Review Article

Advancements and Future Predictions on Diagnostic Approaches towards Cervical Cancer through Nanotechnology-Based Sensors for the Detection of Human Papillomavirus

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ABSTRACT

Cervical cancer has the highest mortality rate worldwide. In the quest for reducing such a high mortality rate, advancements in diagnosis as well as treatment are being undertaken at various scales across the globe. With the recent advancements in the applications of nanotechnology, simple, rapid and inexpensive diagnostic methods for cervical cancer, i.e., human papillomavirus (HPV), especially high-risk oncogenic subtypes 16 and 18 have started to gain attention of health care practitioners. This review outlines the current applications of biosensors for the diagnosis of HPV, as compared to the conventional techniques for measuring HPV that have some limitations. The traditional methods used for cervix cancer are less sensitive, whereas nanotechnology has greatly improved the sensitivity. Due to cancer incidence and mortality growing rapidly worldwide, the prevalence and risk factors are also discussed in this review.

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Introduction

Cervical cancer growing in around 5,00,000 women each year globally has become a serious health issue. Due to no adequate detection methods available in underdeveloped or developing countries this cancer has led to serious increase in mortality and morbidity [1]. According to WHO, in 2018 cervical cancer worldwide number of new cases estimated were 570000 with 311000 approx death cases [2-4]. Thus, it is a health threat globally both for growing and industrialized countries [5, 6]. Approximately one-third of patients diagnosed with cervical cancer die because of recurrence or progression [7]. Cervical cancer develops very slowly and the transfer from precancerous to final cancer stages takes many years [8]. However, accurate early detection can vastly improve success rates of treatment [9]. There are two main categories of cervical cancer, the first being squamous cell carcinoma, which develops on the bottom of the cervix and is the most commonly occurring type. The other is adenocarcinoma, which occurs in glandular cells in the upper part of the cervix [10]. Human papillomavirus (HPV) is a censorious pace in cervical cancer development. HPV is although virtually induced in each type of cervical cancers. This virus usually infects every population, but after 1-2 years, most infections are not detectable [11]. Continuous for long duration viruses are majorly associate to the development of cervical precancer. If not treated, approximately 30% of CIN3 (Cervical intraepithelial neoplasia) leads to the progress of invasive cancer in the upcoming years to centuries [12].

Epithelial cells infected from high-risk human papillomaviruses are associated with cervical cancer [13]. A persistent infection by HPV can be developed by other factors like use of hormonal contraceptives, which can lead to cervical cancer in women [14]. According to International Agency for Research on Cancer (IARC), 12 HPV types (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) are referred to as Group 1 human carcinogens and termed as high-risk HPV types [15]. High-risk HPV types have been identified to be associated with approximately 100% of cervical cancers and also its precursors, according to various large epidemiologic studies [16]. HPV is classified high-risk and low-risk groups based on risk estimates and functional evidence of oncogenic capacity [17]. Around 70% of cervical cancer worldwide occurs due to HPV types 16 and 18, persistent infections of which are associated with most precancer and cancer cells as shown in the (Figure 1) [18, 19]. The overall pervasiveness of high-risk HPV disease is 10.4% and it very well may be as high as 36.5% in some developing nations [20]. HPV infection

determinants, cervical cancer causes include multiparity, sexual practice, use of oral contraceptives and smoking [21]. The risk of developing cervical squamous cell carcinoma is multiplied approximately 400 times following an HPV-16, and 250 times in case of HPV-18 [22].
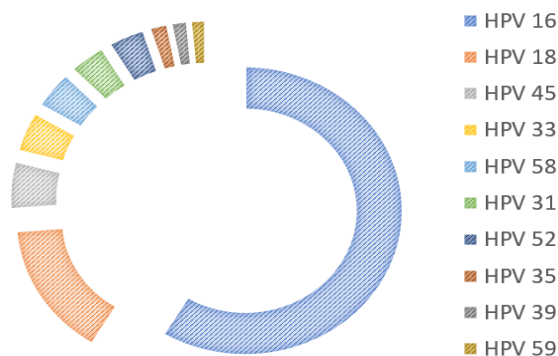

Figure 1: Types of high-risk HPV's causing cervical cancer wherein HPV 16 and HPV 18 play a major role.

