INTRODUCTION

Prostate cancer (PCa) has known to be the second-leading cause of cancer mortality in men (Yeager et al., 2007). It is reported that 85% of the diagnosed PCa cases occur at the age of 65+ years. This is because PCa is an asymptomatic tumour; although it occurs in men at the age of 20 to 30 years, the symptoms appear only when the disease progresses towards advanced stages (Bax et al., 2018).
This delay in diagnosis leads to the undetected progression of cancer to other body parts and hence results in the untimely death of the person. Thus, early detection of PCa is the need of the hour. Prostate-specific antigen (PSA) is the most validated biomarker for early detection and monitoring of the recurrence of PCa (Lindberg et al., 2019; Sattarahmady et al., 2017). It is a protease secreted into the seminal fluid by the prostate epithelium and is detected in the blood through various immunological assays (Carlsson & Lilja, 2019). However, even after PSA testing, confirmatory tests such as biopsy and medical imaging are required. In addition to PSA testing, methods such as nanomaterial-based fluorescent labelling, (Harma et al., 2000; Kumar & kunal Mondal et al., 2020) surface plasmon resonance (SPR; Breault-Turcot et al., 2015) immunoassay and ELISA (Liang et al., 2015) are also reported for PCa detection. These techniques suffer from limitations such as the requirement of expensive equipment, well-equipped laboratories, training staff for the use of expensive machinery and time consumption in the analysis of results. These limitations have been a motivation for us to develop electrochemical DNA-based biosensors as they are cost-effective, easily prepared, and possess higher sensitivity and selectivity towards various targets (Singhal et al., 2017). The present work focuses on designing a DNA-based nanobiosensor for detection of PCa using ZnO-Ts as transducers on paper-based electrodes.

The performance of zinc oxide (ZnO)-based nanostructures in various biological applications has been universally investigated and proven (Mishra & Adelung, 2018; Napi et al., 2019). Particularly, ZnO-based transducers are gaining popularity for the development of diagnostic biosensors (Choi et al., 2010). These transducers possess properties such as high isoelectric point (IEP), ease of fabrication, pH stability, high ionic strength, biocompatibility and other multifunctional characteristics (Wang, 2004). All these features enhance the sensitivity of the biosensors, thereby making ZnO an ideal choice for fabrication. The development of point-of-care (POC) devices demands a synergy of high selectivity, stability and most importantly, the biocompatibility of the transducers (Janotti & Van de Walle, 2009). ZnO having an isoelectric point (IEP) of 9.5 facilitates easier immobilization of the biomolecules with low IEP through electrostatic interactions (Mishra & Adelung, 2018). ZnO nanostructures can be grown using volume tunable manufacturing processes at a low cost. These qualities are desirable in the development of POC diagnostics (Ahmad et al., 2010; Hayat et al., 2014). All these properties of ZnO nanostructures motivated us to choose ZnO for the development of DNA biosensor for the detection of PCa. The semiconducting nano- and microstructures of ZnO are also well suited for DNA biosensors. This is because of their unique properties of reducing the overall potential of the systems as they possess excellent catalytic properties (Wang & Hu, 2009). This further increases the output signal strength corresponding to the analyte concentration, (Papavlassopoulos et al., 2014) or the electron transfer characteristics at the electrolyte–electrolyte interface leading to an enhanced electrical signal. The tetrapodal ZnO nano- and microstructures are rather more advantageous because their complex shape prohibits agglomeration and they also exhibit very low cytotoxicity in contrast to conventional spherical ZnO nanoparticles (Mishra et al., 2013, 2015). Additionally, the flame transport synthesis (FTS) technique is a solvent (reagent) free approach that offers large scale synthesis of these tetrapods with different dimensions, which opens the possibility to upscale the process to meet the industrial standard (Antoine et al., 2012; Mishra et al., 2011). This entails that the ZnO tetrapods which are synthesized by Mishra et al., (2011) can be manifested as an exemplary candidate for POC diagnostics due to their usefulness to be produced at a large scale with cost-effectiveness. The in vitro and in vivo studies based on these tetrapods have already shown their promising potential against herpes simplex viruses (Adelung et al., 2015; Antoine et al., 2012, 2016; Bhatt et al., 2013; Duggal et al., 2017), and however, their roles against various other pathogens need to be explored. Therefore, in the present work, we have used

