

Review Article

Recent Progress in Electrochemical Detection of Human Papillomavirus (HPV) via Graphene-Based Nanosensors

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In the present study, applications of advanced nanomaterials such as graphene oxide (GO), reduced graphene oxide (rGO), and graphene quantum dots (GQDs) as leading and potential candidates toward treating the human papillomaviruses (HPVs) were investigated. In this matter, a comprehensive summary of the formation of GO, rGO, and GQDs will be reported in detail. Continuous efforts have been exerted to develop high-performance biosensors and electrochemical detection systems toward accurate detection of HPV using novel routes. This review paper showed that HPVs appeared in different types and species. These HPVs have many complications on humans, and thus, there are different ways that a person could be exposed to them. Meanwhile, several routes of transmitting the HPVs to human cells are discussed too. Indeed, sexually transmitted diseases could be easily infected by HPVs, although the human immune system can also be boosted to treat this dangerous virus effectively. Some of the HPVs such as HPV-16 and HPV-18 are so dangerous, and HPV DNA could be detected in several vertical cancers. Herein, we reported and summarized some recent progress in electrochemical detection of HPVs using graphene-based nanosensors.

1. Introduction

Human papillomaviruses are viruses with double-stranded and nonenveloped DNA. There are more than 100 types of

this virus, in which more than 40 types of them are transferred through sexual contact, and they can leave disservice effects on the mouth, genitals, or throat. Centers for Disease Control and Prevention (CDC) has specified that HPV is

the most usual type of sexually transmitted infection. There is also a direct link between this virus and creating cervical cancer [1]. HPVs, due to the risk of cancer they caused to creating, are divided into two groups, including high risk and low risk of this virus. The low-risk HPVs, HPV-6 and HPV-11, induce external genital warts, including frequent respiratory papillomatosis and little changes in cervical cells. Through the high-risk HPVs, HPV-16 and HPV-18 are the most dangerous types of HPVs that lead to cervical and anal cancers [2, 3]. Anal cancers caused by HPV are not very common cancer than cervical cancers, but the prevalence of anal HPV infection also occurs in persons with a background of cervical cancer [4]. Methods previously used for the detection of HPV included visual inspection by using acetic acid and Pap smear. Nevertheless, these conventional methods contain several obvious disadvantages: weak rate of specialization and poor sensitivity [2].

In recent years, electrochemical methods for the determination of HPVs have been applied and utilized in many countries. This method includes an electrochemical impedance biosensor array by using gold nanotubes, gold nanosheets, or single-walled carbon nanotubes (SWCNTs) for HPV DNA targets, a voltammetric biosensor for HPV-16 L1 protein by utilizing porous rGO, an amperometric biosensor for detection of HPV oncogene targets using carbon nanoions, and a voltammetric biosensor for HPV-16 antibodies using polyaniline-multiwalled carbon nanotubes (PANI-MWCNTs) [5–8]. Among all of the available methods, DNA hybridization biosensors or genosensors have been widely used for the detection of infectious diseases such as HPV [1]. DNA biosensors showed some advantages including fast detection of disease, ease of work, affordability, and sensitivity of the device. These devices could detect DNA targets from several nontarget species [2]. The basis work of these sensors is immobilization procedures, label-free strategies, and exploitation of redox probes. Nanoplatfoms based on carbon, chiefly graphene and its derivatives, are appropriate structures because of owing characteristics, including mechanical strength and thermal conductivities, and owning the large surface area for use in electrochemical measurements biosensors or genosensors [1]. Graphene nanosheets have a high thermal conductivity of about $5000 \text{ W}\cdot\text{m}\cdot\text{K}^{-1}$, a mechanical strength of about 1.1 TPa, and a large specific surface area of about $2630 \text{ m}^2\cdot\text{g}^{-1}$ [9].

Recently, in the literature, a large number of articles were published using GR derivatives for use in biosensors and medical fields [1, 10]. Huang et al. used ultrasensitive electrochemical DNA biosensors by using GR, Au nanorod (Au NR), and polythionine (PT) for HPV DNA detection. Electrochemical impedance spectroscopy and differential pulse voltammetry were used in biosensors. A glassy carbon electrode is used as an electrode. In this method, HPV DNA could be detected in human serum. Sensitivity was enhanced via the converse of the excellent electric conductivity of graphene/Au nanorod/polythionine and self-assembly of DNA nanostructures. The DNA biosensor showed excellent function for the detection of HPV DNA. The detection limit was $4.03 \times 10^{-14} \text{ mol/L}$. Due to the existence of interference in the sample matrix during the hybridization process

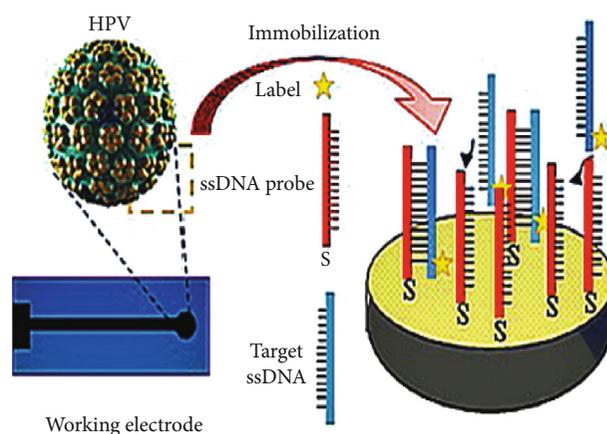


FIGURE 1: The function of the HPV-18 DNA biosensor based on the indicator intercalation detection, DNA hybridization, and immobilization of the ssDNA probe [2].

between DNA targets and capture probes, the differential pulse voltammetry signal was decreased quantitatively in the serum [11].

Farzin et al. used an ultrasensitive electrochemical genosensor by using multiwalled carbon nanotubes by the amine-ionic liquid-functionalized reduced graphene oxide (MWCNTs/ NH_2 -IL-rGO) nanoplatfom for HPV detection. In this work, they used MWCNTs/ NH_2 -IL-rGO-ssDNA as the interface of sense. Anthraquinone-2-sulfonic acid sodium salt monohydrate was used as a redox probe for the ssDNA hybridization. HPV-16 DNA showed a considerable increase in the response of genosensors. Strong interactions that exist between immobilized probe chains and HPV-16 DNA target strand chains led to differential pulse voltammetry (DPV) measurements that could detect the HPV-16 gene. Genosensors could detect very low amounts of HPV-16 DNA concentration. The detection limit was about 1.3 nM (at 3σ) [1].

