Emerging Biosensors for Point of Care Cancer Detection

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ABSTRACT

Cancer is a group of diseases characterized by the six hallmarks namely, self-reliance for growth signals, unresponsiveness to anti-growth signals, evasion of programmed cell death or apoptosis, sustained angiogenesis, infinite replication potential and tissue invasion and metastasis (Hanahan & Weinberg, 2000). The original set has been extended to include two more hallmarks viz. altered energy metabolism pathways and escaping immune-targeted destruction (Hanahan & Weinberg, 2011). Cancer is one of the leading causes for mortality worldwide, with approx. 9.6 million deaths reported globally in 2018 (Bray et al., 2018; Ferlay et al., 2018). The number of deaths has doubled from 1990 to 2016 in India (Dhillon et al., 2018). Its prevalence has been estimated at 18.1 million new cases globally per year in 2018 with 1.15 million new cases per year in India (Ferlay et al., 2019). This number is expected to double by 2030 (Smith & Mallath, 2019). Moreover, cancer treatment puts an enormous financial burden on the patient. The Out of Pocket (OOP) Expenditure can range anywhere from $7500 to $25000 per month for patients with multiple prescribed cancer therapies (Carrera, Kantarjian, & Blinder, 2018). Furthermore, five-year cancer survival rate ranges from as high as 98% in prostate cancer to as low as 9% for pancreatic cancer (Siegel, Miller, & Jemal, 2020).

Keywords: Pancreatic Cancer, Metastasis, Apoptosis.

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Biosensors for Point of Care Testing

Researchers nowadays, are keenly interested in detection of cancer at the very early stage using biosensors because of their high efficiency, sensitivity, accuracy and specificity. In the new era of genomic oncology, genetic biomarkers are becoming the core of cancer biomarkers; that can be identified by the specially designed biosensors.

1.1 Breast Cancer

Breast cancer is very common cancer among women, implicating 2.1 million women each year and also causes greatest number of cancers related deaths. Breast cancer is second most common cancer among all. According to WHO, in 2018 it is estimated 15% of the women died of breast cancer than of all cancer related deaths, that's approximately 627000 in number. Some important biomarkers responsible for breast cancer are BRAC1, BRAC2, CA15-3, CA125, CA27.29, CEA, NY-BR-1, ING-1, HER2 (Human epidermal growth factor receptor), ER (estrogen receptor) / PR (progesterone receptor).

In breast cancer patients, CA15-3 is an important biomarker. Clinically CA15-3 is most often used to monitor patient in cases of advanced breast cancer. It has been seen that the concentrations of CA15-3 increase by 10% in stage1 cancer, 20% in stage 2, 40% in stage 3, 75% in stage 4 of breast cancer. The relationship between CA15-3 levels and breast cancer has shown that the individuals with the values of <30U/mL, before the treatment onset had significantly higher survival times than individuals who had higher levels. For the determination of biomarkers in the breast cancer with high sensitivity and diagnostic accuracy, the biosensors with nanomaterial and their specific characteristics such as metal oxide, metal nanoparticles, nanospheres and integrated nanostructures, such as graphene or reduced graphene oxide, composed of metal oxide and multiwalled carbon nanotubes were provided.

1.1.1 The Detection of Breast Cancer Biomarkers with Microfluidic Immuno–Biochip

The early detection of Epidermal growth factor receptor 2 (EGFr2 or ErbB2) protein with label- free microfluidic immunosensor which is with femtomolar sensitivity. This sensor applies exclusively structured immune-electrode which is made up of porous hierarchical graphene foam modified with electrospun carbon-doped titanium dioxide nanofibers(nTiO2) which is working as an electrochemical electrode.