![FIGURE 1](image) (a) Electrochemical paper analytical device (ePAD) showing the counter electrode (CE), working electrode (WE), and the working region/barrier made by wax, (b) Schematic of deposition/Immobilization of ZnO-Ts, PCa-PDNA (Probe DNA) and PCa-TDNA (Target DNA) on WE of ePADs
these ZnO tetrapods (ZnO-Ts) for the development of DNA biosensor for the detection of target DNA prostate cancer.

The paper electrodes are another highlight of the present work as they were constructed in-house and were economic, disposable and thus possess the potential to develop into a portable POC diagnostic device. The paper electrodes hereafter are referred as electrochemical paper analytical devices (ePADs). Utilizing and manifesting on the cost-effectiveness of ePADs is very helpful in the large scale development of effective POC Ts. These paper-based biosensors have been gaining popularity in clinical analysis (Bougadi & Kalogianni, 2020; Jahanpeyma et al., 2019; Narang et al., 2016, 2017). This is due to their numerous advantages such as versatility, cost-effectiveness, biocompatibility, eco-friendliness, ease of scaling up and easy discard. (Ballerini et al., 2012; Hu et al., 2014; Martinez et al., 2008, 2010) Therefore, the miniaturized paper electrodes were preferred over other electrodes. It is noticeable that the use of ZnO-Ts and ePADs reduced the cost of the sensor to a great extent, thereby making this work a potential which can be translated into a device.

2 | RESULTS AND DISCUSSION

The present work describes a cost-effective fabrication of a genosensor on paper-based electrodes for the electrochemical detection of PCa DNA. The interesting interaction of ZnO tetrapods and the PCa probe DNA (PDNA) is depicted with the help of HAXPES. Our results show that the developed sensor was specific towards the PCa DNA, and the schematic representation of the fabrication of this genosensor on ePADs is shown in Figure 1.

2.1 | Morphological analysis of the synthesized nanomaterials

A typical scanning electron microscopy (SEM) images of the FTS synthesized ZnO tetrapods is shown in Figure 2a. Although they are microscale, their arm surfaces exhibit lots of nanoscale features. The combined nano- and microscale features of these ZnO tetrapods make them very interesting towards different composites and applications. Figure 2b-e represents the TEM of PDNA/ZnO-Ts modified ePADs. The typical TEM image of PCa PDNA coated on ZnO-Ts in Figure 2b shows poor visibility of DNA immobilized on the tetrapodal ZnO. This implies that the electron density imparted by DNA (comprising of C, H, O and N) is extremely low compared to that imparted by ZnO tetrapods. To further investigate the process of immobilization, TEM images at a depth of 2 μm (c), 500 nm (d) and 50 nm (e) have been taken. The presence of a hazy appearance at the edge and in between the nanostructured pods of ZnO clearly establishes that PCa PDNA is present on the ZnO tetrapods, and hence, the process of immobilization is said to be deduced. Furthermore, the immobilization was confirmed with the help of Hard X-ray photoelectron spectroscopy (HAXPES) described in section 2.4.

2.2 | Optimization of the working parameters of the PCa-DNA biosensor

2.2.1 | PCa-PDNA concentration

Cyclic voltammetry was performed to optimize the PCa-PDNA concentration to ensure sufficient hybridization. The electrochemical