Figure 1 shows a schematic illustration of HPV DNA biosensor function according to immobilization and hybridization. The function of the HPV DNA target biosensor is based on the ssDNA probe immobilization and the DNA hybridization. According to this figure, for immobilizing the HPV-18 DNA probe, the electrode was placed in the HPV-18 DNA solution. Interaction between negative charges of the phosphate backbone of the DNA probes and positive charges of amine groups of L-cysteine, used for modification of the electrode surface, caused the development of DNA microarrays. For hybridization of the genomic DNA by using the HPV-18 DNA probe on the electrode, the DNA was heated in a water bath to achieve ssDNA.

According to Europe's patchwork of policies, it reports that HPVs cause death from cervical cancer. Forty-six countries must take precautionary measures for the prevention of deaths that HPVs cause. Participating in national cervical cancer screening and HPV vaccination programs and presenting online information to outstanding political volition are precautionary measures for preventing cervical cancer. Azerbaijan and Belarus are the countries that report more HPV cases [12]. According to reports received and articles published, the necessity of the importance of research and

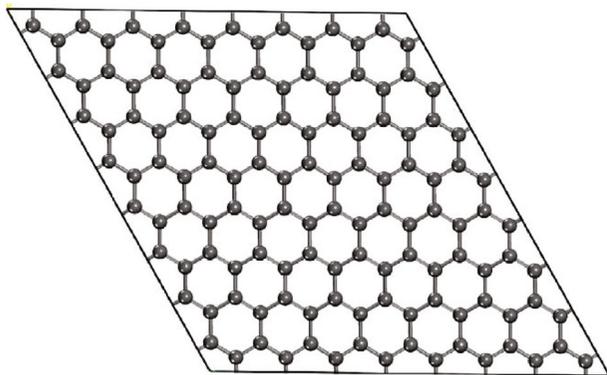


FIGURE 2: Hexagonal arrangement of carbon atoms in graphene sheet [16].

investigation in the field of preventative ways of getting infected with this virus is specified. Also, the ways of diagnosis and treatment of HPVs are significant issues.

In this review, we summarize different routes for synthesizing the GR derivatives such as GO, QGDs, and rGO. These novel materials could be used in biosensors and genosensors for HPV detection. Also, the properties, advantages, and applications of these materials are investigated too. Afterward, papillomaviruses that include HPV, the way of transmitting papillomavirus, different methods of detection, and the role of different E proteins of viruses are investigated.

2. GR for Detection of HPV

GR could be achieved from graphite and is in the categories of the carbon family [13]. Graphene and its derivatives are applicable for biology and medicine because of their exclusive physicochemical properties [14]. Two-dimensional sheet graphene of carbon atoms is located in a densely packed area. As shown in Figure 2, this density is like a home hexagon arrangement [15].

Graphene, due to owning some characteristics, has very many applications. In Table 1, the properties of graphene including characteristics and applications of graphene are shown. Graphene has excellent technical properties including great flexibility, nontoxicity, high amount of density, current transfer, and hydrophobicity.

The hydrophilic properties of graphene prevent its usage in medicine, but GO is hydrophilic due to its oxygen functional groups and is widely used in biomedical applications. Graphene, due to owning electrical conductivity and high potential for electron transfer, has the ability to be used in electrochemical sensors and biosensors. The high amount of density in graphene causes high antibody immobilization, good transfer of electrons, and low detection limits, which increases the sensitivity and repeatability [17].

Graphene is a hydrophobic substance, and the structural modification that takes place on its functional groups to make it hydrophilic and to find biomedical application consists of covalent and noncovalent functionalization. Noncovalent functionalization also increases the graphene properties such as biocompatibility, reactivity, and sensing. In graphene deriv-

TABLE 1: Characteristics and applications of GR [14, 15].

Characteristics	(i) Better mechanical flexibility
	(ii) Great thermal, mechanical, or chemical consistency
	(iii) Environmentally friendly
	(iv) High amount of current
	(v) High amount of chemical inertness
	(vi) High density
	(vii) Very high value of hydrophobicity
	(viii) High optical transmittance
	(ix) Catalytic, electronic, biological, mechanical, thermal, and optical characteristics
	(x) Biocompatibility
	(xi) Quick functionalization
Applications	(i) Usable in genes and little drug delivery of molecules
	(ii) Biofunctionalization of protein
	(iii) Anticancer therapy
	(iv) Biomolecular recognition
	(v) Bioassays
	(vi) Molecular medicine
	(vii) Tissue engineering
	(viii) Biosensing
	(ix) Bone repair of organ regeneration
	(x) Solar cells
	(xi) Supercapacitors

atives such as GO, the formation of hydrogen bonds between GO and water molecules causes the creation of the stable suspension of GO for biomedical applications.

Flexibility properties strengthen the structure and improve adhesion, and this property makes it useful in tissue engineering [18]. Graphene via these characteristics could be utilized in medical and pharmaceutical applications, such as genes, drugs, proteins, cancer therapy, molecules, tissue engineering, and sensors.

2.1. Graphene Oxide. GOs are two-dimensional graphene plates with large surface areas that have oxygen groups on their plates. GO is more biocompatible than graphene and can be used in medical applications because of the presence of oxygen functionalities on its structure such as carboxyl, hydroxyl, and epoxy [19, 20]. GO can interact with materials and can be used in various other applications such as catalysts, bioimaging, drug delivery, biosensors, electronic devices, energy storage devices, and biomedical applications [19, 21]. Figure 3 shows the formation of GO from graphene [22].

GO is produced due to the chemical peeling of graphite layers and is followed by oxidizing of the layers. In other words, the same atoms that exist in carbon layers react with materials that have oxygen functional groups and produce GO [19]. In general, the conversion of graphene to graphene oxide occurs by using some reducing agents, and there are several synthesis methods to produce graphene oxides such as the chemical method, electrochemical method, and microbial method. In chemical methods, chemical reagents that contain chlorate, permanganate, chromate, ferrate, ozone, and peroxide are used. In electrochemical methods, chemical reagents that contain ammonium, chloride, hydroxide,



FIGURE 3: Formation of GO from graphene by oxidation.

TABLE 2: Methods and reducing agents for the synthesis of FLG.

Method	Reducer material	Ref.
Chemical reduction	(1) Hydrazine hydrate	[24]
	(2) Hydrogen bromide	
	(3) p-Toluenesulfonic acid	
	(4) Sodium borohydride	
Bioreduction procedures	(1) Microorganisms	[24]
	(2) Plants	
	(3) <i>Ficus carica</i>	
	(4) Vitamin C	
	(5) Gallic acid	
	(6) Tea solution	
	(7) Bacteria	
	(8) Alanine	
	(9) Black raisins (Zante currants)	

phosphate, nitrate, perchlorate, sulfate, pseudomonas, permanganate, and quartz are used [23]. Different methods for the synthesis of GO are described below.

