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REVIEW

Recent Advances in HPV Detection: From Traditional Methods to Nanotechnology and the Application of Quantum Dots

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Abstract: Cervical cancer, a significant public health concern, demands precise and expeditious detection methods to curb the spread of human papillomavirus (HPV). The early detection of cervical cancer remains a critical challenge in developing reliable and efficient screening tools to meet the demand for controlling cervical cancer. Traditional detection techniques are often cumbersome, costly, and inadequate for on-site HPV testing. Nanotechnology, with its unique electrical, chemical, and optical properties, has emerged as a pivotal component in the development of biosensors for rapid and reliable HPV detection. This article provides a comprehensive review of the advancements in cervical cancer detection, encompassing traditional methods, emerging protocols, and novel quantum dots (QDs)-based approaches for detection. The review examines the application of various nanomaterials in electrochemical and photoelectrochemical biosensors for the diagnosis of cervical cancer, with these innovations offering a significant improvement over conventional approach. Furthermore, we detail the synthesis methods of QDs and their properties, illustrate the substantial enhancement in sensor performance achieved through their applications, and elucidate the improvements and challenges associated with these new protocols while highlighting the potential application prospects of novel QDs technology in HPV detection.

Keywords: cervical cancer, human papillomavirus detection, nanotechnology, quantum dots, biosensors, early diagnosis

Introduction

Cervical cancer ranks as the fourth most prevalent malignancy among women worldwide, constituting a significant menace to women's health.[1] Despite being preventable through vaccination, cervical cancer screening remains indispensable due to limitations in vaccine coverage and immunogenicity.[2,3] The World Health Organization (WHO) recommends nucleic acid detection of human papillomavirus (HPV) as the preferred method for cervical cancer screening,[4] which has drawn scholars' attention to advancements in cervical cancer detection technology.[5] Apart from high-income countries exhibiting screening rates of 84%, women aged 30–49 years residing in upper-middle- (48%), lower-middle- (9%), and low-income countries (11%)[6] may leave generations of girls and women at risk, though global strategies are paralleled with the goal of a 70% screening coverage by 2030.[4] Given the vast population size, there is an urgent imperative to explore innovative methods for promoting cervical cancer screening.[7]

Over the past two decades, traditional diagnostic modality has evolved from a cytology adjunct to a primary co-testing methodology with cytology, establishing an optimized cost-benefit paradigm for cervical cancer screening.[8–10] Persistent infection with high-risk HPV genotypes, particularly HPV16 and HPV18, represents the leading etiological factor in cervical carcinogenesis and precancerous lesions development.[11,12] Molecular detection of these oncogenic

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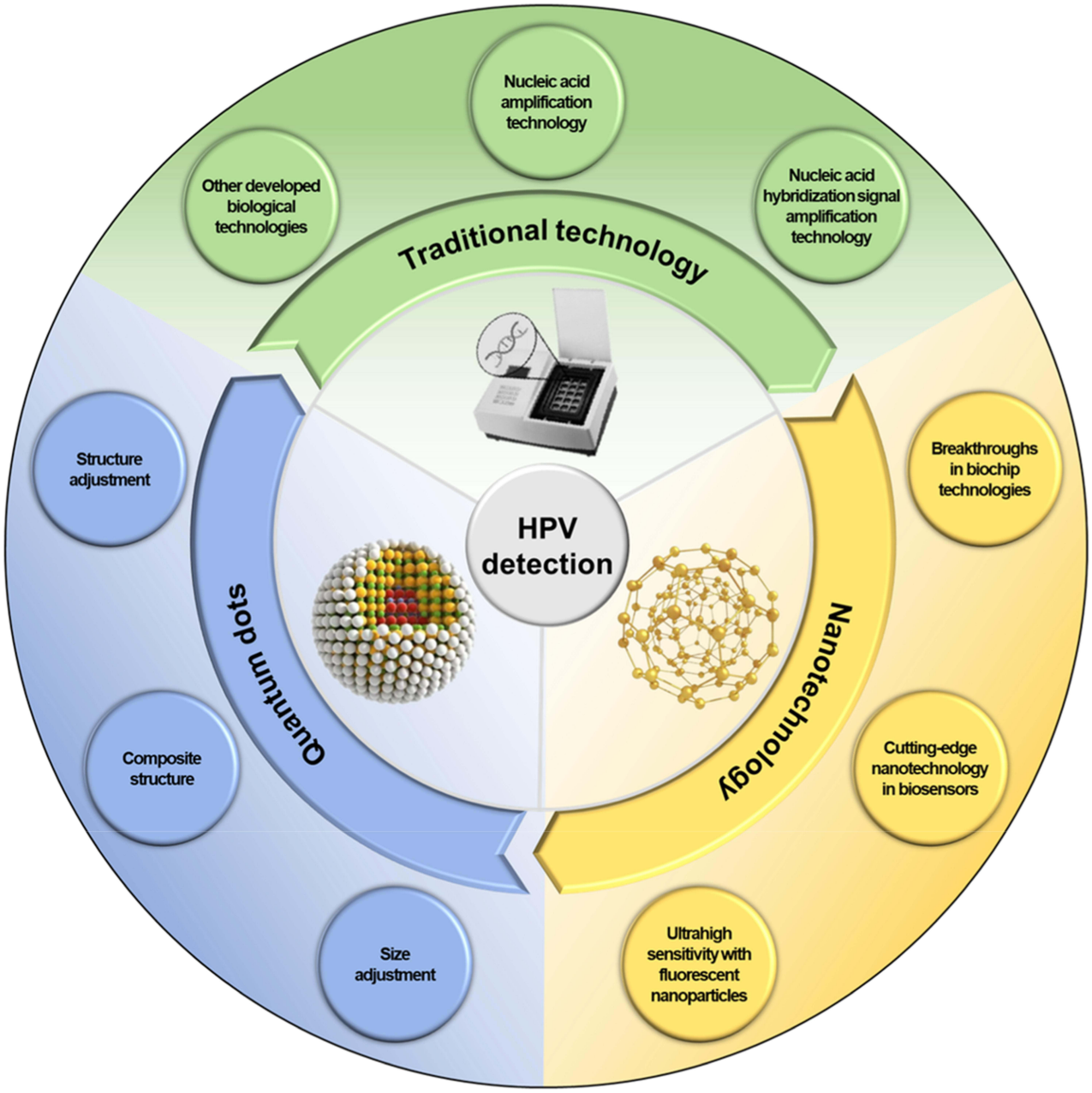
Graphical Abstract



HPV types constitutes a critical component of evidence-based strategies for early cervical cancer detection and precision management.[13,14] The integration of nanotechnology into clinical diagnostics has achieved transformative breakthroughs. In the battle against cervical cancer, timely detection is crucial for effective treatment outcomes. Nanotechnology, with its exceptional sensitivity, precision, and capability for multiplex measurements, has emerged as a potent tool to enhance the early detection of cervical cancer.[15,16]