FIGURE 2  SEM images of flame synthesized ZnO structures at 5 μm showing the tetrapodal geometry of synthesized powder material which has been utilized for fabrication of paper-based prostate cancer sequence sensing device. (b-e) Transmission Electron Microscopy images of PCa-PDNA/ZnO-Ts, at increasing magnification showing the formation of ZnO tetrapods
response was recorded for PDNA concentration from 40 µM to 100 µM. The highest peak current for PDNA concentration of 40 µM was observed in cyclic voltammogram, as shown in Figure 3a. By increasing the concentration of PDNA above 40 µM, the signal strength decreased simultaneously. This change in the signal strength was due to decreased electron transfer rates, which is attributed to increased the resistance offered by the organic layer (PDNA) at the surface of PDNA. Therefore, due to the observed maximum response for the concentration of 40 µM of PDNA, it was chosen as the optimized concentration for subsequent experiments. The immobilization of the PCA-PDNA on ZnO-Ts was due to the electrostatic interaction between Zn²⁺ (in ZnO-Ts) and the negatively charged PDNA. This immobilization of PDNA was further confirmed by HAXPES (described in Section 2.4).

### 2.3 Hybridization time

Since hybridization time is a crucial factor in the construction of a DNA biosensor, the formation of a properly hybridized PDNA and TDNA complex leads to sensitive detection of the TDNA present in the sample. Therefore, optimization of the hybridization time was carried out (Figure 3b) by the preparation of four electrodes immobilized with PDNA to which TDNA was added along with MB. The current response was studied through cyclic voltammetric measurements at various time intervals at a scan rate of 100 mV s⁻¹. After 15 s, a decline in the current response was seen and hence was chosen as the optimum hybridization time of the sensor.

### 2.4 Scan rate

The sensor was also optimized for scan rate by recording electrochemical response for various scan rates from 10 to 100 mV/s. The best anodic and cathodic peak current were observed at 100 mV/s (Figure 4a) and were chosen to be used as the optimum scan rate for further studies. The peak current (Ia) increased linearly as a function of the square root of the scan rate (Bard, 2001; Tyagi et al., 2013) as shown in Figure 4b. The log of the peak current also showed a linear increase as a function of the log of scan rate shown in Figure 4c, exhibiting the diffusion-controlled behaviour of the electrochemical reaction (Figure 4c). The equations presenting linearity in both of these graphs were as follows:

\[
I_a = 6.34 \times 10^{-8} \sqrt{v} - 9.68 \times 10^{-7}, r^2 = 0.97
\]

\[
\log I_a = 0.65 \log v - 7.57, r^2 = 0.94
\]

### 2.5 Electrochemical response at various stages of the modified ePAD

After optimization of the PDNA concentration, hybridization time and scan rate, the electrochemical response of the sensor at various stages of modifications of ePADs was studied using cyclic voltammetry. All the stages of modification of ePADs showed well-defined oxidation and reduction peaks, as evident from Figure 5. However, the current response varied considerably at all these stages. The bare ePADs showed a lower current response of \(6.3 \times 10^{-6}\) A, whereas the ZnO-Ts-coated ePADs exhibited the higher current response of \(8.1 \times 10^{-6}\) A. This was due to the distinctive properties of ZnO-Ts that provided an increased surface to volume ratio due to higher surface area, which thereby increases the conductivity of the sensor. Since the PDNA is non-conducting in nature, hence a decreased current response of \(6.6 \times 10^{-6}\) A was seen. The hybridization of the TDNA with PDNA was further concluded by the drastic decrease in current response to \(2.8 \times 10^{-6}\) A due to the inhibition of the electron transfer of MB after it gets intercalated between the hybridized double-stranded DNA. (Erdem et al., 2000; Singhal et al., 2017, 2018).

The shift in the potential upon modifications with ZnO-Ts, PDNA and TDNA over the surface of ePAD was due to the negative charge on the DNA and the transfer of electrons during hybridization. (Singhal et al., 2017) The role of ZnO-Ts is to transfer the electrons to the PDNA.
from the cationic MB and vice versa during the process of cyclic voltammetry. However, when the surface of ZnO-Ts/ePAD was modified with probe DNA, a decline in the potential, at which this transfer of electrons takes place, occurs, thus making it easier for the MB molecule to undergo reduction. During the hybridization of the PCA target DNA with the complementary probe DNA immobilized on the ZnO-T/ePAD, a restructuration of the molecules occurs which results in higher demand for electrons, making the MB molecule to undergo reduction with less available electrons and hence causing an increase in the reduction potential of the voltammogram.