2.1.1. Green Method for the Synthesis of GO. Ansari et al. used Zante currants to form few-layer graphene (FLG). Zante currants, because of owning some features like the richness in carbohydrates, glucose, fructose, anthocyanins, and polyphenolic, had the great potential to turn GO into FLG. For this aim, a mixture of HNO_3 , graphite flakes, and H_2SO_4 was placed in an ice bath. Then, KMnO_4 was added slowly to the solution. Then, it was exposed to heating in the water bath. Then, an increase and decrease in temperature occurred, respectively. In the following, H_2O_2 was added, and the final solution was centrifuged. The solution was washed with water and placed under vacuum, and GO was produced. Then, the extracted solution of Zante currants was used to reduce graphene oxide and produce FLG. In this way, the GO solution became sonicated. Ammonia solution was added to the extracted Zante currant solution, and the temperature of the solution was increased by a water bath. Then, the solution was washed with water and ether and finally formed FLG [24]. In addition to the method mentioned above, there are different methods for the synthesis of FLG that are given in Table 2.

2.1.2. Hummers' Method for the Synthesis of GO. Galande et al. obtained GO by oxidized graphite powder by using the modified Hummers' method. At first, graphite powder was heated and graphite became laminated. Afterward, using

the modified Hummers' method, GO was dissolved in deionized water and then ultrasonicated. Then, the pH of the solution changed by adding acid and alkaline solution. Indeed, diluted H_2SO_4 and KOH were added to the solution. This showed that the structure of GO was related to change in pH [8]. Chen et al. produced GO with Hummers' method. At first, NaNO_3 and H_2SO_4 were added to graphite and they were mixed in an ice bath. Then, KMnO_4 was added to the solution in an ice bath under stirring. Then, the solution was transferred to a water bath that its temperature was higher than before. Afterward, sonicated water was added to form a paste and stirred under heating. Then, pure water and H_2O_2 were added and the color of the solution changed. To remove excess materials, the solution was washed and filtered. The achieved cake was mixed with water and sonicated. Also, the solution was centrifuged several times. GO was obtained from the sediment and dried in air. GO solution was achieved by dried GO in water that was sonicated in ultrasonication [9].

2.1.3. Tour Method for the Synthesis of GO. Habte and Ayele synthesized GO using the Tour method by surveying different parameters on synthesis conditions. Graphite powder was oxidized with a mixture of potassium permanganate and $\text{H}_3\text{PO}_4/\text{H}_2\text{SO}_4$. Parameters such as the amount of concentration, reaction temperature, and reaction time were varied to study the degree of oxidation of graphite to graphene oxide. Results showed that at constant concentration and time, the rate of oxidation increased by increasing temperature.

It is found that as the time rate increased, the oxidation capacity also increased. But as the reaction time increased, it became overoxidized. Also, the oxidation capacity of graphite oxide increased as the amount of the oxidizing agent increased due to the nature of potassium permanganate that is a well-known strong oxidizing agent [25]. Sali et al. investigated the effect of the GO synthesis method on the performance and properties of polysulfone-GO mixed matrix membranes. GO was prepared via the Tour method by mixing graphite with $\text{H}_3\text{PO}_4/\text{H}_2\text{SO}_4$. Then, KMnO_4 was added to the prepared solution. The reaction's temperature was kept constant for three days. The mixture was centrifuged, and at last, graphite oxide particles were washed with UP water and centrifuged. GO which was synthesized with the Tour method produced GO with more efficiency in enhancing the mechanical properties of the PSF membrane and with a larger sheet size [26].

2.2. Graphene Quantum Dots. Graphene quantum dots (GQDs) or graphene oxide quantum dots (GOQDs) are zero-dimensional carbon nanomaterials, such as graphene and graphene oxide nanomaterials. The methods used for the synthesis of GQDs or GOQDs contain top-down and bottom-up methods [15, 27]. Top-down methods to achieve GQDs/GOQDs contain the hydrothermal method, acidic oxidation, oxidative cleavage, solvothermal method, microwave-assisted or ultrasonic-assisted process, and electrochemical oxidation. These methods cut materials such as graphene sheets, graphene electrodes, graphite powder, graphene oxide, carbon fibers, and black carbon into quantum size [15, 27, 28]. Bottom-up methods for producing GQDs/GOQDs contain the hydrothermal method, microwave-assisted hydrothermal method, citric acid carbonization, metal-catalyzed method, and soft template method [15, 27]. GQDs are the small size of carbon nanomaterials with a mean average size of less than 100 nm. GQDs have many applications because of their characteristics such as the low amount of their toxicity to the environment, inexpensive cost of their final preparation, good solubility of GQDs in solvents, and some excellent properties such as their excellent technical properties and owing oxygen functional groups [28]. Different methods for the synthesis of GQDs are described below.

2.2.1. Synthesis of Graphene Quantum Dots by the Electrochemical Method. Ahirwar et al. used graphite as an electrode in the electrochemical method to prepare GQDs/GOQDs. By changing the composition of the electrolyte, different GQDs/GOQDs were produced. Performing a reaction in the stoichiometric state led to the production of GQD3, and changes in the concentration of alkaline hydroxide resulted in the production of GQD1, GQD2, and GQD4 [15]. Li et al. used the electrochemical approach for nitrogen-doped GQDs with oxygen groups to improve device applications. To prepare nitrogen-doped GQDs, the solution of tetrabutylammonium and acetonitrile was used. N-GQDs were produced by voltammogram scanning. Then, N-GQDs were dissolved in the solution of tetrabutylammonium and acetonitrile. Indeed, they prepared N-GQDs by use of graphene sheets as raw material to show their ability in electrocatalytic applications [29]. GQDs have many applications and benefits. The schematic illustration of the process is shown in Figure 4 [15].

Li et al. used the electrochemical method to prepare GQDs. Electrochemical reactions occurred in PBS by CV scan. Graphene films were used as electrodes. To obtain GQDs and increase their hydrophobicity properties, the electrode reacted with O_2 plasma [30].

2.2.2. Synthesis of Graphene Quantum Dots by the Ultrasonic Method. Zhuo et al. used the ultrasonic method to prepare GQDs for application in bright photoluminescence. For producing GQDs, graphene was mixed with H_2SO_4 and HNO_3 . Then, the solution was sonicated. Afterward, the mixture was heated in a furnace under exhaust gas to remove excess and concentrated amount of H_2SO_4 and HNO_3 . Then, the

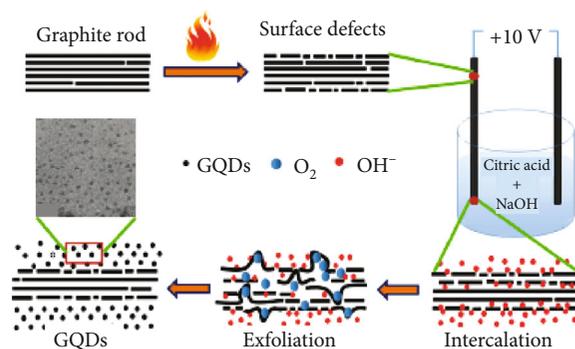


FIGURE 4: Schematic illustration of the production of graphene quantum dots by the intercalation of OH^- ions, O_2 production, and exfoliation process.