Because of the brilliant biocompatibility, the applications which are ideal for the electrochemical sensor are high reaction kinetics, anatase nTiO2 and good stability for proteins. The 3D and the porous features of graphene foam allow nTiO2 to penetrate and attach to the surface of graphene foam by physical adsorption. The graphene foams combine with the functional nTiO2 which yields in large surface area, high charge transfer resistance, and porous access to sensing surface by the analyte, which results in new possibilities for the development of electrochemical immunosensors. The antibody of ErbB2 on the graphene foam – nTiO2 composite gets covalently immobilized by the enabling of EDC-NHS chemistry. To get an immunosensor's design, the working electrode was designed to hang above the gold counter electrode in a microfluidic channel. The immunosensor went through electrochemical impedance spectroscopy and differential pulse voltammetry to quantify breast cancer biomarkers. The high sensitivities of the two methods are 0.585μA μ/M cm/2 and 43.7kΩ μ/M cm/2 in which wide concentration range of target ErbB2 antigen from 1×10 ¯15 M to 0.1×10¯6 M and from 1×10¯13 M to 0.1×10¯6 M respectively. Usage of the anti ErbB2, results in high specificity, even in the presence of ErbB3 and ErbB4. Many applications will be derived from the integration of graphene foam – nTiO2 composite into microfluidic devices, in the field of electrochemical detection of chemical and biological species.

1.1.2 Early Diagnosis by DNA Biosensors Based on AuNPs

For early detection breast cancer biomarkers by electrochemical detection of HER2, two different DNA modified gold nanoparticles and graphene oxide loaded on glassy carbon electrodes were prepared. In this electrochemical DNA biosensor, a "sandwich type" detection strategy was employed and its response was measured by Amperometric detection. For the sensitive detection of the breast cancer biomarker ErbB2 and the control marker CD24, the electrochemical signal enhancement was achieved via gold nanoparticles and graphene oxide system. The modified graphene oxide was described by using Fourier transform infrared spectroscopy, transmission electron microscopy, Raman spectroscopy, scanning electron microscope, UV-visible spectroscopy and energy-dispersive X-ray spectroscopy. Some important steps involved in modification of glassy carbon electrode with gold nanoparticles, graphene oxide and DNA probes, target and reporter probe were electrochemically characterised using electrochemical impedance spectroscopy and cyclic voltammetry.

The detection limits of 0.16nM and 0.23nM were obtained with sensitivity 378nA/nM and 219nA/nM for ErbB2 and CD24 respectively by using Amperometric detection of a horse radish peroxide label.

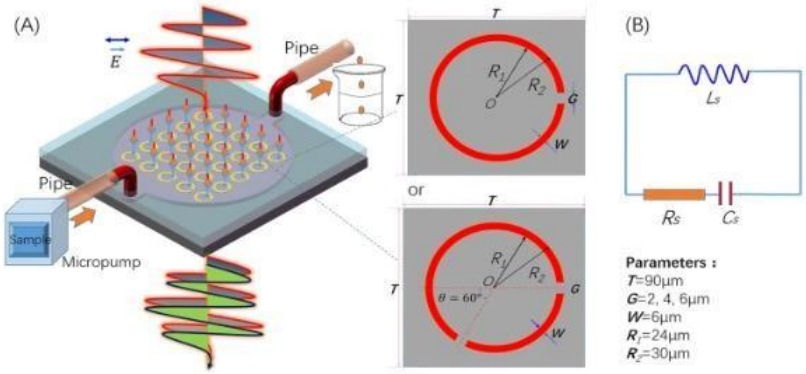
1.2 Liver Cancer

Liver cancer is considered to be the fourth most lethal cancer globally, and hepatocellular carcinoma (HCC) accounts for 75–85% of liver cancer cases. The most approachable HCC biomarkers are (1) the AFP, its isoform lens culinaris agglutinin-reactive fraction of alpha- fetoprotein (AFP-L3); and (2) des-γ-carboxy prothrombin (DCP). However, there are many other molecules that might be taken into account, including glypican 3 (GPC3), glutamine synthase (GS), heat shock protein 70 (HSP70), cytokeratin 19 (CK19), Golgi protein 73 (GP73), midkine, osteopontin (OPN), squamous cell carcinoma antigen (SCCA), Annexin A2, fibroblast growth factor 3/4 (FGF3/4), micro-RNAs (miRNAs), Long non-coding RNAs (lncRNAs), circulating tumor cells (CTCs), cell-free DNA (cfDNA), and other biomarkers.