2.6 | HAXPES analysis

The interactions of ZnO-Ts with PDNA and hybridization of TDNA with PDNA were analysed by the binding energy spectra of ZnO-Ts, PDNA/ZnO-Ts and duplexed bioelectrode (TDNA-PDNA/ZnO-Ts) recorded at the synchrotron beam line. High-resolution spectrum for ZnO-Ts and PDNA/ZnO-Ts for Zn 2p and O 1s are shown in Figure 6. The binding energy spectrum of Zn (Figure 6a) reveals two characteristic binding energies at 1022.56 and 1045.76 eV corresponding to \(2p_{3/2}\) and \(2p_{1/2}\), respectively. (Wu et al., 2016) The difference of 23.2 eV between the binding energies corresponds to the presence of Zn\(^{2+}\) valence states. A comparison of the binding energy of \(2p_{1/2}\) of ZnO-Ts (Figure 6a) with that of PDNA/ZnO-Ts (Figure 6b) shows a significant change of about 0.6 eV from 1045.7 eV to 1045.1 eV. Similarly, the binding energy of \(2p_{3/2}\) also changes by ~0.4 eV on the coating of PDNA on ZnO-Ts. The shifts in the binding energy of 2p orbitals of Zn with PDNA. Figure 6c and Figure 6d shows the binding energy spectrum of O 1 s for ZnO-Ts and PDNA/ZnO-Ts, respectively. Figure 6c shows only one binding energy peak at 532.21 eV, corresponding to ZnO. (Parthasarathy et al., 2016) However, a close perusal of the O 1 s spectra of PDNA/ ZnO-Ts (Figure 6d) shows

**FIGURE 4** a, Optimization of the scan rate at which PCa-PDNA modified ePAD hybridized with the PCa-TDNA. b, Calibration plot showing the linearity of the anodic peak current as a function of the square root of the scan rate \(v\). c, Calibration plot of the log of anodic peak current as a function of the log of scan rate

**FIGURE 5** Cyclic voltammogram showing response at various stages of ePAD in 0.1 M sodium phosphate buffer (pH 7.8) containing 1 \(\mu\)M of MB
additional peaks at 530.9 eV and 539.9 eV corresponding to C = O and C-OH, respectively. This is due to the presence of the nucleotide bases: guanine and thymine in the PDNA. The most intense peak obtained at 532.18 eV represents the presence of (PO4)2− bonding, (Stypczynska et al., 2014) close to the O 1 s for O of ZnO. The binding energy of O 1 s at 532.1 eV is not altered after the attachment of PDNA to ZnO-Ts, which indicates that Zn of ZnO-Ts interacts with PDNA and O of ZnO does not interact with PDNA.

For a more detailed analysis, Figures S1–S4 exhibit the resolved photoemission spectra with reliable distinction and assignment of various components in all core-level spectra. Figure S1 represents the wide energy spectrum scans for all three stages of samples (ZnO-Ts, PDNA/ZnO-Ts and TDNA-PDNA/ZnO-Ts). Figures S2–S4 represent the Gaussian-fitted peaks for individual elements viz. Zn 2p, O 1 s and N 1 s for samples PDNA and TDNA.

2.7 | Analytical performance of the biosensor

On a close perusal of cyclic voltammograms, a decrease in the response signal of MB with increasing concentration of TDNA in the range of 1 pM to 50 μM was observed (Figure 7a).