TABLE 3: Applications and synthesis methods of GQDs.

Applications	(i) Bioimaging
	(ii) Sensing
	(iii) Catalysts
	(iv) Photovoltaic devices
	(v) Fluorescence performance
	(vi) Light-emitting diodes
	(vii) Laser
	(viii) Electrochemical sensing
	(ix) Photovoltaics
	(x) Photocatalysis
	(xi) Solar cells
	(xii) Photodetectors
	(xiii) Batteries
	(xiv) Drug carriers
	(xv) Drug delivery
	(xvi) Fuel cell
	(xvii) Optoelectrical detector
	(xviii) Antimicrobial materials
Synthesis methods	(i) Hydrothermal method
	(ii) Microwave-assisted hydrothermal method
	(iii) Soft template method
	(iv) Metal-catalyzed method
	(v) Liquid exfoliation method
	(vi) Electron beam lithography method
	(vii) Solvothermal treatments
	(viii) Ultrasonic shearing
	(ix) Electrochemical oxidation
	(x) Oxygen plasma treating
	(xi) Ozonation peroxide method
	(xii) Pyrolysis
	(xiii) Pyrolysis combined with chemical oxidation

achieved solution dissolved in water and filtered with the membrane. Finally, GQDs were produced [31].

Applications and different synthesis methods of GQDs are given in Table 3. These methods have advantages such as low-cost synthesizing strategies, low toxicity, good amount of solubility in water and organic solvents, high chemical and thermal stability, biocompatibility, stable PL (photoluminescence), highly accessible surface areas, oxygen-rich functional

TABLE 4: Combinations to prepare GQDs/GOQDs and type of quantum dots produced.

Electrolyte combination	Types of quantum dots produced	Ref.
(1) Citric acid (2) Alkaline hydroxide	GQD1-GQD2-GQD3-GQD4	[15]
Tetrabutylammonium perchlorate in acetonitrile	N-GQD3	[29]



FIGURE 5: Green synthesis of GQDs by using natural starch [32].

groups, unique fluorescence, and surface grafting. GQDs have absorbed massive attention because of their excellent properties such as surface, molecular, electrical, and mechanical properties. Another reason for absorbing enormous attention is the high potential applications in imaging, sensing, catalysts, energy fields, and other areas. GQDs have many other ideal properties and applications shown in detail below.

Different combinations to prepare GQDs/GOQDs and the type of quantum dots produced are given in Table 4. In the electrochemical method, the electrolyte used is a combination of several materials and also a combination of electrolytes that have a direct effect on the type of GQD that could be produced. Two types of electrolyte combinations and types of GQDs are shown below.

2.2.3. Synthesis of Graphene Quantum Dots by Green Synthesis. Chen et al. used green synthesis by using natural starch, water, and gold nanoparticles to synthesize GQDs. In the synthesis process, GQDs were produced with the green method by using starch and water. $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ was added to the GQD solution. AuNPs were made only by mixing GQDs and $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ without any surfactants and reducers. Gold nanoparticles (AuNPs) also were used to stabilize GQDs. The illustration of the process is given in Figure 5. According to this figure, starch was dissolved with water and heated at 60°C for 15 min. The obtained starch solution was heated at 180°C for 8 hours by hydrothermal reaction. Then, the solution was centrifuged and filtered for removing excess materials for 30 min. At last, GQDs were obtained. AuNPs were also produced by mixing obtained

GQDs and Au(III) without using any reducing agents or surfactants [32].

2.2.4. Synthesis of Graphene Quantum Dots by the Hydrothermal Method. Roy et al. used raw plant leaves by the hydrothermal method to produce GQDs. Due to the use of plants in this method, because of owning a large amount of hydrocarbons and nitrogen-containing groups in plants, there was no need to add water or a reducing agent or organic solvent. The leaf extract and hot water were dissolved together, and the rest of the solids were removed by centrifugation. Then, the solution was sonicated. Afterward, this solution was reacted in an autoclave at high temperatures. For removing extra material, the solution was centrifuged. Then, the obtained GQD solution was dialyzed and dried to obtain pure GQDs [33]. Dong et al. used single-walled carbon nanotubes to produce GQDs by the hydrothermal method. For this work, SWCNTs reacted with HNO_3 and were oxidized; then, the solution was heated under hydrothermal reaction. The structure of SWCNTs was changed and produced by graphene nanosheets. Afterward, graphene nanosheets reacted with HNO_3 and released and obtained GQD1 and GQD2 that could dissolve in water [34]. Zhao used GO and HNO_3 as the raw material and oxidant, respectively, to synthesize GQDs by the hydrothermal method. The change in the concentration of nitric acid had a direct effect on the size of the GQDs. GQDs were produced by using GO and nitric acid. For the synthesis of GQDs, they obtained GO from graphite by adopting the modified Hummers' method. GO was used for producing GQDs by the top-down method in three steps. At first, GO was refluxed with

HNO_3 . The reaction continued until the color of the solution darkened. Then, the solution was centrifuged and cooled. Then, the sediment of the solution was washed with water and centrifuged, which was detected as oxidized graphene oxide. Then, the obtained oxidized GO was dissolved in water and heated in an autoclave hydrothermally. After that, the suspension was centrifuged, and a light brown supernatant and black deposit were detected as oxidized GO and oxidized rGO. Then, the supernatant was dialyzed with water to remove acids and excess materials. The obtained solution was GQD1. The sediment of the solution was refluxed with HNO_3 . The reaction continued until the color of the solution changed to light blue. Then, the solution remained at room temperature and was dialyzed with water to remove the acids that were used and excess materials. Finally, GQD2 was obtained [35]. Pan et al. used graphene sheets to synthesize GQDs with strong blue fluorescence. For blue fluorescence, deoxidization of GO sheets under thermal reductions produced graphene sheets in nitrogen gas. By using H_2SO_4 and HNO_3 in sonication, graphene sheets were oxidized. Owing these groups caused graphene sheets to be soluble in water. Then, the solution was washed with water to remove excess materials. Then, the purified solution's pH rose. The resulting solution was heated in a source of poly(tetrafluoroethylene). Then, the temperature of the solution decreased to room temperature. After separating the larger nanoparticles, GQDs were obtained. Also, green fluorescent GQDs were produced under high temperatures and strong alkaline. In other words, GQDs were obtained from rGO with the hydrothermal method by using O_3 . In this work, GO was produced from the modified Hummers' method. GO also was prepared for diluted graphite oxide solution that was sonicated. The homogenated solution was centrifuged to remove excess materials. Afterward, using hydrogen peroxide and ozone produced ozonized graphene oxide. The solution was dissolved in a solution of hydrogen peroxide and water. Then, the mixture was modified with ozone. rGO was obtained by deoxidization of ozonized graphene oxide by heating the solution. Then, the pH of the solution was decreased, and the solution was sonicated. To achieve ozonized rGO, the solution was placed in an ice bath under sonication. Then, the solution was heated in an autoclave containing poly(tetrafluoroethylene). After using filtration, GQD was obtained [36].