Quantum dots (QDs), known as semiconductor nanocrystals, are composed of nanoparticles that typically range in size from 1 nm to 100 nm, closely aligning with the exciton Bohr radius.[17] Unlike traditional enzyme-linked immunosorbent assays and organic fluorescent dyes, QDs offer a broader and more effective excitation spectrum,[18] along with

a narrow and symmetrical emission spectrum. They also exhibit high fluorescence quantum efficiency,[19] excellent photochemical stability,[20] flexible surface chemistry, and the ability to be biologically modified. Among various traditional and novel HPV detection technologies, QDs stand out as a remarkable fluorescent marker due to their superior specificity, heightened sensitivity, and enhanced fluorescence stability.[21]

Overall, this review provides a thorough examination of recent advancements in HPV detection, covering both conventional strategies and cutting-edge approaches that incorporate various nanotechnologies, particularly the integration of QDs. It examines the advantages and disadvantages of traditional methods and highlights the exciting potential of QD technology for HPV detection.

Traditional Methods for HPV DNA Detection

The HPV genome is intricately organized into three principal functional regions: the early transcription region (E region), the late transcription region (L region), and the long control region (LCR). The E1 and E2 genes within the E region are instrumental in viral replication and transcription, respectively.[22,23] Overexpression of E6 and E7 can immortalize cells by interfering with host cell cycle, inhibiting cell apoptosis, causing gene instability and affecting telomerase activity, which leads to the occurrence of cervical cancer.[24,25] The L1 and L2 genes encode structural proteins of the viral capsid, pivotal for HPV infectivity.[25–27] In the context of persistent HPV infection, integration of the viral genome into the host's genomic DNA may lead to the disruption and loss of regions such as L1, E1, and E2, while the E6 and E7 regions are often preserved.[25,28] This observation suggests that detection methods focusing on the E6 and E7 regions could offer a more specific assessment of HPV DNA, regardless of its integrated state.[29] Current HPV molecular detection technologies in clinical practice are developed into nucleic acid hybridization signal amplification, nucleic acid amplification technologies, and other developed biological technologies.[30]

Nucleic Acid Hybridization Signal Amplification Technology

Nucleic acid hybridization signal amplification technology represents a direct method for identifying HPV nucleic acid sequences in clinical samples, circumventing the need for PCR amplification. This approach minimizes the risk of contamination, although it falls short in discerning specific HPV genotypes. The domain encompasses three principal techniques: hybrid capture technology, enzyme digestion signal amplification technology, and branched DNA signal amplification technology.

Hybrid Capture Technology

Hybrid capture technology's detection principle is based on hybridization of the target HPV genomic DNA with the corresponding RNA probe, which is captured by the corresponding antibody on a microplate. Finally, HPV detection is carried out by enzyme-linked secondary antibody and catalytic substrate chemiluminescence.[31–33] A pioneering clinical trial of cervical cancer screening published in The New England Journal of Medicine demonstrated that the second-generation hybrid capture (HC2) product has significantly better clinical detection sensitivity in detecting grade 2 or 3 cervical intraepithelial neoplasia (CIN2 or CIN3) than the traditional cytological detection (94.6% vs 55.4%), but its specificity is slightly lower than that of conventional cytological detection (94.1% vs 96.8%).[34] In addition, studies have shown that HC2 undergoes certain cross-reactivity with certain non-targeted and non-carcinogenic HPV genotypes.[35] After optimizing probes, this series of products can effectively reduce potential cross-reactivity between HPV genotypes. There is no need for a professional PCR laboratory to use hybrid capture technology for testing work, which has the potential to penetrate into grassroots medical units and remote areas and has great application prospects.

Enzyme Digestion Signal Amplification Technology

Enzyme digestion signal amplification technology's detection principle is to detect specific target nucleic acid sequences through two synchronous isothermal reaction signals and amplification technology. Based on this technology, Cervista™ HPV HR was developed and approved by the US FDA in 2009.[36] A comparative study between Cervista and HC2 for detecting high-risk HPV in cervical lesions showed that the consistency rates of positive and negative percentages were 90.8% and 64.5%, respectively. Cervista and HC2 methods have the same sensitivity of 90% in detecting cervical lesions of CIN2, with specificity of 47% and 43%, respectively.[37,38] Enzyme digestion signal amplification technology excels in

its good specificity, providing the capability to semi-quantitatively assess mRNA transcription levels. Despite these strengths, the method encounters limitations, notably its inability to discriminate between specific HPV subtypes and the complex procedural steps involved, which can be a deterrent in clinical settings seeking streamlined operations.

Branched DNA Signal Amplification Technology

The representative product of branched DNA signal amplification technology for HPV detection is QuantiVirus™ HPV E6/E7 mRNA test reagent developed by DiaCarta. This reagent adopts the principle of nucleic acid sandwich hybridization technology and can directly detect HPV mRNA in liquid-based samples.[39,40] Studies have shown that the positive rate of E6/E7 mRNA detection is notably higher than that of HC2 detection (75.3% vs 62.3%). HPV mRNA detection and HC2 detection demonstrate equivalent sensitivity to high-grade cervical intraepithelial neoplasia (CIN2+), reaching a rate of 82.4% (14/17). However, the specificity of HPV mRNA detection for CIN2+ is significantly lower than that of HC2 detection.[39] Leveraging the precision of branched-chain DNA signal amplification technology, Kodia Biotechnology has crafted a qualitative detection kit targeting 14 high-risk HPV mRNA species with commendable specificity and simplified pretreatment process. Nonetheless, the methodology faces a trade-off, as the detection protocol involves several incubation cycles that may be perceived as cumbersome and time-intensive.[40]

Nucleic Acid Amplification Technology

HPV nucleic acid amplification technology mainly includes PCR amplification or constant temperature amplification, which first enriches a portion of the DNA sequence in the HPV genome, and then detects it by fluorescent probes, reverse dot hybridization, gene chips, flow cytometry fluorescence hybridization, and other methods.

Real-Time Fluorescence PCR Technology

Real-time fluorescence PCR technology involves the incorporation of fluorescent markers into the PCR reaction system, allowing for the real-time monitoring of DNA amplification through the accumulation of fluorescence signals. This method enables the simultaneous amplification and detection within a closed tube system, culminating in the quantitative analysis of the target sample using standard curves. It offers high sensitivity and specificity for the detection of specific HPV types and provides several operational advantages, including full automation, expedited detection times, high throughput, and the capability to measure viral load. Moreover, it significantly minimizes the risk of contamination from amplification products.[41–43] However, the technology faces constraints due to the finite number of fluorescence channels on real-time PCR instruments, which limits the typing detection scheme within a single tube. To overcome this, multiple HPV types must be tested in grouped sets across multiple reactions, compensating for the instrument's limitations.