The reason behind this decrease in the current response lies with methylene blue (MB), which was used as the hybridization indicator in the present study. MB reacts with the free guanine bases present in the ss probe DNA (PCa-PDNA). This interaction between MB and PDNA leads to an increase in the current response. However, when hybridization occurs between the PDNA and the complementary TDNA, there are no free guanine bases present as the guanine bases pairs with the cytosine of the complementary DNA (as per the standard Chagas rule of complementary base pairing in DNA). Thus, MB gets intercalated between the double-helical DNA strands, thereby leading to a decrease in the current response upon hybridization. Therefore, a decreased current response was observed upon hybridization of the TDNA. A similar analysis has been reported earlier as well. (Silvestrini et al., 2015; Tak et al., 2014) The cyclic voltammetric response was cross-confirmed by performing electrochemical impedance spectroscopy (EIS) studies at PDNA modified ePADs and by varying the TDNA concentrations from 1 pM to 50 μM (Figure 7b). The resistance charge transfer (Rct) value increased upon increasing the TDNA concentration due to the increased hindrance in the electron flow upon the hybridization of the TDNA with PDNA. Thus, the results of EIS confirmed the CV response. The fabricated sensor offered a wide linear range (1 pM to 50 μM) applicability with a sufficiently low detection limit for PCa-DNA detection. As per the visual evaluation method, (Sengul, 2016) the limit of detection (LOD) was calculated to be 1 pM.

2.8 | Analysis of sensors selectivity and stability

The sensors specificity was checked by comparing the signal response of non-complementary DNA coated on PCa-PDNA/ZnO-Ts/ePADs and that of PCa specific TDNA coated on PCa-PDNA/ZnO-Ts/ePADs. Figure 8 presents the cyclic voltammogram wherein the PDNA and the hybridized non-complementary DNA (NC-DNA) showed a nearly similar response. The hybridized PCa-TDNA, however, showed a significant decrease in response due to the hybridization between the PCa-PDNA and PCa-TDNA. Since the guanine of PDNA is no more available for interaction with MB,
it results in a decrease in the current response. This confirmed that the sensor did not respond to the non-complementary DNA, making our sensor selective towards the PCa-TDNA. The stability of ePADs was checked by periodically measuring the ability of the PCa-PDNA-coated ePADs to hybridize with the PCa-TDNA. The ePADs were stored in the refrigerator at 4°C during the stability measurements. The current response of the ePADs remained the same until the 9th day. However, a significant increase in the current response was observed after the 10th day. This could be attributed to the decreased stability of the probe DNA thus limiting its stability for 8-10 days.

2.9 | Comparative analysis

A comparative analysis of all the electrochemical DNA and a few immunosensors developed for the detection of prostate cancer have been tabulated in Table 1. All the immunosensors developed so far (Dai et al., 2019; Farzin et al., 2019; Gutiérrez-Zúñiga & Hernández-López, 2014; Karami et al., 2019; Li et al., 2005; Okuno et al., 2007; Seenivasan et al., 2017; Soukka et al., 2003; Zhao et al., 2019) for detection of PSA possess limitations such as high costs (associated with development of antibodies) and chances of cross-reactivity persist. Moreover, some sensors used complicated synthesis procedure (Gutiérrez-Zúñiga & Hernández-López, 2014; Li et al., 2005) which further increases the cost of the sensor making them highly expensive. Contrarily, DNA sensors were more specific and cost-efficient. However, the DNA biosensors developed so far for PCa DNA detection used expensive electrodes (such as GCE) and also the precursors/salts required for the synthesis of the interface (nanomaterials) were quite expensive. Our sensor when compared with these DNA sensors was inexpensive (due to the use of ePADs and ZnO-Ts) and was easy to handle. Further, the specific drawbacks associated with these sensors have been described in Table 1. The present work stands out from all the previous sensors as it is very cost-effective, disposable and portable.

A comparative analysis of all the electrochemical DNA and a few immunosensors developed for the detection of prostate cancer have been tabulated in Table 1. The drawbacks associated with these sensors have also been described. The present work stands out from all the previous sensors as it is very cost-effective, disposable and portable.

3 | CONCLUSIONS

The current work presents the development of a paper-based platform for the detection of DNA of prostate cancer. Zinc oxide tetrapods as signal enhancing interface makes the present sensor as one of its unique kind as they are simple to produce and the process can be easily upscaled to fulfil the industrial requirements. The ZnO also has an added advantage of having a high isoelectric point (9.5). This property makes it easier for low