2.2.5. Synthesis of Graphene Quantum Dots by the Chemical Method. Moon et al. synthesized $\text{ZnO}@$ graphene quantum dots with a chemical method for multifunctional applications. In other words, they improved photovoltaic yield by using $\text{ZnO}@$ graphene quantum dots with octylamine. For the synthesis of GO in this work, they use graphite powder that was mixed with sulfuric acid and nitric acid. Then, the solution was ultrasonicated by keeping the temperature constant. The solution remained at room temperature until changes in the color of the solution occurred and became darker. Then, the solution was washed with solvents such as water and ethanol multiple times and centrifuged. Finally, the solution was dried. For the production of $\text{ZnO}@$ graphene core-shell quantum dots (ZGQDs), at first, GO was dissolved

in *N,N*-dimethylformamide and sonicated for better dispersion. Also, zinc acetate dehydrates were dissolved in dimethylformamide (DMF) and GO was gradually added to the prepared solution under stirring. Then, the temperature of the solution was increased by heating and remained constant. When the color of the solution changed, the solution was washed with ethanol multiple times and centrifuged. Finally, the solution was washed with water. At last, the solution was dried, and ZGQDs were obtained. Continuing their work, they synthesized octylamine-functionalized ZGQDs [37].

2.2.6. Synthesis of Graphene Quantum Dots from GO by Photo-Fenton Reaction. Zhou et al. used GO to produce GQDs. GO reacted with the Fenton reagent ($\text{Fe}_2/\text{Fe}_3/\text{H}_2\text{O}_2$) under ultraviolet light and produced GQDs. To obtain GQDs, GO, H_2O_2 , and FeCl_3 were mixed, and the pH was reduced. The reaction was also dialyzed to remove iron ions, H_2O_2 , and small-sized molecules. For the better impact of peripheral carboxylic groups on the photoluminescence properties in reaction, the produced GQDs were reduced in dried tetrahydrofuran by using the reducer agent LiAlH_4 to reduce groups to hydroxyl [38].

2.3. Reduced Graphene Oxide. GO is a form of graphene. rGO is a form of GO that is reduced by eliminating its oxygen functional groups. The commercial method for the synthesis of rGO uses hydrazine or hydroquinone, which are used as a reducing agent for rGO synthesis. These materials cause a rapid decrease and a high amount of rGO. However, these chemicals are corrosive and dangerous. The green synthesis is healthier, more comfortable to access, environmentally friendly, and inexpensive. Green reactants such as ascorbic acid and carrot root are used to produce RGO [39–42].

rGO has the same properties as GO, such as mechanical, electronic, and conductive properties [43]. Decreasing the GO can make a big difference in its structure and properties, which is why the reduced GO mode has attracted so much attention. The reduction of GO could progress the GO properties such as electrical conductivity [44]. rGO, because of owning GO properties and achieving better properties than GO, due to reduced functional groups, gained very desirable applications in several fields of electronics, biologics, catalysts, membranes, and environments [43].

3. Papillomaviruses

Papillomaviruses have genomes that are double-stranded, which is classified in the papilloma family [45]. Papillomavirus genomes are divided into three areas including the early region, late region, and long control region [46]. The early region consists of open reading frames such as E1, E2, E4, E5, E6, and E7 that have applications such as transactivation of transcription, replication, transformation, and viral adaptation to different cellular environments. The late region is responsible for coding for the L1 and L2 capsid proteins and has the ability to form the structure of the virion and simplify the packaging and maturation of viral DNA. The upstream regulatory region contains sequences that could

control transcription and replication of viral DNA [47]. Papillomaviruses are a group of heterogeneous viruses; each type of them is associated with a specific lesion [48].

They enter the body by contact with small lesions on the skin and stimulate the body to induce cell proliferation, including the epithelial skin and mucous membranes [49]. Papillomas or warts are often benign, but due to environmental and genetic factors, some of them make a change in humans in the form of malignancy. Neoplasia of papillomaviruses is dependent on anogenital cancer, cancer of the upper respiratory tract in humans, skin cancer related to rabbits and humans, cancers of the upper alimentary tract and bladder in cows, oral cancer in dogs, and cancer of the alimentary tract and bladder in humans [50]. Most papilloma infections, after several months with activation of the cell-mediated immune response, directed against antigen viruses, can be cleared [51].

4. Human Papillomavirus

Papillomaviruses of humans are the cause of warts and are found in all human populations, and sometimes, the infection caused by them can lead to cancer [48, 51]. These viruses pollute epithelial tissue stem cells at a pluristratified cutaneous or mucosal site [52]. The human papillomavirus (HPV) includes sexually transmitted infections [53]. HPV is a large family of viruses and is the most common sexually transmitted infection. In most cases, HPV infection has no symptoms, and it is self-limiting [54]. This infection is associated with the benign or malignant proliferation of neoplasms in humans [55]. Risk factors for HPV infection include host factors, sexual history-related factors, viral factors, and substance use-related factors. Host factors include age, safety of the system, infection of the human immunodeficiency virus, infection with other sexually transmitted diseases, deficiency of micronutrients in the body, and genetic polymorphisms. Sexual history-related factors include the number of partners for sex, the status of the newer and older partners, the status of partners that have partners, the use of birth control pills, and the use of condoms. Viral factors that include types of HPV communicate with people who are infected with HPV and the loading of viruses. Substance use-related factors include using alcohol, using cigarettes, and using illicit drugs [56]. Among the viral factors that can play a role in the progress of cancer, the types of HPVs including HPV E7 and HPV E6 transcription factors are very important in causing cancer [57]. There are many types of HPVs that some of them are currently known to leave adverse effects on the genital. It is the leading cause of cervical cancer [54]. HPV infection is in the category of viral infections that could leave some effects on internal and external areas of the body. Depending on the type of risk that HPV causes, they could lead to infection warts or cancers; different effects of this virus are the effect on the skin and mucous membranes, the effect on genital areas of men and women, and the cervical, vaginal, vulvar, penile, and anal cancers [3, 58]. HPVs are very special viruses. They have some particular characteristics in the molecular range. Recognition of viruses helps to detect and understand the behavior of viruses and find solutions for

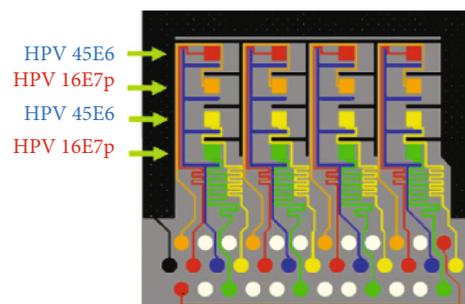


FIGURE 6: Electrode spots of electrochemical detection of HPV16E7p and HPV45E6 [53].

the debarment and treatment of disease. Some properties of HPV are known as small diameter (nm), nonenveloped, and double-stranded DNA [59].

