the functionalized carbon nanotubes enables it to bind with the antibodies covalently [22]. Surface activation mechanism is shown in the Fig. 2.

### 2.3. Immobilization of the antibodies

The sensor surface was washed using PBS solution before the immobilization of the antibodies. Followed by this step, the immobilization of the antibodies was done by incubating the modified sensor with 0.5 μl of 7 mg/ml CA-125 antibodies in PBS for 2 h. The incubation process was done at 4 °C. The sensor surface was rinsed using PBS solution and approximately 1 μl of ethanolamine was added on top of the modified sensor for 1 h to block the non-reacted groups on the sensor surface. The sensor was then cleaned with PBS and dried with nitrogen gas.

### 2.4. Addition of the antigens

The biosensor is integrated with the PDMS microfluidic channel on top through which the antigens solutions are meant to be passed. A drop of CA-125 antigens solution (5 μL) having 560 μg/ml concentration is placed on the inlet of the hydrophilic PDMS channel. The antigens solution flows through the microfluidic channel due to capillary effect and interacts with the antibodies which are exposed on top of the sensor platform. The schematic model of the biosensor is shown in Fig. 3.

### 2.5. Measurement of capacitance

The capacitance is measured at all the stages having different layers. All the measurements were taken using two point probe station and the dielectric parameters were calculated by using Agilent 4284A Precision LCR meter. The Fig. 4 shows the probe station used in this experiment for capacitive measurements. The scanned frequency was in the range between 10 kHz and 100 kHz with a step of 10 kHz at each succession. The capacitance was calculated for (a) bare electrodes, (b) insulation of the electrodes by SAM layer; (c) after addition of carboxylic functionalized Carbon Nanotubes, (d) after immobilization of the CA-125 antibodies and (e) after conjugation of CA-125 antigens and antibodies. All the measurements were taken at 100 mV amplitude with the DC voltage at 0.5 V.

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