FIGURE 7 (a) Cyclic voltammogram verifying the ability of the PCa-PDNA modified sensor to hybridize with various concentrations of PCa-TDNA ranging from 1 pM to 50 μM in 0.1 M sodium phosphate buffer (pH 7.8) containing 1 μM of Methylene Blue. (b) Nyquist plot verifying the ability of the PDNA modified sensor to hybridize with various concentrations of PCa-TDNA ranging from 1 pM to 50 μM (c) Calibration curve of TDNA/PCa-PDNA/ZnO-Ts modified electrodes as a function of the log of TDNA concentration and anodic peak current (d) Calibration curve of TDNA/PCa-PDNA/ZnO-Ts modified electrodes as a function of the log of TDNA concentration and Rct.
isoelectric point bearing DNA to be immobilized over ZnO-Ts via electrostatic force of attraction. The sensor also depicted the widest linear range so far from 1 pM to 50 μM and also the lowest detection limit of 1 pM. The use of ePADs also adds to the advantage of being economic, disposable and the ability of producing in-house. All these features clubbed together to make the present work highly suitable for the development of point-of-care devices. This method/platform after integration with suitable electronics and testing with real patient samples holds the potential towards providing a technology that could actually be used on the bed-site of the patients affected with prostate cancer.

4 | EXPERIMENTAL

4.1 | Apparatus

Cyclic voltammetry (CV) measurements were performed using a Potentiostat/ Galvanostat (Autolab, Eco chemie, The Netherlands model: AUT83785) driven by Nova 1.11 software using a two-electrode system. Electrochemical impedance spectroscopy (EIS) was done on Precision Impedance Analyzer, 6500B Series from Wayne Kerr Electronics Ltd., UK. All electrochemical experiments were performed at standard temperature (25°C). SEM was performed at Kiel University using UltraZiess microscope at 10 kV. TEM was done using FET Tecnai TF-20 high-resolution transmission electron microscope at Kusuma School of Bioscience, IIT Delhi. Hard X-ray photoelectron spectroscopy (HAXPES) at beamline BL-14, Indus-2 (Jagannath et al., 2018) which is a synchrotron radiation facility, was used to measure X-ray photoelectron spectroscopy. The excitation energy was 4.311 keV, and the system was equipped with Hemispherical analyser and a detector system (Phoibos 225, Specs make). A typical pressure of 5 × 10⁻⁹ mbar was available at the experimental facility.

4.2 | Chemicals

Monosodium dihydrogen orthophosphate (NaH₂PO₄), disodium hydrogen phosphate (Na₂HPO₄) and methylene blue (MB) were obtained from Sigma Aldrich. The solutions were made in doubly distilled water (DW), and other chemicals were of analytic reagent grade.

4.3 | Probe DNA (PDNA) and target DNA (TDNA)

The probe DNA, target DNA and non-complementary DNA primers were based on GenBank prostate cancer database design and were synthesized by GCC Biotech as lyophilized translucent films. (Tezerjani et al., 2016) The sequences of the primers were as follows:

Probe DNA (PDNA): 5’-TGA GCT CAG CAG ATG AAA GG -3’

Target DNA (TDNA): 5’-TCT TTG TAC TTT TCT CAG TGC CTT TCA TCT GCT GAG CTC A-3’

Non-complimentary DNA (nDNA): 5’- AAA GGG CAG AAG GTC TGT TCT CAC-3’

These primers were dissolved in Tris-EDTA (TE buffer) buffer of pH 8.0 to formulate the stock solutions of 100 μM. The standard procedure of dissolving 1 M NaH₂PO₄ and Na₂HPO₄ was used to prepare 0.1 M sodium phosphate buffer (SPB). Methylene blue (1 μM) was prepared in SPB, and this final buffer was used to perform further electrochemical measurements.

4.4 | Synthesis of Zinc Oxide Tetrapods (ZnO-Ts)

The nano- and microscale ZnO-Ts have been synthesized by the recently introduced flame transport synthesis (FTS) approach at Kiel University (Mishra et al., 2011, 2015). The FTS process was a solvent-free strategy that involved commercially available Zn microparticles and sacrificial polyvinyl butyral (PVB) powders. The Zn microparticles and PVB were mixed in a weight ratio of ~1:2 in a ceramic crucible, which was burned at a high temperature of ~900°C for 30 mins in normal ambient in a muffle furnace. After the growth process, the white tetrapodal ZnO powder was carefully harvested and used for desired studies.