Reverse Dot Hybridization Technology

Reverse line blot (RLB) method employs biotin-labeled PCR amplification products that hybridize with probes fixed on nitrocellulose or nylon membranes, and generates detection signals through chemical colorimetry.[44,45] This technique offers broad coverage for the identification of HPV genotypes and is characterized by a stable hybridization process, coupled with a robust adaptability to diverse sample types. However, RLB technology encounters some challenges, including a heightened susceptibility to contamination from PCR amplification products, a multi-step procedural requirement, and the potential for subjective bias in the interpretation of colorimetric results, which could introduce human error into the diagnostic process.

Gene Chip Technology

Gene chip technology employs universal primers to amplify the HPV E1 gene, followed by denaturation of the amplified product. Simultaneously, a multitude of probe molecules are affixed onto solid-phase supports, generating a two-dimensional DNA probe array designed to hybridize specifically with the labeled amplification products. HPV genotyping is subsequently accomplished through enzyme-labeled colorimetry or fluorescence labeling methods.[46–49] This technology offers the capability to simultaneously identify multiple HPV types and concurrent infections, meeting the requirements of high-throughput, rapid analysis, and minimal sample volume. It is tailored to clinical needs with its sensitivity and specificity aligning well with HPV typing, featuring stable fluorescence signal interpretation and clear, actionable results. Nevertheless, gene chip technology faces certain limitations. The cost of DNA chips is relatively high, and the necessity for a gene chip scanner for

analysis elevates the overall detection expense. Moreover, the HPV viral load usually cannot be determined using this method. A comprehensive summary of the specific products of molecular assay for HPV detection is provided in Table 1.[50–75]

Other Developed Biological Technologies

RNAscope is an innovative method for RNA in situ hybridization that combines the benefits of traditional RNA in situ hybridization techniques and fluorescence in situ hybridization (FISH) technology to visualize the transcription of individual RNA molecules. Unlike PCR technology, RNAscope can confirm whether the HPV DNA content exceeds a specific threshold, allowing for a more straightforward and intuitive screening of HPV infections.[76] This technique addresses several challenges, including the inability of PCR to provide location data, non-specific binding observed in immunohistochemistry, the limitation of protein detection only, and the restricted availability of antibodies.

With the progress of molecular biology, the importance of isothermal amplification methods—those that do not depend on temperature cycling instruments—has increasingly been recognized by researchers. Recombinase polymerase amplification (RPA) technology is particularly significant due to its straightforward and rapid nature, yielding results within approximately 30 minutes. This method does not necessitate sophisticated laboratory environments or equipment; rather, it requires only a simple heating device,[77] which enables individuals without extensive professional training to conduct the tests. The sensitivity of RPA can reach levels of 100 to 101 copies per reaction.[78] Nonetheless, as the entire RPA process occurs at a constant temperature, there may be instances of non-specific amplification.

The field of nucleic acid detection is currently focused on three primary research areas: CRISPR/Cas9, CRISPR/Cas12, and CRISPR/Cas13. Within the CRISPR/Cas12a system, two essential components are the guide RNA (gRNA) and the Cas12a proteins.[79] Recently, a novel CRISPR-Cas12a-mediated colorimetric detection platform was developed for MPXV and HPV DNA sensing by applying probe DNA to reprogram the catalytic properties of MoS2 QDs, featuring subpicomolar detection limits, high specificity/sensitivity and applicability in human sera biosamples, with its colorimetric results analysable via a smartphone platform.[80] The CRISPR/Cas system employs various signal transmission methods, with the most classic being real-time fluorescence detection, lateral flow chromatography strips, and hydrogels. Although a portable constant temperature fluorescence detector can measure the fluorescence intensity during the reaction, the viral load in the original sample was low. Transferring it to the CRISPR/Cas12a system for detection can significantly enhance sensitivity; however, this also raises the risk of aerosol contamination.

Nanotechnology in the Diagnosis of Cervical Cancer

In the late 1990s, nanotechnologies advanced clinical diagnosis, notably with the use of gold nanoparticles (AuNPs). The nanogold-mediated catalyzed reporter deposition (CARD) technique has demonstrated superior diagnostic performance compared to conventional polymerase chain reaction (PCR), exhibiting 90% detection sensitivity for HPV-positive cervical cancer specimens versus only 20% for PCR.[81] This breakthrough catalyzed the application of nanotechnology in virus detection.

Ultrahigh Sensitivity with Fluorescent Nanoparticles

The surface of the nanoparticle is modified with antibodies or ligands with biomarkers related to cervical cancer, which can be targeted by biomarkers to achieve visualization or electrochemical signal detection. In a groundbreaking study, Palantavia et al developed an ultrabright fluorescent mesoporous silica nanoparticle for the early detection of cervical cancer. The nanoparticle, loaded with Rhodamine 6G at a concentration significantly higher than its aqueous solubility, was labeled by folic acid for the specific targeting of cervical cancer cells, which overexpress folate (FA) receptors. The research revealed that the fluorescence intensity from pre-cancerous cervical cells was substantially greater than that from normal cells. This novel approach also enabled significantly better sensitivity (95–97% vs 30–80%) and maintained specificity (94–95%) compared with current clinical tests, indicating a promising method for the enhanced diagnosis of early-stage cervical cancer.[82,83] Recently, a colorimetric nanosensor based on AuNPs for detecting high-risk HPV 16 and 18 was developed, showing high specificity (77.8%–87.3%) and excellent negative predictive value (>96%) in clinical evaluation of 173 patients.[84] The innovative use of more nanoparticles with various biomarker-specific targeting offered a significant advancement in the field of cervical cancer diagnostics, with the potential to revolutionize early detection methods and improve patient outcomes.

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Table 1 Specific Products of Molecular Assay for HPV Detection