4.1. Different Methods of Human Papillomavirus Detections.

Methods previously used for the detection of HPV included visual inspection with acetic acid and Pap smear. New methods compared to traditional methods have some advantages, such as fast detections. Nowadays, different methods are used for the detection of HPV, such as signal amplification assays, nucleic acid hybridization, nucleic acid amplification, and hybrid capture [59, 60].

Civit and Frago used electrochemical biosensors for HPV detection. For the detection of two types of HPVs including HPV16E7p and HPV45E6, they used electrochemical genosensor arrays, which have many effects on the skin. Electrode arrays contained gold electrodes. Electrode arrays were placed in a microfluidic cell. Electrode arrays were installed on a polycarbonate fluidic chip. At first, arrays were sonicated in a solution consisting of acetone and isopropanol and then washed with water. Then, the arrays were cleaned by applying differential potential in a solution of H_2SO_4 . At last, electrodes were washed with water and dried by using nitrogen gas. Then, the electrodes were modified using the immobilization of the probe and the backfiller DT1 in the KH_2PO_4 aqueous solution. At last, the electrodes were washed by using a solution containing PBS with Tween and water under stirring and dried with nitrogen gas [53]. Figure 6 shows electrode spots that are used for the detection of HPV16E7p and HPV45E6.

For DNA detection of both HPVs, the sandwich-type model was used. At first, HPV targets in the solution of PBS and Tween were shed on modified gold electrodes and incubated. The sensors were washed in a solution containing PBS and Tween under stirring; then, the solution was dried with nitrogen gas. Subsequently, hybridization was done by spotting probes in PBS and Tween. Then, they were incubated. Afterward, the arrays were hybridized, washed with PBS-Tween, and dried in nitrogen gas. For electrochemical detection, electrode arrays were modified and assembled on the microfluidic cell. This process is done in the microfluidic system by using the TMB substrate [53]. Figure 7 shows a schematic illustration of the electrode modification, hybridization process, and electrochemical detection [53].

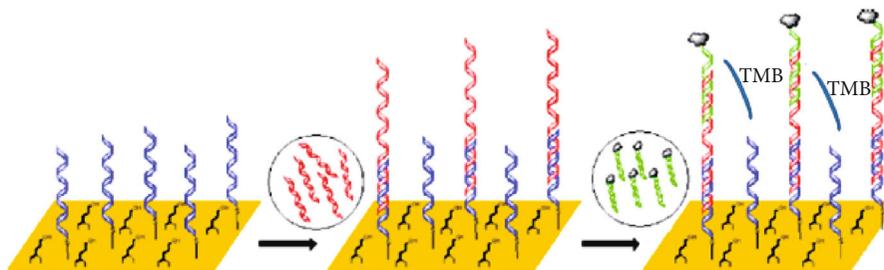


FIGURE 7: Schematic illustration of the developed assay based on the coimmobilization of the probe, hybridization process, and electrochemical detection.

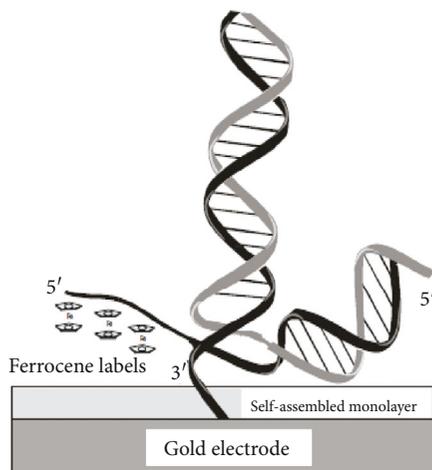


FIGURE 8: eSensor DNA detection system.

HPV activates oncogenes E6 and E7, and this causes the development of cervical cancers. In general, it is challenging to diagnose HPV infections, and there are some tools to diagnose it [53]. One of the tools is based on molecular cognition; in this method, HPV DNA was detected. In other words, genomic DNA was extracted by identifying the posterior capsular rent. In this method, the diagnosis has been complicated due to the high mutation rate [61, 62].

Another method used for detecting HPV is using electrochemical biosensor diagnosis. In this method, the DNA target hybridizes in the sample and produces an electric current in the solution. Electrochemical biosensors have progressed since they were introduced. Electrochemical biosensors have certain benefits that lead to the high potential application of them. Advantages of electrochemical biosensors used for HPV detection are as follows: affordability, simplicity, excellent sensitivity, possibility of microfabrication technologies, adaptability with mass manufacturing, use of low-density DNA chips, and possibility of portability [53, 63]. The named advantages lead to electrochemical biosensors becoming conventional instruments in HPV detection.

Vernon et al. designed two eSensor chips for the detection of HPV. Each sensor contained specific probes. In this method, they used eSensor chips, gold electrodes, reference electrodes, and auxiliary electrodes. The electrodes were connected to a connector on the lip of the chip. Probes were achieved by using disulfide-coated chips. For the design of the HPV target, an HYBsimulator was used. Signal probes

were synthesized by using modified adenine that contained a ferrocene substitution. For sample extractions, samples containing HPV were selected. Samples were coded. With the help of the DNA Mini Kit, DNA was extracted. Then, the concentration of DNA increased [63]. Figure 8 shows the illustration of eSensor for DNA detection that is also used for HPV DNA [63].

Electrochemical approaches are used for HPV detection. For example, the electrochemical impedance spectroscopy (EIS) technique is used for DNA detection. One of the approaches is the use of DNA biosensors. In this method, if the electrode surface is covered with nanostructured materials, a significant active area is created to connect to probe molecules. The advantages of using DNA biosensors include high specificity to target sequences, potential for the detection of genetic disorders, and potential for the detection of bacterial infections [64]. DNA biosensors are very important in detection fields, especially for the detection of disease that their genes create disease. The main features of these biosensors are simple functionality, fast, and simple detection. Also, high sensitivity and unique detection of DNA biosensors lead to their extensive progress [65].