While HPV infections are mostly decipherable in a few months, several persist and indicate viral oncogenes inactivating p53 and Rb, causing genomic stability, collection of somatic modification and combining of HPV into the host genome in some cases [23]. There are four different stages in the emergence of cancer from HPV: Stage I involves acquisition of HPV. The persistence of infection marks Stage II. Stage III is linked with premalignant diseases, and Stage IV is marked by the development of cancerous cells as represented in (Figure 2) [22]. The information that persevering infection with cancer-causing HPV types is the fundamental cause in setting off the advancement of cervical malignant growth has opened new doorways for primary and secondary prevention. The usage of the two strategies for anticipation can make carcinogenic happening and demise to a great extent avoidable [24].
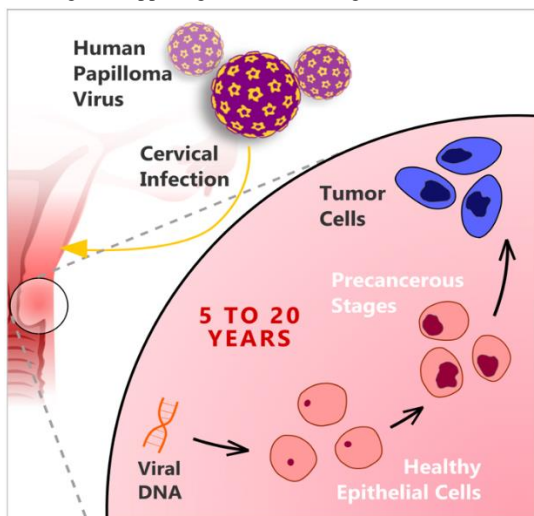

Figure 2: A normal cervix undergoes precancerous stage and finally turns to cancer detected cervix. When a normal cervix gets infected by HPV infection, it exists over a prolonged period (above one year). These infected cells gradually lead to precancerous lesion in the cervix. This spreads very quickly and undesirably harming the cervix and causing cancer.

In 90-100% cases of cervical cancer detection, HPV DNA can be detected in accordance with epidemiological controls in cervical specimens from women [25]. Also, HPV DNA is detected much more frequently in women having cervical neoplasia, when compared to women having a normal cervical cytologic detection [26]. Test of HPV DNA is an effective step in prevention of developing cervical cancer and its precursors. Hence, detection assays of HPV DNA are being clinically validated with cervical scraping specimens for the purpose of screening of cervical cancer [27]. Even to detect the human papillomavirus DNA in cervical specimens, various epidemiological studies are progressing based on DNA technology [28]. The early detection of the existence of these infections has been a significant tool to prevent a malignant growth, advancement and molecular detection method have likewise improved in recent couple of years. Conventional molecular methods are reliable and sensitive. It is still not feasible to establish these methods in public health in developing nations as they have a high cost. It is important to evolve and analyse other techniques for fast and low cost diagnosis of HPV in order to systematize the public health for aiding the early detection of cervical malignant growth [29].