Products	Type of Detection Technology	Target	Genetic Testing	Genotyping	Product Certification	Ref.
Xpert® HPV Test	Real time fluorescent PCR	DNA	Detect 13 high-risk types	Partial typing	CE	[50]
Onclarity™ HPV Assay	Real time fluorescent PCR	DNA	Detect 14 high-risk types	Partial typing	FDA	[51, 52]
Cobas® HPV Test	Real time fluorescent PCR	DNA	Detect 14 high-risk types	Partial typing	FDA	[53]
Sansure biotech HPV Diagnostic Kit	Real time fluorescent PCR	DNA	Detect 23 types	Complete typing	NMPA	[54, 55]
Liferiver® HPV Genotyping Real Time PCR Kit	Real time fluorescent PCR	DNA	Detect 14 high-risk types	Partial typing	NMPA	[56]
BioPerfectus Multiplex Real Time HPV Assay	Real time fluorescent PCR	DNA	Detect 21 types	Complete typing	NMPA	[57]
Linear Array® HPV Genotyping Test	Reverse dot hybridization	DNA	Detect 37 types	Complete typing	CE	[58, 59]
INNO-LiPA® HPV Genotyping Extra II Assay	Reverse dot hybridization	DNA	Detect 32 types	Complete typing	CE	[60, 61]
YanengBIO HPV Genotyping Kit	Reverse dot hybridization	DNA	Detect 23 types	Complete typing	NMPA	[62]
Hybribio HPV GenoArray Diagnostic Kit	Reverse dot hybridization	DNA	Detect 37 types	Complete typing	NMPA	[63]
Digene HC2 hPV DNA Test	Hybrid capture	DNA	Detect 13 high-risk types	Unclassified	FDA	[61, 64]
DALTONbio® HPV Nucleic Acid Detection Kit	Hybrid capture	DNA	Detect 14 high-risk types	Partial typing	NMPA	[65]
Anyplex™ II HPV HR Assay	Melting curve analysis	DNA	Detect 14 high-risk types	Complete typing	CE	[66]
Zeesan MeltPro® Assay	Melting curve analysis	DNA	Detect 14 types	Complete typing	NMPA	[67]
Tellgenplex™ HPV DNA Test	Flow cytometry fluorescence hybridization	DNA	Detect 27 types	Complete typing	NMPA	[68]
SureX® HPV Genotyping Test	PCR and capillary electrophoresis	DNA	Detect 25 types	Complete typing	NMPA	[69]
QuantiVirus™ HPV E6/E7 mRNA Test	Branched-DNA	mRNA	Detect 14 high-risk types	Unclassified	CE	[70]
PapilloCheck® HPV-Screening Test	Gene chip	DNA	Detect 24 types	Complete typing	CE	[71, 72]
Cervista™ HPV HR Test	Enzyme digestion signal amplification	DNA	Detect 14 high-risk types	Unclassified	FDA	[73]
APTIMA® HPV Assay	Transcription mediated amplification	mRNA	Detect 14 high-risk types	Partial typing	FDA	[74, 75]

Cutting-Edge Nanotechnology in Biosensors

The unique properties of nanotechnology of biosensors platform emerged as a highly promising tool, offering rapid and precise diagnostics for cervical cancer. A recent study by Wang et al introduced a novel electrochemiluminescence (ECL) sensor for HPV16 DNA detection, utilizing Eu^{3+} doped polydopamine nanoparticles (PDA:Eu NPs). To enhance sensor sensitivity, a hydrogel reductive copper (I) particle catalyst was integrated, inducing more hydroxyl radicals for signal strengthening. By employing a T7 exonuclease-mediated cycling process, the sensor demonstrated a linear response to target DNA concentrations from 1 nmol/L to 100 nmol/L, with a limit of detection (LOD) of only 0.6 nmol/L.[85] Another highly sensitive ECL biosensor, named "signal-off" for HPV16 detection, was developed. This biosensor utilized a novel PCN-224/nano-zinc oxide composite enhanced by polyacrylamide to improve water solubility and stability. Signal amplification is achieved through exonuclease-based cycling cleavage and hybridization chain reaction (Figure 1A).[86]