Recently, specially designed metamaterials in the Terahertz range are being widely used as biosensors for liver cancer detection. It works on the principle of resonating frequencies, where the frequency range of Terahertz Wave (0.1THz to 10 THz) matches with the vibrational frequency of biomolecules released from biomarkers. The intensity of peak gives the estimation of the concentration of the biomarkers in the body. This technique provides the promising results, moreover, it a label-free, non-contact and non-destructive inspection technique on the specific biomolecules. To obtain the sharp peaks, the metamaterials can be designed as asymmetric structures, which can cause fano resonance; provided the structure can resonate and eliminating the substrate effect. Because of the limitation of the THz sensors to work on dry or semi-hydrated sample due to strong water absorption at THz range, Microfluidic chips are integrated with biosensors providing advantages such as low cost, less sample requirement, higher accuracy and rapid testing. The metamaterials are designed with antibody specific to antigen of biomarker in the aqueous phase.

Designing of Metamaterial Biosensor Microfluidic Chip: The metal-split-ring resonators(MSSRs) and poly-(dimethylsiloxane) microchannel form the basic structure of biosensor. The dimethylsiloxane (PDMS) form the microfluidics layer over the metamaterial to prevent the loss of THz wave transmission.

The THz wave which is electrically polarised is launched on the THz chip in integration of microfluidics chip, where the electric field parallel to MSSRs excites the inductive-capacitive resonance, which induced the current in the nearby MSSRs. This leads to electric energy and magnetic energy transfer between the inductance and capacitance. The chip detects the strong electrical field generated among the dielectric gap and provides us the signal. The metamaterial was fabricated on highly resistive silicon substrate. The designing involves 4-6 µm width(W) of split ring, inner radius and outer radius of range 24-26 µm and 28-30µm respectively. The size of gap can be changed from 2µm to 6µm based on the accuracy requirement.



(**A**) The sketch of THz metamaterials biosensor chip integrated with microfluidics; (**B**) Equivalent circuit with {RLC}s for the SRRs.

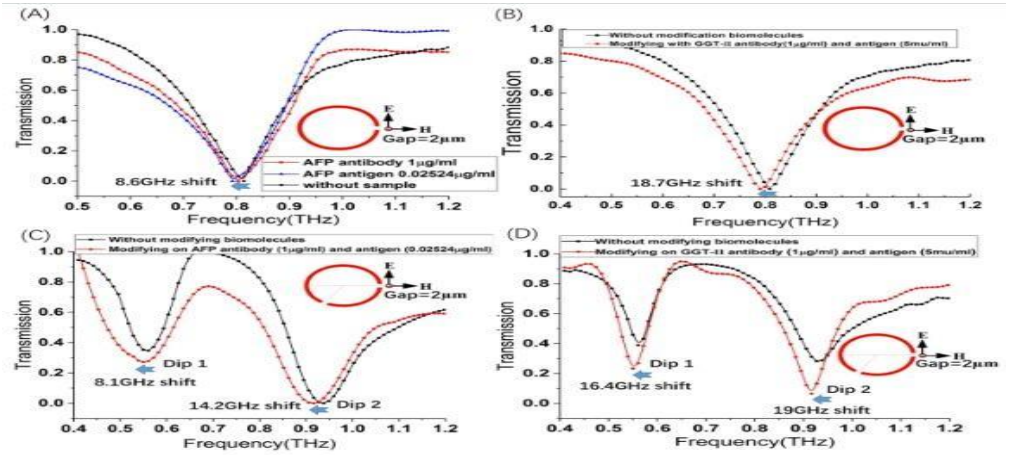
RESULTS

Liver cancer antibody Alpha fetoprotein (AFP) (1 µg/mL solution in PBS buffer) were coupled to the surface of the SRRs through chemical reaction between amidogen beside the alkaline aminophenol (Arg and Lys) of IgG and the active carboxyl; and maintaining 16 hours at 4 °C. AFP antigen or Glutamine transferase isozymes II (GGT-II) antigen with different concentrations was injected into the chamber to incubate for more than 40 min. The reflection spectra or transmission spectra were recorded through THz-TDS setup during different time. The MSSRs biosensors integrated with microfluidics is based on THz spectral dip frequency shifts, which can be changed with refractive index. A figure of merit (FOM) obtained by dividing the sensitivity by the resonance frequency line width is widelyused to characterize SRRs sensing capabilities. FOM is described as:

FOM=S/FWHM

where, FWHM repents full width at half maximum.