Traditional techniques are insufficient in accuracy and efficiency to screen cancer at initial level. Thus, there is significant requirement of developing new methods to quickly detect and develop the infection [30]. Therefore, biosensors are considered to be safer in handling and in stability [31]. In the recent years, a research is done in the field of analytical electrochemistry leading to the fabrication of biosensors which have the potential to analytically characterize these in selectivity, reliability, sensitivity, easy fabrication and use with low cost [32]. Even nanotechnology is considered to play a crucial role in the biosensor development. With the use of nanomaterials in biosensors their sensitivity and performance has enhanced [33]. The advantages of nanotechnology-based biosensor over the conventional techniques available are shown in (Figure 3). In this review we discuss the future aspects possible for the detection of cervical cancer using nanotechnology-based DNA-enabled sensors and their advantages over various conventional methods present currently.
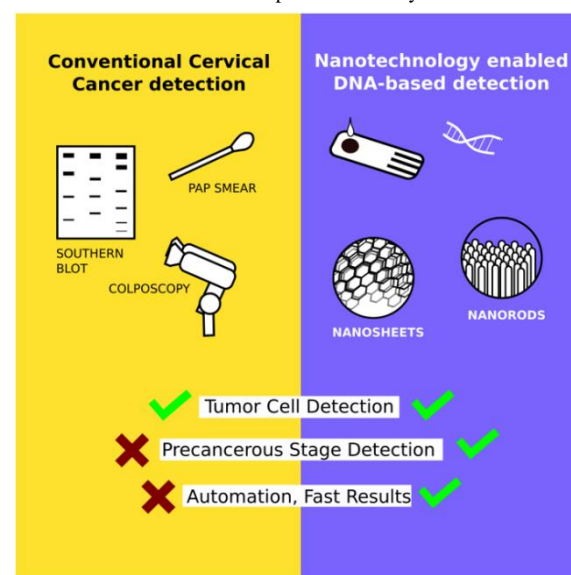

Figure 3: Benefits of using nanomaterials-based biosensors for the detection of cervical cancer in comparison to conventional methods.

Prevalence of Cervical Cancer Worldwide

The pervasiveness of this deadly infection is increasing gradually all over the globe as about 18.1 million new malignant growth cases have been known in 2018. The dominance of cervical malignancy has been altogether found in low- and middle-income nations as this cancer positions fourth for both occurrence and mortality; on the other hand, there are no viable screening methods present [34]. Cervical cancer has become the third most prevalent type of cancer. It's also the second most common in developing and underdeveloped countries [35]. The total number of cases of cervical cancer worldwide are estimated at 265,700 out of which 86.7% of cases are observed in less developed regions of the world [36]. A detailed study of cervical cancer mortality and incidence by sub-regions worldwide is shown in the (Figure 4). This cancer is mainly diagnosed in countries in Melanesia, sub-Saharan Africa, South-Eastern Asia and South America. Highest regional in mortality and incidence is sub-Saharan Africa as illustrated and with rates raised in Eastern Africa, (Malawi has become the country having the highest mortality and incidence rate), Southern Africa and Middle Africa. The rates of incidence are lower (7 to 10 times) in countries involving Northern America, New Zealand/Australia and Western Asia (Iraq and Saudi Arabia) with up to 18 times varying mortality rates [4].
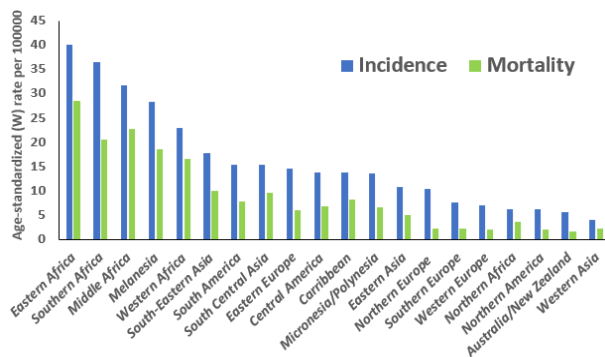

Figure 4: Cervical cancer cases by world regions for Incidence and Mortality Age-standardized Rates in 2020. Source: GLOBOCAN 2020.

Around 123,000 women in India are identified having cervical cancer each year, with 55% mortality rate. Age groups most affected by this cancer range between 55-59 years in India [37]. While in developed countries 68% to 84% undergo the Pap Smear test, only 2.6% to 6.9% of women in India receive the test, while accounting for a total of one-fourth of all cervical cancer cases worldwide [38]. It can be roughly estimated that out of 53 Indian women, 1 will be diagnosed with cervical cancer, while in a developed country, the likelihood is closer to 1 diagnosis in 100 women [39].