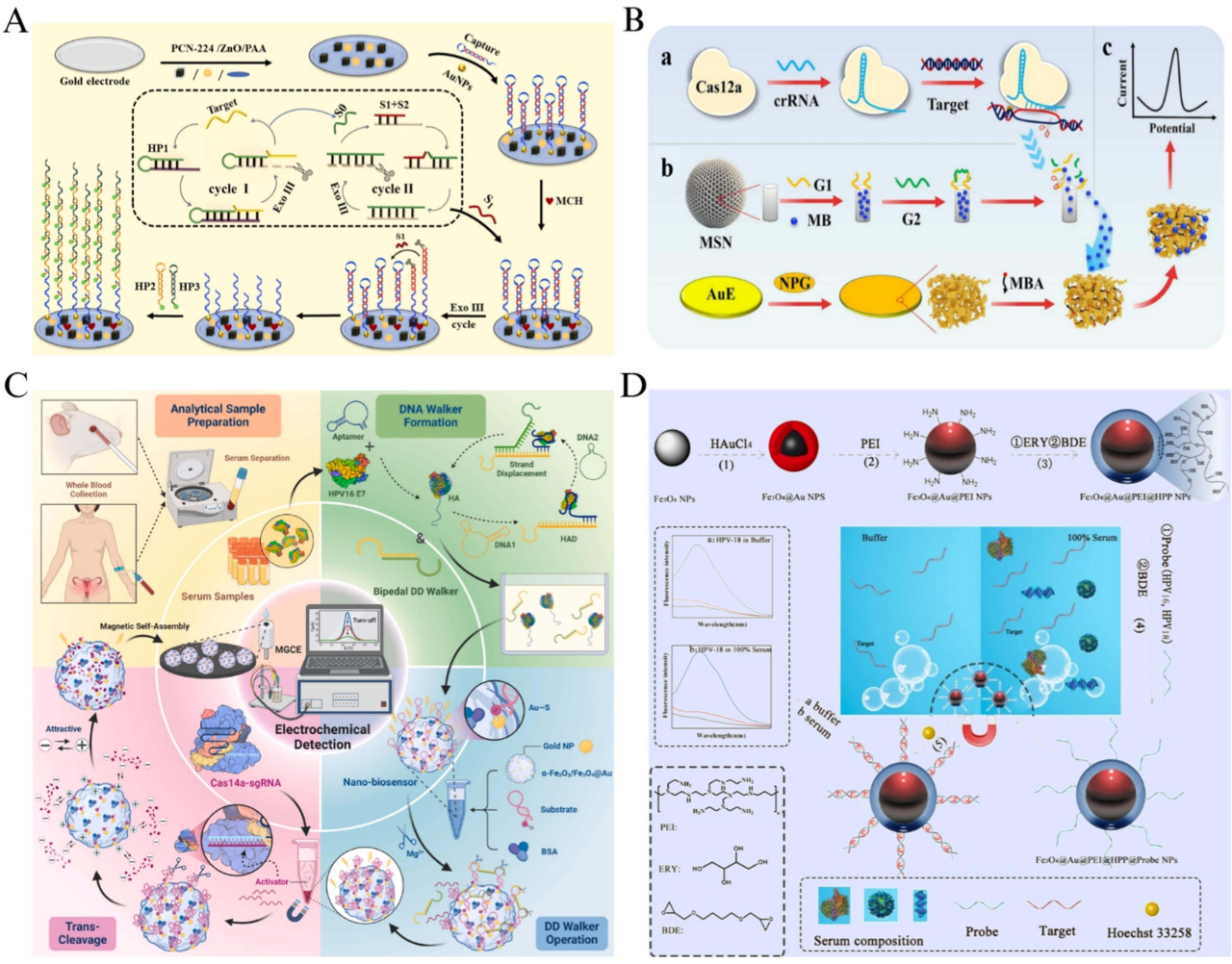

Figure 1 (**A**) Schematic diagram for the ECL biosensing platform based on PCN-224/ZnO nanocomposites coupled with cyclic amplification and chain reaction for HPV-16 assay. Reproduced from Wu D, Dong W, Yin T, et al. PCN-224/Nano-Zinc oxide nanocomposite-based electrochemiluminescence biosensor for Hpv-16 detection by multiple cycling amplification and hybridization chain reaction. Sensors and Actuat B Chem. 2022;372:132659. © 2022 Elsevier B.V. All rights reserved.[86] (**B**) Illustration of the modularized electrochemical sensing strategy based on CRISPR/Cas-mediated controllable MB release/enrichment system for ultrasensitive determination of HPV-16, including target recognition module (a), signal amplification module (b), and signal transduction module (c).Reproduced from Wang H, Niu Y, Liu H, et al. A modularized universal strategy by integrating CRISPR/Cas with nanoporous materials for ultrasensitive determination of nucleic acids. Chem Eng J. 2025;506:160065. © 2025 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.[88] (**C**) Schematic diagram of an electrochemical nanobiosensor with CRISPR/Cas14a system integrated with bipedal DD walker to detect HPV16 E7 serum samples. Reproduced from Yue Y, Liu M, Ma M, et al. CRISPR/Cas14a integrated with DNA walker based on magnetic self-assembly for human papillomavirus type 16 oncoprotein E7 ultrasensitive detection. Biosens Bioelectron. 2025;272:117135. © 2025 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.[89] (**D**) Schematic illustration of the fabrication procedures of Fe_3O_4@Au@PEI@HPP NPs and the strategy of HPV genotype detection in buffer or in 100% serum based on Fe_3O_4@Au@PEI@HPP NPs. Reproduced from Chen L, Liu M, Tang Y, et al. Preparation and Properties of a Low Fouling Magnetic Nanoparticle and Its Application to the HPV Genotypes Assay in Whole Serum. ACS Appl Mater Interfaces. 2019;11(20):18637–18644. Copyright © 2019 American Chemical Society.[91]

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A novel study presented a highly sensitive ECL biosensing strategy for HPV16 DNA detection, utilizing CRISPR/Cas12a to regulate Pdots-DNA binding and enhance ECL emission through the local surface plasmon resonance effect of AuNPs, achieving a detection limit of 3.2 fM.[87] Recently, Wang et al (2025) developed a modularized electrochemical sensing strategy that integrates CRISPR/Cas12 with nanoporous materials, enabling ultrasensitive nucleic acid detection. This strategy achieved a detection limit of 0.41 fM for HPV16 and demonstrated universality in detecting HPV18 (Figure 1B).[88] In a similar vein, Yue et al (2025) created a label-free electrochemical platform that combines CRISPR/Cas14a with DNA walkers and magnetic self-assembly. This innovative approach allows for the ultrasensitive detection of HPV16 E7, with a detection limit of 67.17 fg/mL and recovery rates of 98.46%–115.78% in serum samples (Figure 1C).[89] Additionally, Wang et al presented a label- and modification-free Cas12a-based ECL biosensor, which can detect HPV16 DNA at an ultralow concentration of 0.63 pM in approximately 60 minutes, showcasing great potential for point-of-care diagnostics.[90] These developments highlight the power of combining advanced technologies like CRISPR systems with nanomaterials to enhance detection capabilities.

Based on electrochemical genosensor array for the simultaneous and sensitive detection of high-risk HPV DNA sequences,[92–94] the optimization of novel nanocomposite materials provided a promising platform, such as a 3-aminopropyltriethoxysilane (APTES) modified gold electrode coupled with a super sandwich structure,[95] a graphitic nano-onion/molybdenum disulfide (MoS_2) nanosheet composite,[96] a novel nano-composite of Perylene Tetra carboxylic acid functionalized copper nanoparticles and reduced graphene oxide (Cu-PTCA/rGO),[97] and rGO and DNA nano-biohybrid-coated carbon screen-printed flexible electrodes (CSPEs).[98]

Breakthroughs in Biochip Technologies

In recent advances, a biochip allows hundreds of samples to be analyzed simultaneously with high efficiency, thereby making it possible for detecting multiple HPV subtypes.[99] A DNA microarray system utilizing a bipolar integrated circuit photodiode array (PDA) chip, through a gold nanoparticle-mediated silver enhancement technique, improved the detection limit from 1.2 nM to 30 pM and expanded the range of detectable DNA concentrations by adjusting the silver development time.[100] Chen et al's study presented an innovative nanoparticle probe, featuring gold magnetic particles modified with polyethyleneimine and hyperbranched polyether polyol, which exhibited low fouling and high stability in complex biological systems, and have been successfully applied for the sensitive and selective fluorescence detection of high-risk HPV genotypes 18 and 16 in buffer and whole serum, demonstrating strong linearity and low detection limits (Figure 1D).[91]

QDs for HPV DNA Detection

Types of QDs

QDs, as intricate assemblies of atoms and molecules, can be crafted from a single semiconductor material or a combination of multiple semiconductors.[101] Predominantly, QDs are composed of atoms from IIB-VI, III–V, or IV–VI groups of the periodic table that have 1~10 nm size dimensions, with CdY (Y represents S, Se, Te) being a common choice, alongside composite and multilayer structures.[102–104] QDs mainly encompasses four primary types: mononuclear, core-shell, doped, and alloyed. Mononuclear QDs, characterized by a high density of surface defects, typically exhibit low quantum yield and inferior luminescence stability. In contrast, the latter three categories of QDs have been engineered to address and ameliorate these deficiencies. QDs transcend conventional notions of "dots", as they consist of hundreds to thousands of atoms, yet confine their internal electron motion to a very limited spatial scale.[101] This unique confinement endows QDs with distinctive physical and chemical properties, most notably their exceptional optical characteristics. These attributes have catapulted QDs to the forefront of applications in in vitro diagnostics and live cell imaging, underscoring their broad potential and value in the biomedical sciences.[105–107]

Preparation of QDs

The preparation of QDs has undergone significant refinement over the years, converging on two predominant methodologies: physical and chemical approaches, with the latter frequently taking precedence in contemporary practice. Within

the chemical domain, two principal strategies have emerged for the fabrication of QDs: one is to synthesize them in organic systems using colloidal chemistry, and the other is to synthesize them in aqueous solutions.

Metal Organic Compound Synthesis Method

The metal-organic compound synthesis method refers to the method of preparing QDs based on the high-temperature cracking reaction between organic compounds and inorganic metal compounds or organic metal compounds in the presence of ligands. This approach is a significant breakthrough in the early field of QD research. In a seminal work in 1993, Bawendi et al[108] pioneered the synthesis of highly luminescent CdSe QDs, employing dimethyl cadmium ($Cd(CH_3)_2$), trioctylphosphine selenide (TOPSe), and trioctylphosphine oxide (TOPO) as precursors within a coordinated solvent system. The insolubility of CdSe nanoparticles in methanol allows for the acquisition of nanoparticles with favorable quantum yield through a simple centrifugation process post-methanol addition. Nonetheless, this technique is encumbered by several significant limitations, including intricate procedural steps, the challenging control of reaction conditions, and the use of highly toxic and flammable precursors. These constraints have impeded the broader adoption of this method in the synthesis of CdSe QDs, suggesting that researchers need to make improvements in synthesis methods and reaction reagents to synthesize QDs with higher quality.