Larger the value of FOM, precise will be the results. The results of AFP and GGT II at 2 µm are shown in the graph since we know, lower the gap, higher will be precision. The testing results of the two AFP and GGT II show in agreement with the experimental results. However, the accuracy can be enchanced using advanced Metamaterials operating on fano resonances.



The typical tested results of liver cancer biomarker. (**A**) The results for AFP of SRRs with a 2 µm gap; (**B**) The results for GGT-II liver cancer marker testing of SRRS with a 2 µm gap; (**C**) The results for AFP of SRRs with 2 gaps (2 µm); (**D**) The results for GGT-II liver cancer marker testing of SRRS with 2 gaps (2 µm).

1.1 Lung Cancer

Lung cancer is the leading cause of cancer-related deaths, with mortality being closely associated with smoking habits (Barta, Powell, & Wisnivesky, 2019). The common symptoms include coughing up blood, weight loss, fever, fatigue and anorexia among others. The main problem associated with LC is its misdiagnosis as tuberculosis in countries where TB is prevalent. This is due to the fact that both diseases share common symptoms (Prabhakar, Shende, & Augustine, 2018). However, it can be distinguished on the basis of patient's medical history and whether the person smokes or not as TB can occur in non-smokers. Moreover, TB

Yet another biosensor which prove effective are the microcantilever sensors for liver cancer detection. In microcantilever, a cavity was designed in the free end of the cantilever for local body immobilization. This local immobilization approach reduces the adsorption induced variation of Stiffness constant, k in comparison to the whole surface adsorption.

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A novel cantilever array-based bio-sensor was batch-fabricated with IC compatible MEMS technology for precise liver cancer biomarker detection, that is piezoelectrically driven into vibration and those vibrations can be detected by Wheatstone bridge. The resonance frequency depends on two factors- stiffness constant,k and mass, m.

BIOMARKER DETECTION: For the detection of Biomarkers various concentrations of antigens were taken into account. The figure depicts the measured frequency shift of locally immobilized anti-AFP levers to the various concentrations of antigen AFP, and a linear regression was obtained with a relative uncertainty less than 5%.

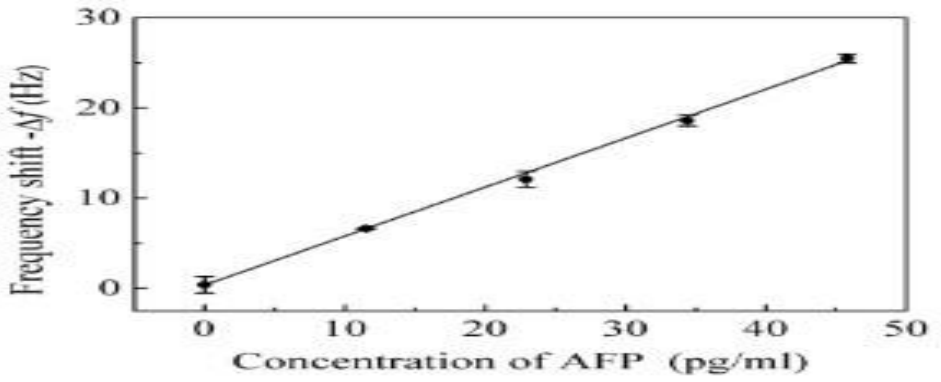

Figure 2: The relative resonance frequency shift versus AFP antigen concentration of 0-50 pg/ml.