Risk Factors of Cervical Cancer

Over 99% cases of cervical cancer stem from HPV infection as already discussed. Factors increasing the risk and susceptibility to cervical cancer involve excessive smoking and for long period, having sexual encounters at an early age, multiple sexual partners, long-term usage of oral contraceptives, weaker than usual immunity, contraction of STDs (sexually transmitted diseases) [40]. Cervical cancer related mortality can be reduced if awareness regarding early symptoms can be increased, some of which are:

i.　　Bleeding during or after intercourse.
ii.　　Bleeding after menopause.
iii.　　Bleeding in between periods.
iv.　　Pain in lower abdominal.
v.　　Abnormal vaginal discharge [41, 42].

Diagnosis of Cervical Cancer Using Nanomaterials-Based Biosensors

As from 2001, the Food and Drug Administration (FDA) of US has accepted five testing modalities for HPV detection/screening which are Hybrid Capture 2 HPV DNA test (sensitivity from 63.6%-100%), Cervista HPV HR test (sensitivity from 92.8%-100%), Cobas 4800 HPV test (sensitivity from 71.1%-99%), the Aptima HPV assay (sensitivity from 55.3%-100%), and BD Onclarity HPV assay (sensitivity from 85.7%-100%). These assays have several limitations like cross-contamination of samples resulting in false-positive reports, does not allow particular HPV identification, are time utilizing, expensive instrumentation usage requiring skilled professionals for data analysing [43, 44]. Therefore, these techniques are less economical, restraining them into clinical settings implementation [45].

Various tests used in the detection of cervical cancer such as Pap smear or occult blood detection can be helpful in differentiating between healthy and diseased cells or tissues, but these conventional methods prove ineffective at detecting disease at early or precancerous stages [44]. The conventional methods available for HPV detection with their advantages and limitations are explained in (Table 1). Nanotechnology has emerged as a powerful diagnostic method for various cancers and is developing promising biosensor platforms for the ultrasensitive detection of HPV [46]. In the recent past, nanoparticles modified using specific nucleic acid probes have been used for the detection of HPV DNA sequences. Various biosensing platforms such as electrochemical, DNA-based, optical, and magnetic biosensors have been introduced for the detection of HPV. Some are discussed below.

Table 1: Advantages and disadvantages of various screening methods for cervical cancer.

S. No.	Screening Techniques	Advantages	Disadvantages
1.	Pap smear	• Helps find cervical cancer before it spreads when it is easier to treat. • Early detection may mean less treatment. • History of widely accepted • Well trained and professional are needed. • High specificity	• May be a false positive/false negative or even lead to death due to overdiagnosis. • Long time is needed for results to come • Systems requires time communication ensurity of test reports and follow-up of patients • Requires laboratory quality assurance • Less sensitivity

2.	HPV test	• Can detect nearly all cases of dysplasia. • More effective overall due to better detection of detecting high-grade dysplasia.	• Likely to detect changes of abnormal cell which are not cancerous cells. • Thus, leading to unnecessary treatment, including surgery of cervix.
3.	Visual screening for cervical neoplasia Visual inspection with Lugol's iodine (VILI)	• VIA and VILI are relatively simple and less costly • Results are available immediately.	• High provider variability • Lower specificity • Lack of standardization • Frequent retraining needed
4.	Cytology	• The accuracy and specificity of the test are usually over 90%.	• It is uncomfortable and painful. • Under poor conditions its sensitivity can be as low as 38%.
5.	Liquid-based cytology (LBC)	• Less time is required for samples	• Expensive instruments • Less sensitivity and specificity
6.	HPV DNA testing	• Easy and simple test • High specificity	• Results not immediately available with high cost. • Complex laboratory requirements and specimen. • Low specificity

Using MEMS technology, Polyaniline-multiwalled carbon walled nanotube film (PANI-MWCNT) were polymerized on platinum electrode arrays (IDA) for the detection of HPV. Because of the functional conductivity of PANI-MWCNT immobilized Ag peptide aptamers were used as affinity capture reagent. HPV-16-L1 was implanted as probe for the detection of HPV16 antibody (Ab) on the IDA. This technique added benefits such as reagentless and multiple detection of complex formation of antigen-antibody on well-conducting electrode interface of PANI-MWCNT [47].