TOPO is the most commonly used solvent for synthesizing colloidal nanocrystals. Common methods include preparing CdSe nanoparticles by mixing $Cd(CH_3)_2$ and TOPSe through nucleation, followed by maturation, annealing, and selective deposition to ultimately separate high-quality CdSe QDs. The particle size can be controlled by changing the temperature.[109] Furthermore, Zhang et al[110] developed a new method for producing hyperbranched Co_2P nanocrystals with uniform size, shape, and symmetry using TOPO as a solvent and phosphorus source. The morphology of the nanocrystals can be controlled from a layered structure to a hexagonal symmetric structure by changing the concentration of surfactants. The synthesis of colloidal small-size CdS QDs is typically fraught with challenges, including low particle yields and the concurrent formation of byproducts such as precursor compounds (PCs) associated with magic-size clusters (MSC). However, Li et al[111] have illuminated a novel pathway in the field. Their work demonstrates that the introduction of TOPO can effectively fragmentize the PCs, thereby facilitating the nucleation and growth of small-size QDs at room temperature. This innovation presents a groundbreaking method for the production of small-size QDs, eliminating the issue of PC coexistence and significantly enhancing particle yield (Figure 2A).

Aqueous Inorganic Synthesis Method

QDs synthesized via organic methods are often limited in their solubility, being soluble primarily in specific non-polar or weakly polar organic solvents. This limitation hinders their direct application in aqueous environments, which are prevalent in many analytical and biological systems. To surmount this, the surface of QDs must be modified with appropriate ligands, enabling their transfer into the aqueous phase for further analysis and application. In this context, the exploration of direct aqueous-phase synthesis of QDs is of paramount importance, promising to expand the versatility and applicability of QDs in scientific research and clinical diagnostics.

The direct synthesis of QDs in the aqueous phase offers several unparalleled advantages, including operational simplicity and cost-effectiveness compared to the intricate organic phase synthesis methods. It is characterized by high repeatability, minimal environmental impact, controllable surface charge and properties, excellent biocompatibility, suitability for mass production, and the ease of introducing functional groups. Consequently, this approach has emerged as a prominent research topic.[113,114] Pioneering work by Sondi et al[115] successfully synthesized CdSe QDs at room temperature by rapidly mixing aqueous solutions of either sodium selenide or selenourea with those of cadmium chloride in the presence of aminoglycan as stabilizing agent. While the luminescence efficiency of the resulting QDs is relatively low, and the preparation time for red fluorescent QDs is extended, the method's unparalleled advantages for biological applications make the refinement of this aqueous phase synthesis technique highly significant. Currently, the direct synthesis of water-soluble QDs in aqueous phase predominantly employs water-soluble thiol reagents, such as acetic acid (TGA) and propionic acid (MPA), as stabilizing agents. These thiol compounds are capable of coordinating and binding with the metal cadmium on the QD surface, effectively repairing surface defects and enhancing the stability of the QDs. Concurrently, the functional groups present in thiol reagents, including NH_2, COOH, and OH, act as functional