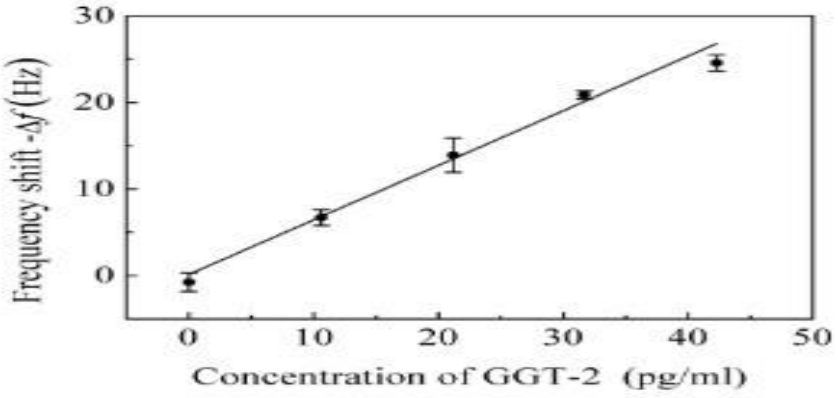

Figure 3: The relative resonance frequency shift versus GGT-2 antigen concentration of 0-50 pg/ml.

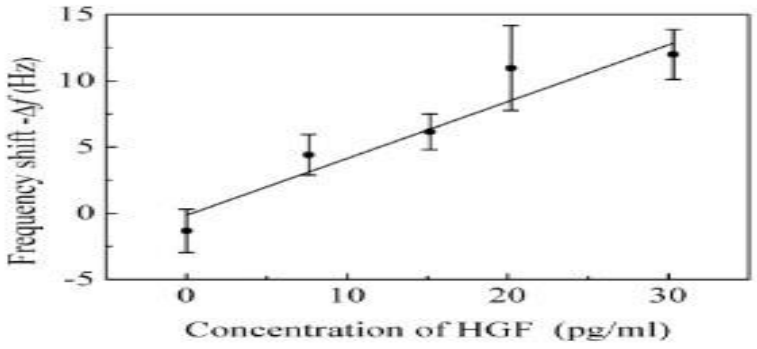

Figure 4: The relative resonance frequency shift versus HGF antigen concentration of 0 30 pg/ml.

Eliminating the effect of k can help detecting the various liver cancer biomarkers more efficiently.

can act as starting point for LC (Prabhakar et al., 2018). Lung cancer can be further classified into LCT, SCLC and NSCLC based on histology (Roointan et al., 2019).

Biomarkers and biosensors can be employed for early diagnosis of lung cancer due to their fast response time and high sensitivity. The biomarkers can be broadly classified into genetics and epigenetics biomarkers and protein-based biomarkers. Some of the common biomarkers utilized are CEA or Carcinoembryonic Antigen, NSE or Neuron-specific enolase, CYFRA21- 1, VEGF, Annexin-II and miRNAs like miR-205, miR-210, miR-708 (Roointan et al., 2019).

1.1.1 Modified Surface Plasmon Resonance based Biosensors

SPR based biosensors have received a lot of interest lately due to their high sensitivity, non- invasiveness and real time results analysis. A label-free, optical SPR works on the principle of detection of changes in refractive index when analyte molecules bind to the immobilized biorecognition element on the surface of biosensor chip (Piliarik, Vaisocherová, & Homola, 2009). These biosensors are cost-effective, portable, and easy-to-use with high specificity and accuracy. Moreover, their specificity and sensitivity can be greatly enhanced by making slight modifications.

Chiu and Yang constructed a novel SPR biosensor using carboxyl-MoS_2 nanocomposite film (instead of using just MoS_2) for detection of LC biomarker CYFRA 21-1. This modification resulted in a fifteen-fold enhancement in the binding affinity K_a and more than two-fold enhancement in SPR angle shift. The LOD of this biosensor (=0.05 pg/mL) was found to be 10^4 times more sensitive than traditional ELISA (=0.60 ng/mL) (Chiu & Yang, 2020).

Miti et al. developed a miRNA biosensor based on localized SPR. This biosensor used HCR or Hybridization Chain reaction for amplification. It works on the principle that two DNA hairpins, in the presence of target sequence, will initiate assembly. This can then be successfully detected using SPR. This biosensor was successful in detecting LC biosensor miRNA-17 and the overall procedure was completed within an hour (Miti et al., 2020). SPR based biosensors hold a lot of potential to be widely adopted as POC diagnostic devices for detection of various types of cancer biomarkers.