For the detection of HPV16, a biomicrosystem which consists of 98 biosensors based on the monoclonal antibody (mAb) 5051 was fabricated. The mAb 5051 immobilization was conducted onto a self-assembled monolayer of 4-aminothiophenol on a polymethylmethacrylate substrate with gold nanolayer. The manufacture of this biomicrosystem is easy and also carrying out 98 tests *in situ* simultaneously [48]. An electrochemical label-free and enzyme-free graphene/Au nanorod/polythionine (G/Au NR/PT) modified glassy carbon electrode (GCE) is constructed. As graphene helps to increase surface area and electrical conductivity of the electrode wherein Au NRs enhancing the immobilization of the probe DNA and also ability for hybridization. Thus, proposing a biosensor with good selectivity and detection limit and HPV DNA can be detected from human blood using this electrochemical technique [49].

A novel "signal-on" electrochemical DNA-based sensor was designed based on a sandwich-hybridization employing pyrrolidinyl peptide nucleic acid probes (acpcPNA) which helped in the detection of HPV 16 and 18 simultaneously. The advantage of this sensor was it comprised of an unlabeled acpcPNA capture probe (P1) and an AQ-modified acpcPNA signaling probes (AQ-P2). Thus, this sample helped for the detection of the HPV16 (SiHa) and HPV18 (HeLa) positive the human cancer cell lines [50]. A prGO-MoS$_2$ modified electrode based electrochemical sensor was designed for the detection of HPV16. A composite matrix was formed which was considered to be very

fascinating feature for the modification done with aptamers for the selective sensing. The use of MoS$_2$-rGO hybrid helped to increase its structural compatibility and enhanced electrochemical properties [45]. An inkjet printing technique was used to develop a novel paper-based electrochemical biosensor which used modified electrode consisting anthraquinone-labeled pyrrolidinyl peptide nucleic acid (acpcPNA) probe (AQ-PNA) and graphene-polyaniline (G-PANI) for the detection of HPV16 DNA at the initial stage by measuring Electrochemical Impedance Spectroscopy (EIS). From this method a new inexpensive and disposable device was developed [51].

A label-free calorimetric assay was developed which demonstrated interaction of Sso7d [a double-stranded DNA (dsDNA) binding protein] and Cys-Sso7d with DNA and Au NPs for the fast and specific detection of HPV types of 16 and 18 genes. In this DNA analysis the label-free Cys-Sso7d/Au NP probe as compared to other nanoparticle-based biosensors does not need modified complementary probe. Thus, providing great speed, sensitivity and specificity with cost effectiveness, this assay helped in the detection of high-risk HPV and to improve the accuracy of cervical cancer screening [52]. EIS of the GC-GNS modified electrode was compared with GE. The nanostructured electrode based on the gold sheets has the potential in the biosensing applications as they can increase the active area and surface roughness of the modified electrode. This biosensor also showed good selectivity with one base pair mismatch and outstanding detection limit showing great advancement in the diagnosis and clinical analysis of HPV DNA [53].

A quality sensor for fast identification of the HPV 16 which is related with the presence of cervical malignant growth was fabricated. The detection is on voltammetric determination of HPV 16 DNA by utilizing interdigitated electrodes with titanium dioxide nanoparticles modification. This gene-based sensor has a detection limit of ~ 0.1 fM [54]. An impedimetric HPV DNA-based biosensor dependent on gold nanotubes (AuNTs) in label free detection was developed. The AuNTs improved nanoporous polycarbonate (AuNTs-PC) template as biosensor

platform modified by electrodeposition technique. The biosensor showed the linear range of 0.01 pM to 1 μM with a detection limit of 1 fM [55]. Amplified fluorescent sensor ultra-modified with ultrathin 2D MXene Ti_3C_2 nanosheets (Ti_3C_2 NSs) for HPV-18 detection shows a low limit of detection of 100 pM with increase specificity. Besides, the created DNA sensor can be utilized to decide PCR enhanced HPV-18 from cervical scratches tests. It features ultrathin Ti_3C_2NSs as a likely possibility for development of fluorescence DNA biosensors with incredible performance [56].