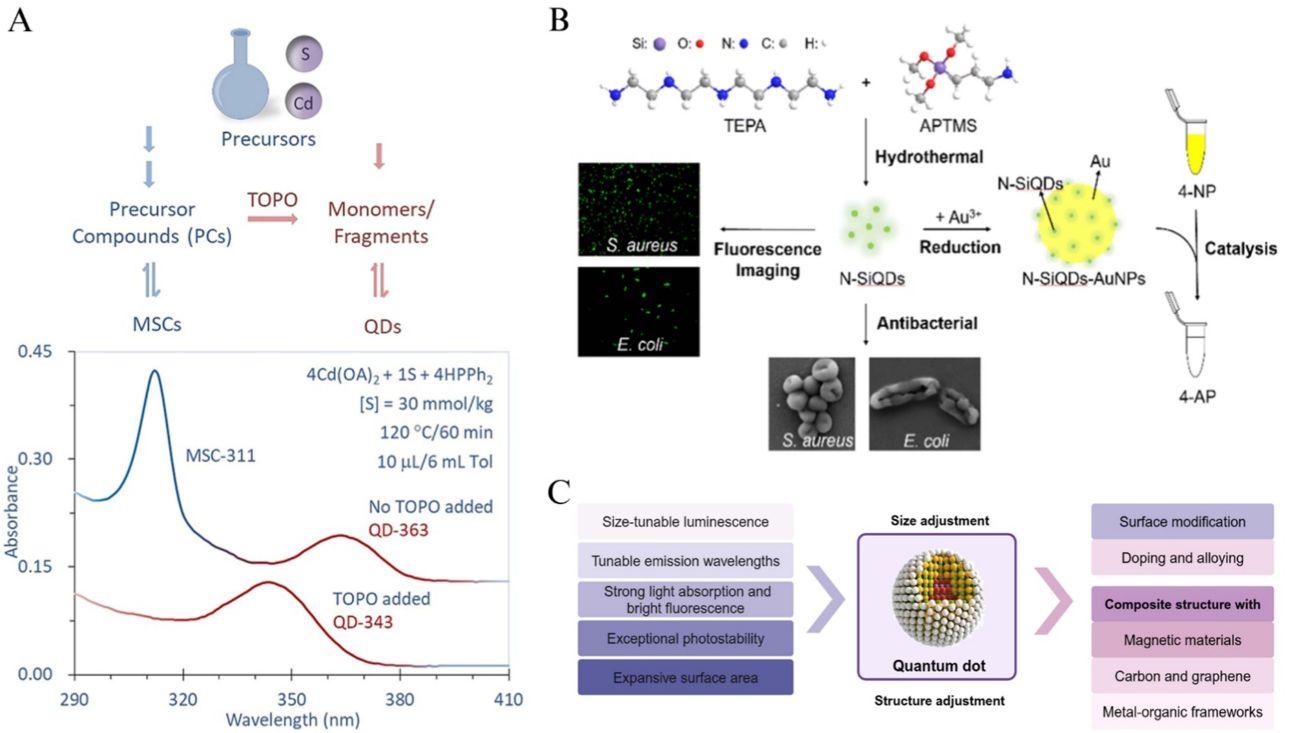

Figure 2 (**A**) Schematic drawing for our comprehension on the use of TOPO to fragmentize the PC that has formed in a prenuclation stage sample to facilitate the nucleation and growth of colloidal small-size CdS QDs with enhanced particle yield and without the coexistence of the PC and/or MSCs. Reproduced from Li L, Zhang J, Zhang M, et al. Fragmentation of Magic-Size Cluster Precursor Compounds into Ultrasmall CdS Quantum Dots with Enhanced Particle Yield at Low Temperatures. Angew Chem Int Ed Engl. 2020;59(29):12013–12021. © 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.[111] (**B**) Schematic diagram of the synthesis and multifunctional applications of N-SiQDs. Reproduced from Wang YF, Pan MM, Song YL, et al. Beyond the fluorescence labelling of novel nitrogen-doped silicon quantum dots: the reducing agent and stabilizer for preparing hybrid nanoparticles and antibacterial applications. J Mater Chem B. 2022;10(36):7003–7013. © 2022 Royal Society of Chemistry.[112] (**C**) This schematic represents the key features of QDs that are critical for HPV detection, emphasizing their size-adjustable luminescence, tunable emission for wavelength specificity, and robust photostability. It also illustrates the integration of QDs with various materials to form composite structures, enhancing their application in biosensing through magnetic separation, carbon and graphene interfaces, and metal-organic frameworks.

modification groups, which significantly improve the water solubility of the QDs.[116] In recent years, methods for preparing water-soluble QDs using other types of reagents as stabilizers have emerged. Wang et al[117] successfully prepared CdTe:Zn^{2+} QDs with strong fluorescence performance, low biological toxicity, and good biocompatibility by using glutathione as a stabilizer and incorporating Zn^{2+} ions through an aqueous inorganic synthesis method. Besides, Yang et al[118] successfully prepared $CdTe_xSe_{1-x}$ core-shell QDs modified with mercaptopropionic acid and L-cysteine using a high-temperature hydrothermal synthesis method. High-quality $Cd_xZn_{1-x}Se$ and $Cd_xZn_{1-x}Se$/ZnS core/shell QDs were prepared by using a one-step hydrothermal method, while the fluorescence properties and stability were significantly enhanced after capping with a ZnS layer. The prepared products have low cytotoxicity and can also detect Hg^{2+} ions with high sensitivity and selectivity.[119] These studies[113,118–120] also indicate that the core/shell/shell QD structure with doping in the shell layer is a versatile method for synthesizing and ameliorating doped QDs.

Notably, hydrothermal synthesis has been extensively utilized for fabricating multifarious QDs, catering to a multitude of applications. For instance, Paul et al[121] introduced a one-pot hydrothermal synthesis of gelatin quantum dots (GeQDs) with high photoluminescence quantum yield and remarkable stability, which were successfully applied for cell imaging across various clinical cell types, demonstrating their potential as non-toxic biomarkers for stable and long-term fluorescent imaging. Regarding carbon dots (CDs), Xian et al's study successfully synthesized red fluorescent CDs with high quantum yield and monochromaticity using a hydrothermal method, revealing a logarithmic correlation between their aggregation-induced emission wavelength and concentration.[122] Furthermore, Gao et al[123] ulteriorly proposed nitrogen-doped CDs as a versatile ratiometric fluorescence probe for the visual detection of hypochlorite and thiosulfate, which offers high sensitivity, selectivity, and biocompatibility, paving the way for the development of efficient fluorescent probes for visual detection and biomedical applications. Intriguingly, sulfur quantum dots (SQDs)

have emerged as promising candidates, distinguished by their low toxicity and exceptional luminescent capabilities. Most recent research by Shen et al synthesized red-light-emitting SQDs with high fluorescence efficiency and stability via a one-step hydrothermal process, demonstrating their potential as luminophores for fluorescence and ECL analysis, as well as for bio-labeling and imaging applications, by leveraging the use of an etching agent to tune emission and resorcinol to enhance ECL intensity.[124] Wang et al[125] presented a novel H_2O_2-assisted top-down synthesis of SQDs with high photoluminescence quantum yield and color tunability, enabling the creation of down-conversion white light emitting diodes with excellent color rendering, and highlighting the potential of these eco-friendly, water-soluble SQDs as luminescent materials derived from abundant precursors. Silicon quantum dots (SiQDs) have thoroughly proven their utility, underscored by their fluorescent properties. Wang et al's research presented the one-pot hydrothermal synthesis of nitrogen-doped silicon quantum dots (N-SiQDs) with multifaceted applications, including bacterial imaging due to their biocompatibility and fluorescence, the synthesis of N-SiQDs-stabilized gold nanoparticles with enhanced catalytic performance, and intrinsic antibacterial activity against both Gram-positive and Gram-negative bacteria, thereby expanding the horizons for SiQDs in nanocomposite and biomedicine applications (Figure 2B).[112]

Application of QDs in HPV DNA Detection

Conventional virus detection methods often suffer from low sensitivity and specificity, long time consumption, and high cost. Rapid, efficient, selective, and sensitive virus detection remains problematic. As shown in Figure 2C, QD has a series of remarkable characteristics, making it more suitable for detecting HPV.[126–130] A summary of the QDs-based developed biosensors for HPV detection is provided in Table 2.

The main application of QDs in HPV DNA detection is presented in various forms, such as fluorescence sensors, electrochemical sensors and the emerging carbon and silicon quantum dot detection photoelectric analysis, fluorescence analysis, electroanalysis and colorimetric analysis, etc.[134,135,137–139] The function of quantum dots mainly depends on its structural regulation, including the surface of quantum dots, doping and alloying of quantum dots, and composite structure of quantum dots (Figure 2C). Innovative applications of QDs are also combined with traditional methods in situ hybridization and PCR synergies. The application of QDs-based detection technology in the diagnosis and monitoring of cervical cancer has demonstrated its versatility and efficiency, positioning it as a pivotal tool for future screening and diagnostic procedures.

Size Adjustment of QDs and HPV Detection

The luminescence mechanism of quantum dots reveals that their fluorescence properties are intrinsically linked to the size of the energy gap. Specifically, the width of the energy gap between the valence band and the conduction band is directly influenced by the size of the QDs. An innovative photoelectrochemical (PEC) biosensor, engineered with precise quantum size control to harness quantum confinement effects, was developed for the detection of HPV16. This was achieved through the CRISPR-Cas12a (Cpf1)-mediated disassembly of a Z-scheme heterojunction, leveraging the biosensor's unique response to the targeted viral DNA. Li et al perfectly exploited the sensor's ability to combine biomolecular recognition with photon-to-electron conversion capabilities and designed a PEC biosensor that can detect HPV16 by controlling the quantum size, thus improving the PEC reaction performance (Figure 3C).[140]