Campos-Ferreira et al. used electrochemical DNA biosensors for HPV-16 detection. In this work, HPV oligonucleotides were diluted with water and selected as the HPV-16 probe, HPV-16 target, and noncomplementary DNA. Then, the DNA extracted was amplified. Afterward, L-cysteine was electrodeposited on the surface of the gold electrode. Afterward, the HPV-16 probe was motionless on the electrode surface with the cysteine film that was formed. Then, the solution of the HPV-16 target was added to the electrode with a motionless HPV-16 probe while incubating. For the hybridization of DNA with HPV-16, the extracted DNA was heated in a water bath and then immersed in ice to obtain ssDNA. Then, it was added to the surface of the modified electrode. To form hybridization of the extracted DNA and HPV-16 probe, they reacted under stirring. Also, before and after hybridization, methylene blue was used. For analysis of the electrochemical signal, the differential pulse voltammetry method was used as a redox probe [66]. A schematic illustration of the overall performance of biosensors is shown in Figure 9. Target analytes connected to a biomolecule were immobilized on a surface. The converter transforms the one detected into a measurable signal.

Wang et al. used the electrochemical method, for the detection of hepatitis B and papillomavirus DNA, by using

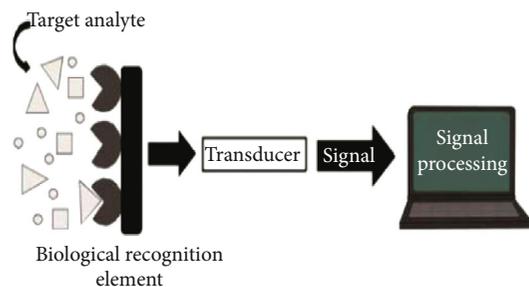


FIGURE 9: General operation principle of biosensors [67].

the SWCNT array that was coated with gold nanoparticles. By immersing the wafer into the solution containing Fe-Mo catalysts, they could be loaded on one side of the SiO₂ with the Si substrate. To obtain laminar flow, the wafer was placed in the center of a quartz tube. The mixture was heated in the atmosphere of hydrogen and argon. Then, ethanol vapor was achieved by bubbling H₂ in ethanol at ambient temperature. Gold nanoparticles were deposited electrochemically in place of SWCNT arrays using the amperometric method by applying the differential potential in the HAuCl₄ solution. The SWCNTs with Au electrodes were incubated in the ssDNA probe. Then, the electrode was washed with water to remove the unassembled probe ssDNA. Then, the SWCNTs/Au/ssDNA electrode was immersed in the target ssDNA solution, and hybridization was done in a water bath under heating. Afterward, the electrode was washed with phosphate buffer to remove the unlinked target ssDNA. In this work, EIS measurements were done by using an Autolab PGSTAT30 electrochemical workstation, with ordinary electrodes consisting of the saturated Ag or AgCl reference electrode, platinum foil auxiliary electrode, and SWCNTs with Au or DNA-modified SWCNTs with Au working electrode. In this work, the madding random SWCNT array was easier [64]. Chekin et al. used nucleic aptamer modified porous rGO (prGO) with MoS₂-based electrodes for treatment. They used glassy carbon (GC) electrodes modified with prGO and MoS₂ for the detection of L1, the major capsid protein of HPV. They created an electrochemical sensor whose electrode had a covalent operation. The electrode used in it had an Sc5-c3 aptamer. The RNA aptamer was targeted against the HPV-16 L1 protein. In this work, for the production of prGO, at first, rGO was produced from graphene oxide by using hydrazine reduction. Hydrazine hydrate was added to graphene oxide; then, the solution was heated. Afterward, reduced graphene oxide was precipitated. Then, the one produced was filtered with membranes, washed with water and methanol, and dried in the oven. Afterward, reduced graphene oxide was dissolved in H₂O₂ and sonicated. Then, the mixture was refluxed. Finally, the obtained solution was filtered and dialyzed to remove excess materials. GC electrodes were varnished with alumina powder, which was drop-casted with prGO, and then dried. Then, the MoS₂ solution was drop-casted and dried. The obtained GC/prGO/MoS₂ electrodes were immersed in PBS, and the voltage difference was applied between them. Then, electrodes were immersed in a solution containing mercaptoundecanoic acid and poly(ethylene glycol) methyl

ether thiol. HPV-16 L1 aptamers were immersed in a solution of EDC with NHS in PBS. They were motionless by activating the first carboxyl groups. Porous carbon is commonly used as an electrode for sensitive bioelectrochemical sensors for HPV treatment. Porous carbon materials show exciting features that could be used in electrochemical sensors and connected to electrodes. These porous carbon materials have some advantages over other carbon materials such as residing porosity, high specific surface areas in the electrode, reinforcement of the sensitivity of the sensor, diffusion of the analyte via interconnected pores, high mechanical strength, excellent chemical stability, potential for immobilization of many ligands, fast sensing, excellent electrochemical properties, and integrity of the porous structures [59].

Braga et al. claimed that their invention of biosensors for the detection of infection and associated maladies relates to a biosensor for the determination of infection and possible neoplasia associated with it. The biosensor is invented wherein the pathogenic agent is the HPV. For the determination of HPV, the state of progression of HPV infection and/or neoplasia is derived from said infection [68].

Aspermaier et al. used reduced graphene oxide-based field-effect transistors (rGO-FET) for the sensing of HPV. The highly selective and sensitive detection of the HPV-16 E7 protein was due to characteristics of pyrene-modified rGO functionalized with the RNA aptamer Sc5-c3. The aptamer-functionalized rGO-FET allowed for monitoring the aptamer-HPV-16 E7 protein binding in real time with a detection limit of about 100 pg mL⁻¹ (1.75 nM) for HPV-16 E7 from five blank noise signals [67].

Mahmoodi et al. used ultrasensitive electrochemical DNA nanobiosensors for the detection of HPV-18. The good performance of the nanocomposite of MWCNTs and rGO biosensors was achieved through hybridization of the DNA probe-modified screen-printed carbon electrode (SPCE) with the extracted DNA. The performance of this biosensor was evaluated using synthetic DNA and DNA extracted from HPV-18 patients. Due to the use of rGO/MWCNT, L-cysteine to modify the surface of the electrode, and Au nanoparticles, the biosensor was also able to detect very small quantities and a large range of analytes [2].

In Table 5, traditional methods of analysis with the methods based on electrochemistry are compared.

4.2. The Way of Transmitting Papillomavirus. The cell cycle of papillomaviruses directly depends on the differentiation of epithelial cells. It is believed that infections cause papillomavirus by microtrauma lesions that exist in the epithelium and basal cells, which are exposed to the virus. Papillomavirus at first pollutes the epithelial cells of the basal layer, which is the only layer that is actively distributed in the epithelium [74]. Papillomavirus receptors entering the epithelial cells are not functionally defined, but the protein complex such as integrin alpha-6 beta-4 as a receptor is considered. This integrin is initially expressed during wound healing, and this is a logical candidate for receptors of this molecule for the entry of the HPV. However, various studies have not shown the mediation of this molecule to enter the virus. After entry of the virus into cells, HPV genomic DNA cells multiply in

TABLE 5: New methods compared to traditional methods have some advantages.