In this study an electrochemical biosensor DNA-based, for the initial detection of HPV-18 using nanocomposite of reduced graphene oxide (rGO) and multiwalled carbon nanotubes (MWCNTs) electrodeposited on screen-printed carbon electrode and modified with Au nanoparticles (AuNPs) measured using differential pulse voltammetry technique [57]. Table 2 summarizes the above-mentioned biosensors with their specific oncogenes to detection of HPV with very high sensitivity.

Table 2: Summarizes the nanoparticles modified platforms with specific oncogenes to detect HPV with their high sensitivity.

S.No.	Gene Specific for HPV	Nanocomposite and electrodes used	Detection limit	Sensitivity	Linear Range	Reference
1.	HPV16	platinum surface/polyaniline-multiwalled carbon nanotube composite	490pM	1.75 ± 0.2 μA nM^{-1}	10nM-50nM	[47]
2.	HPV16	4-aminothiophenol, on a polymethylmethacrylate substrate with a gold nanolayer	NR	NR	NR	[48]
3.	HPV16 and HPV18	graphene/Au nanorod/polythionine	4.03×10^{-14} mol/L	NR	1.0×10^{-13}	[49]
4.	HPV16 and HPV18	acpcPNA probe/gold deposited SPCE	0.1-1000nM	NR	0.5nM-100nM	[50]
5.	HPV 16	porous reduced graphene oxide/Molybdenum disulfide	1.75pM	2.35-0.76 (ng mL^{-1})	3.5pM-35.3pM	[45]
6.	HPV16	graphene-polyaniline (G-PANI)	2.3nM	NR	10nM-200nM	[51]
7.	HPV 16 and HPV 18	CysSso7d/Au NP (gold nanoparticles)	1.00ng mL^{-1}	85.7%/100.0% and 85.7%/91.7%	to 1.00×10^3 ng mL^{-1}	[52]
8.	HPV16	gold nanosheets	0.15pM	NR	1pM-1μM	[53]
9.	HPV16	titanium dioxide nanoparticles	~ 0.1fM	$40x 10^{-4}$ (AM^{-1})	10fM-10μM	[54]
10	HPV16	AuNTs-PC	1fM	0.01pM	0.01pM to 1μM	[55]
11.	HPV18	Ti_3C_2 nanosheets	100pM	NR	1.0nM and 50nM	[56]
12.	HPV18	rGO and MWCNTs	0.05fM	NR	0.01fM to 0.01nM	[57]

*NR= Not reported

Conclusion

In the coming future, with the help of biosensors, HPV can be detected at an early stage with greater precision. Although a number of reliable techniques are available for the detection of cervical cancer still for the screening of cervical cancer, but they have some major drawbacks for instance, expensive, long assessment duration, skilled professionals to handle the instrument. Cytologic assessments for this cancer lack in specificity and sensitivity, providing false positive results many a times. Hence, there is a rapid need to develop a simple and accurate detection method for this cancer. Thus, development of biosensor for the diagnosis of HPV has explored new approaches leading to overcome these limitations.

Nanotechnology has been widely concentrated to improve the administration of cervical malignancy and utilized for the determination of HPV to build affectability. Furthermore, the use of various nanomaterials (Quantum dots, carbon nanotubes, magnetic nanoparticles, etc.) has added significant results like low limit of detection-based electrochemical-based DNA biosensors. In addition, provides POCT of HPV in a pocket friendly way and making it convenient to use. Therefore, convenient, accurate, and reliable methods with easy and rapid detection of cervical cancer are emerging. In the upcoming years, biosensors with nanomaterials can be a great assist in the detection of HPV. This review summarizes the recent developments in the detection of cervical cancer in biosensors because of some loopholes existing in the present screening technologies available. The

worldwide prevalence of cervical cancer is also discussed making it the fourth most mortality-causing cancers and leading in Asia.

Acknowledgement

Conflicts of Interest

None.

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