4.5 | Fabrication of electrochemical paper analytical devices (ePADs)

Screen printing method was used for the fabrication of the paper electrodes. In this method, a screen or stencil with electrodes
| Sensor Type | Interface Used | Detection Range | Limit of Detection | Drawbacks | Ref. |
|-------------|----------------|-----------------|-------------------|-----------|-----|
| Immuno      | Europium(III)-chelate nanoparticles | 0.5 ng L⁻¹ to 5 μgL⁻¹ | 0.21 g L⁻¹ | Costly, difficult to store and use, chances of cross-reactivity persist | Soukka et al. (2003) |
| Immuno      | N-AuNPs/anti-PSMA Ab | 6 cells/40 μL | Nanomaterial synthesis required expensive salts, difficult to store and operate, persistence of cross-reactivity | Seenivasan et al. (2017) |
| Immuno      | CdTe@SiO2-Ab2 | 0.01 to 5 ng mL⁻¹ | 0.003 ng mL⁻¹ | Costly, difficult to store and use, cross-reactivity can persist | Zhao et al. (2019) |
| Immuno      | MWCNT/His-rGO/anti-PSA Ab | 10 fg mL⁻¹ to 20 ng mL⁻¹ | 2.8 fg mL⁻¹ | Interface fabrication required expenses and time, low temperature storage, cross-reactivity with other proteins in blood | Farzin et al. (2019) |
| Immuno      | Pd/NH₂-ZIF-67/anti-PSA Ab | 100 fg mL⁻¹ to 50 ng mL⁻¹ | 0.03 pg mL⁻¹ | Costly, low temperature storage difficult to store and operate | Dai et al. (2019) |
| Immuno      | AuNPs/Ab₁ | 0.01 – 20 ng mL⁻¹ | 0.009 ng mL⁻¹ | Use of antibodies made the sensor expensive with use of expensive salts as signal amplifier, low temperature storage, lower shelf life | Karami et al., (2019) |
| Immuno      | GME/MHA+EG3SH | 0 ng mL⁻¹ to 100 ng mL⁻¹ | 0.51 ng mL⁻¹ | Tedious and expensive fabrication process, difficult to store and operate, chances of cross-reactivity persist | Gutiérrez-Zúñiga & Hernández-López (2014) |
| Immuno      | n-type In₂O₃ nanowires and p-type CNT | 100 pg mL⁻¹ to 100 ng mL⁻¹ | 5 ng mL⁻¹ | Long and expensive synthesis procedure, use of expensive and high maintenance antibodies | Li et al. (2005) |
| Immuno      | SWNT/MEA | - | 0.25 ng mL⁻¹ | High costs, high maintenance conditions of antibodies, chances of cross-reactivity | Okuno et al. (2007) |
| DNA         | PCW/TGA/AuNPs/CHT/GCE | 1.0 × 10⁻¹⁴ M to 1.0 × 10⁻⁶ M | 3.1 × 10⁻¹⁵ M | Complicated synthesis procedure leading to an increased cost of sensor | Tezerjani et al., (2016) |
| DNA         | LBL Chitosan/ MWCNTs | 10 × 10⁻¹⁵ M to 10 × 10⁻⁹ M | 0.128 × 10⁻⁹ M | Time-consuming, expensive electrode fabrication with expensive synthesis procedures | Soares et al. (2019) |
| DNA         | RGO/FET | 10 × 10⁻¹⁵ M to 1 × 10⁻⁹ M | 100 × 10⁻¹⁵ M | Nanomaterial synthesis required expensive salts, difficult to store and operate | Cai et al. (2014) |
| miRNA       | SWCNT/ den-Au | 0.01 × 10⁻¹⁵ M to 1 × 10⁻⁹ M | 0.01 × 10⁻¹⁵ M | Long and expensive synthesis procedure, use of expensive and high maintenance miRNA | Sabahi et al. (2020) |
| DNA         | ZnO-Ts/ePAD | 1 pM to 50 μM | 1 pM | Inexpensive, disposable, portable, easy to handle, fast response time, easy to store, easy to prepare, stability for 8–10 days | Present work |
which was already printed on the face of the screen was first selected. The other components used in the construction process were conductive carbon ink and paper as the substrate. The conductive carbon ink was heated at a temperature of 45°C until a paste-like composition was observed. The ink was then placed on the screen/stencil and was further pressed using a squeegee leading to the printing of electrodes on the surface of the paper. This process leads to the development of printed electrodes within the paper device with a ready to use configuration. In this manner, around 200-300 electrodes were printed and dried for about an hour at room temperature. The electrodes printed contain a two-electrode system consisting of a working electrode (WE) and a counter electrode (CE). Finally, wax was used to create a hydrophobic barrier around the WE and CE hence defining the working region on the electrode. The wax was first heated, and then melted, so that it spreads horizontally leading to the formation of hydrophobic barriers. These electrodes were further used during the entire experimental process.