1.2 Prostate Cancer

Prostate cancer is the second most common cancer among men. Generally, the tumour formed is latent i.e. it does not mature and affects the patient's life. Thus, it is very important to identify whether the tumour is latent or not as early identification can help save lives. Despite the

limited specificity and high rate of overdiagnosis, Prostate Specific Antigen (PSA) is the most commonly used biomarker for the detection of prostate cancer. To counter this issue new prostate cancer antigen have been proposed. These biomarkers have higher accuracy of PSA in the management of early Prostate Cancer Antigen. Some of the commercially available Prostate cancer antigens are: PCA3 score, four-kallikrein panel and Prostate Health Index. There are also new emerging biomarkers such as PSA glycoforms, microRNAs, TMPRSS2: ERG fusion gene, androgen receptor variants, circulating tumour cells, and PTEN gene. These biomarkers can change the early management of Prostate cancer. Using these biomarkers biosensors can be designed. These biosensors help in diagnosis of cancer.

1.2.1 Bioconjugation Single use biosensor

Detection of Prostate cancer is done most commonly with the help of PSA despite its high rate of false positive tests. Apart from PSA, alpha-methylacyl-CoA racemase (AMACR) is also highly expressed biomarker in the Prostate cancer cells.

The novel bioconjugate single use Biosensor is capable of detecting both PSA and AMACR in undiluted human serum. The preparation of biosensor via bioconjugation mechanism does not take much time and can be prepared just prior to the test. This type of biosensor is accurate, single use and cost effective. It also requires very small quantity of test medium coupled with its short preparation time it becomes a very attractive choice for detection of biomarkers produced by prostate cancer cells.

Detection of AMACR is conventionally done with the help of ELISA, chemiluminescence immunoassay, radioimmunoassay, and fluoro-immunoassay. Even though these methods have fairly high accuracy the process is fairly laborious, expensive and time consuming. Thus the cost effective biosensor technology became more popular for detection of biomarkers in early stages of disease monitoring. The most common way to detect AMACR is using Square Wave Voltammetry to directly detect AMACR using aptamers immobilized on a polymer substrate.

Bioconjugation mechanism conjugate two or more molecules into a single molecule which contains properties of all the components. This method makes for zero-length linkage between the protein and the electrode element of the biosensor. Bioconjugation also has improved clinical applications as it shortens the preparation time and enhances the coverage of biosensor surface. The interaction between antigen and antibody is the biorecognition mechanism. Anti- PSA and Anti-AMACR were modified with N-succinimidyl S-acetylthioacetate(SATA) with the help of bioconjugation mechanism. This leads to a thiol linked anti-PSA or anti-AMACR

which directly links to the thin gold sheet electrode element of the biosensor during incubation. Preparation including the incubation takes about one day.

Thus, the use of bioconjugation in preparation and pulse voltammetry in I measurement results in a single use, cost effective and highly sensitive and selective biosensor for detection and management of early-stage prostate cancer with the help of PSA and AMACR biomarkers. This type of biosensor is proving it self to be a very effective diagnostic tool for the screening application of prostate cancer.

1.2.2 Graphene based biosensor

Graphene is playing an important role in the biosensor field with remarkable physical, optical, electrochemical and magnetic properties.

The use of graphene can significantly enhance the electrochemical signal of various electrodes because of various properties such as high electrochemical surface area, descent conductivity, high electron transfer rate, and a broad range of chemical functional groups on the surface of graphene. There are certain limitations in the use of graphene as it has irreversible self-agglomerations, non-specificity, less colloidal stability repeatability and poor reliability.

Based on graphene two types of biosensors can be made, firstly, graphene material based on GO, GQDs and rGO are assembled in the surface of the biosensor, mainly the electrode or transistor channel, to construct a novel biosensor interface with improved molecular receptors. Based the increased specific surface area and unique π–π orbital interactions on the interface excellent biosensor performance can be achieved. Secondly graphene materials can be applied as carriers for construction of novel nanocomposite. This is approach is very suitable for sensitive protein biomarker analysis because of its unique catalytic and chemical reactions and biosensor signal amplification.

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DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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