Methods	Advantages	Disadvantages	Ref.
Electrochemical detection	(i) Sensitivity (ii) Specificity (iii) Rapid response (iv) Simplicity (v) Without prolonged experimentation processes (vi) Without purification requirements (vii) Low cost (viii) Stability (ix) Using a wide range of transduction mechanisms such as electrical, electrochemical, optical, and mass-based mechanisms (x) Small sample volume (xi) Small size (xii) Small sample volume	(i) The need for the application of redox-active markers in the sample solution (ii) Thermal denaturation	[2, 69–71]
Signal amplification assays	(i) High sensitivity to genotyping (ii) Lower false-positive rate (iii) FDA-approved test (hc2) (iv) Quantitative	(i) Was not designed to genotyping individual (ii) Patented and licensed and technologies	[72]
Nucleic acid hybridization assays	(i) Presence of HPV in association with morphology (ii) Southern blot is the gold standard for HPV genomic analysis	(i) Southern blot and hybridization cannot be degraded (ii) Relatively large amounts of purified DNA, time-consuming, and low sensitivity	[72]
Nucleic acid amplification assays	(i) Multiplex analysis (ii) Very high sensitivity (iii) Flexible technology (genotype and viral load)	(i) Contamination with previously amplified material can lead to false positives (ii) Lower amplification signals of some HPV genotypes	[72]
Pap smear test	(i) Enabling easy testing	(i) Expensive instrumentation and expert analysis (ii) Dedicated instrumentation (iii) Exhaustive labels (iv) Low specificity and sensitivity (v) Time-consuming and complex operation (vi) Limited resources and personnel	[69, 73]

the nucleus, and the number of copies is increased by about 50-100 copies per cell. When infected cells start to divide from the basal layer, one of the daughter's cells migrates and begins to differentiate. In other words, viral genomes were divided into daughter cells that remain in the nucleus after cell division. Producing reproductive viruses by daughter cells allows infected cells to remain in the basal layer for several periods of time [52, 75].

4.3. Genetic Characteristics of HPV. The HPV genome is divided into three areas in terms of function. The first area is the early area, which encodes the proteins (E1–E7) and is involved in the replication of viral DNA. The second area is the late (L) region, which has the ability to encode the structural proteins (L1 and L2), and is involved in virion assembly. The third area is the most noncoding part and is named the long control region (LCR), and it is involved in the transcription and replication of viral DNA. The viral E proteins have the ability to transcribe from the early promoter called p97;

for example, in HPV-31, the viral L proteins have the ability to transcribe from the late promoter called p742 [76]. In the HPV's life cell cycle, the viral genomes are expressed from different polycistronic mRNAs transcribed from a strand of single DNA. The life cycle of HPV is related to the distinction program of the host cell that infected the keratinocyte, and the production of virion particles was restricted to distinct suprabasal cells [77]. Figure 10 shows the HPV-16 DNA genome. In this figure, the early genes, late genes, and LCR are described. Early genes promote p97, also shown by an arrow [78].

4.4. Virus Proteins and Their Role. The E1 protein is one of the viral proteins that only has enzymatic activity. This protein regulates viral DNA replication. It can make changes in cell gene expression. E1 proteins of HPV-16 and HPV-18 can regulate the expression of genes with the responsibility of reproduction, migration, and metastasis that could create cancers [79]. E2 protein has a full length and is a necessary

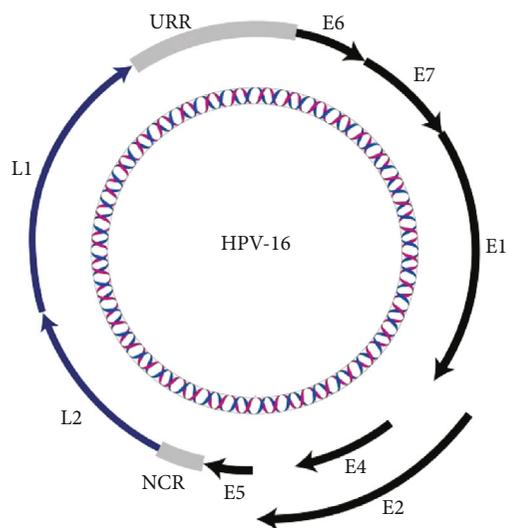


FIGURE 10: Schematic representation of the HPV-16 double-stranded circular DNA genome.

protein with the regulatory form encoded by all papillomaviruses. E2 proteins are the main proteins that could adjust the transcription of the papillomaviruses. E2 proteins activate or suppress transcription processes; this action occurred by absorbing cellular agents into the genome. E2 proteins have the ability to bind the sequence motifs located in the viral genome and then induce the activation or suppression of transcription. E2 proteins bind to viral genome sequence patterns frequently within the upstream regulatory region (URR) of the viral genomes, and thus, transcription is activated or suppressed in this way [80].

5. Future Perspectives

Many countries are expected to be equipped with HPV prevention facilities and apply diagnostic and treatment methods in the next years. Recent results show that the use of electrochemical detection methods is more accurate than traditional Pap smear methods.

A powerful electrochemical biosensor should have user-friendly properties together with high-performance components. Graphene-based materials such as GO, rGO, and GQDs, due to owning environmentally friendly properties, biocompatibility, high reactivity, sensing, and quick functionalization, are good candidates for use in electrochemical biosensors and genosensors. Advancement in graphene-based materials for electrochemical sensors for HPV detection should be continuously researched because it promises great advantages and exposures; in any case, testing ought to be ongoing to guarantee human safety. The electrochemical biosensor method is the most incentive for the progress of future HPV detection because of its capacity to create fast and accurate measurement information; however, a lot of research can be done to increase its outstanding capacity.

In comparison with conventional techniques, these electrochemical sensors for HPV detection demonstrate more favorable applications to improve human health.

6. Conclusion

The present study described the image of HPV and the diseases the viruses could cause. The potential of DNA biosensors, for example, biosensors that use graphene derivatives, could detect HPV. Graphene derivatives, such as graphene oxide and graphene quantum dots, due to their potentials, such as thermal, mechanical, or chemical stability, biocompatibility, easy access, and large surface areas, are good candidates for use in biosensors or genosensors. The functions of these biosensors are by using DNA extracted from HPV samples and synthetic DNA. The most hazardous type of this virus, such as HPV-16 and HPV-18, causes dangerous diseases such as cervical and anal cancers. Some factors, such as personal health, should be thoroughly observed to avoid getting this disease.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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