4.6 | Immobilization of probe DNA on ePADs (PDNA/ZnO-Ts/ePAD)

The synthesized ZnO-Ts (5 mg/mL) were drop-deposited (10 µL) on the circular area of the working electrode (WE) of the ePAD. The ePAD was then dried on a hot plate at 60°C. Hereafter, the ePAD is referred to as ZnO-Ts/ePAD. After modification with ZnO-Ts, the WE was immobilized with the probe DNA (PDNA) of the PCa. For this, 5 µL of the PDNA was dropped over the ZnO-Ts coated WE of ePAD and kept at 3°C for 2 h. Hereafter, the modified ePAD is referred to as PDNA/ZnO-Ts/ePAD. This PDNA modified electrode was used further for the detection of the target DNA.
immobilization of the PDNA was confirmed by cyclic voltammetry (CV) and XPES.

4.7 | Hybridization of the target DNA on PDNA/ZnO-Ts/ePAD nanobiosensor

The different concentrations of PCa TDNA (1 pM to 50 µM) were detected by dropping a mixture of PDNA/TDNA and MB over the working region of the sensor, and the confirmation of hybridization was performed using cyclic voltammetry. Since the hybridization time is a crucial parameter for DNA-based biosensor, several experiments for hybridization time between PDNA and TDNA were carried out. Six electrodes, each having a fixed concentration of PDNA (50 µM) immobilized on ZnO-Ts/ePADs, were prepared and incubated with complimentary TDNA (80 µM) for different time intervals of 5, 10, 15 and 20 s. The prepared ePAD is now referred to as TDNA/ZnO-Ts/ePAD.

4.8 | Procedure for specificity and stability analysis

The stability analysis of the ZnO-Ts/ePAD modified with PDNA was performed by storing PDNA/ZnO-Ts modified ePADs at 4°C. The current response in CV was measured periodically upon the addition of the PCa-TDNA. The selectivity analysis of the PDNA/ZnO-Ts modified ePAD was performed by exposing it simultaneously to complementary and non-complementary target samples. The catch here was that the concentration of the non-complementary nucleotide sample used was kept three orders higher than that of the complementary sample in order to determine the selectivity of the sensor. This was done to show that the sensor response is highly selective to the target sequence of only PCa even if the non-complementary target is present in higher quantity. The probe modified electrodes were exposed, and the current response in CV was studied.

4.9 | Sensing principle

Methylene blue (MB) is a cationic dye that is widely used as an indicator to detect and study the process of hybridization between the probe and target DNA. (Md et al., 2016) Since MB has the property to interact with the exposed and free guanine bases of the single-stranded DNA, it is used extensively as a redox probe in electrochemical sensors. The MB molecule upon interaction with guanine leads to an increase in the electrochemical response (Figure 9a). However, when the process of hybridization takes place between the probe and target DNA, the molecules of MB get inserted in between double helix and thus face stearic hindrance. This molecule of MB is now said to be intercalated, and this intercalation is confirmed by a decrease in the current response (Figure 9b) of the sensor. (Erdem et al., 2000; Singhal et al., 2017, 2018).

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