Structure Adjustment of QDs and HPV Detection

Surface Modification of QDs

Through the use of the photoelectric analysis technique, AuNPs' surface can accumulate metallic silver. The target DNA will ultimately be quantified into the corresponding electrical signal after a sequence of steps.[142] The Au NP-modified Keggin-type polyoxometalate (SiW_{12})-grafted CdS quantum dots (SiW_{12}@CdS QDs) demonstrated exceptional selectivity and sensitivity in target DNA detection, attributed to its outstanding PEC response (Figure 3A and B).[141] Sun et al's work presented a novel photoelectrochemical biosensor array (PEBA) platform for HPV genotyping that utilizes TiO_2@AuNPs and CdS QDs-labeled DNA probes. The presence of HPV targets triggers a conformational change in the probes, altering the PEC signal and enabling sensitive detection with a low limit of 0.1 copies/μL across a linear range for nine HPV types (Figure 4).[134] Researchers have crafted an ultrasensitive nanobiosensor for the detection of HPV18 by leveraging the

Table 2 A Summary of the QDs-Based Developed Biosensors for HPV Detection

QDs-Based Detection System	Detection Type	Detection Target	Detection Range	Detection Limit	Country	Year	Ref.
CdTe QDs/NiTPP composite sensor	FD	HPV16 HPV18	0.1–40 nM 0.05–40 nM	62 pM 40 pM	CHN	2024	[131]
CQDs/APTES/Au	ECD	HPV16	100 fM-100 nM	0.73 fM	CHN	2024	[132]
Cys-GQDs LOC-Genosensor	ECD	E6 and E7 oncogenes	-	26 fM (79.6 fM for quantification)	BR	2024	[133]
SiW_{12}@CdS QDs	PEC	HPV16	15–130 nM	0.8 nM	CHN	2023	[134]
Zn-doped MoS_2 QDs	ECL	HPV16	0.1–200 nM	0.03 nM	CHN	2020	[135]
PDDA-GO/GQDs/DNA-gold NPs/target DNA electrode	ECL	Target DNA	1.0 pM-1000 nM	0.1 pM	CHN	2019	[136]
MGPs-DNA target-CdTe/ZnSe core/shell QDs	FD	L1-HPV and HPV16 E6	-	0.0125 ng/μL	CZ	2019	[137]
FRET using CdTe QDs	FRET	HPV18	1.0–50.0 nM	0.2 nM	IR	2017	[138]

Abbreviations: APTES, 3-aminopropyl)triethoxysilane; CQDs, Carbon quantum dots; Cys, Cysteamine; ECD, Electrochemical detection; ECL, Electrochemiluminescence; FD, Fluorescence detection; FRET, Fluorescence resonance energy transfer; GO, Graphene oxide; GQDs, Graphene quantum dots; SiW_{12}, Keggin-type polyoxometalate; LOC, Lab-on-chip; MGPs, Magnetic glass particles; MoS_2, Molybdenum disulfide; NPs, Nanoparticles; PDDA, Poly (diallyldimethylammonium chloride); NiTPP, Porphyrin derivatives; PEC, Photoelectrochemical; QDs, Quantum dots.

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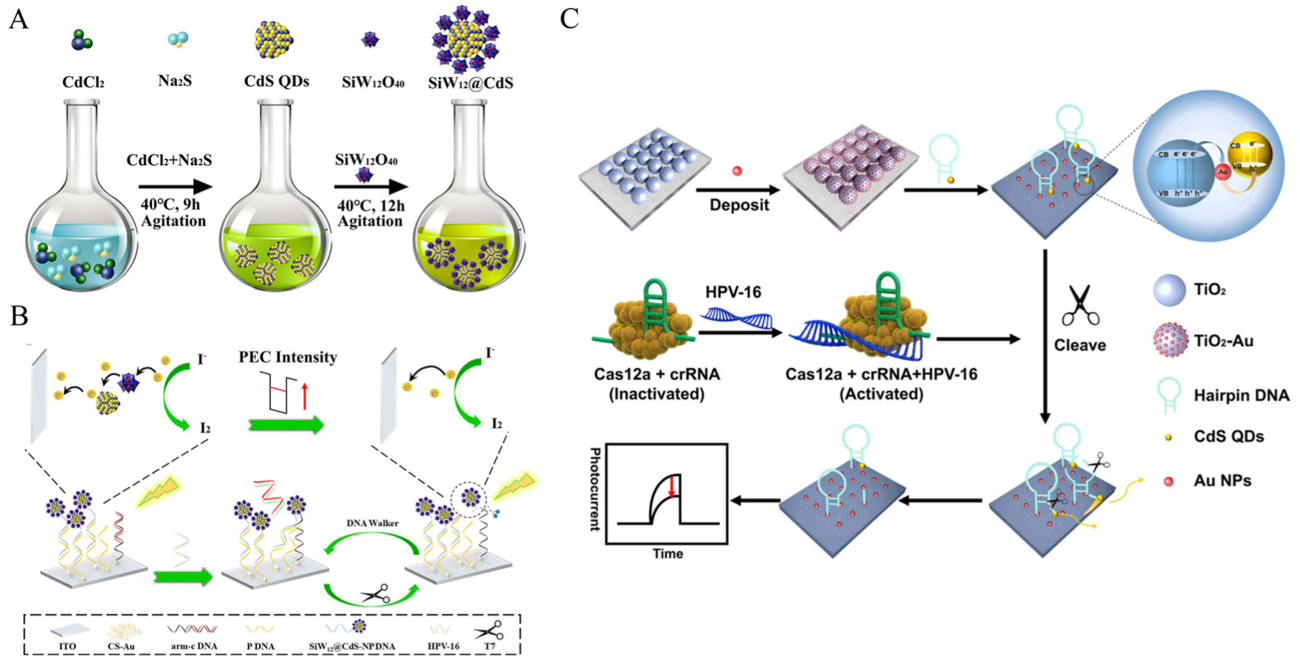

Figure 3 (**A**) Schematic illustration of the synthesis of POM@CdS QD composites. (**B**) Schematic illustration of the PEC sensor for detecting HPV 16 DNA. Reproduced from Cheng Y, Sun C, Chang Y, et al. Photoelectrochemical biosensor based on SiW12@CdS quantum dots for the highly sensitive detection of HPV 16 DNA. Front Bioeng Biotechnol. 2023;11:1193052. Copyright © 2023 Cheng, Sun, Chang, Wu, Zhang, Liu, Ge, Li, Li, Sun and Zang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY).[141] (**C**) Schematic illustration of CRISPR-Cas12a-based PEC biodetection toward HPV-16 by disassembly of Z-Scheme heterojunction among TiO_2, Au NPs, and CdS QDs. Reproduced from Li Y, Zeng R, Wang W, et al. Size-Controlled Engineering Photoelectrochemical Biosensor for Human Papillomavirus-16 Based on CRISPR-Cas12a-Induced Disassembly of Z-Scheme Heterojunctions. ACS Sens. 2022;7(5):1593–1601. Copyright © 2022 American Chemical Society.[140]

synthesis of water-soluble CdTe QDs. These dots were functionalized with amino-modified oligonucleotides to form QDs-DNA conjugates that, upon interaction with target DNA and a Cy5-labeled oligonucleotide, assemble into sandwich hybrids. This assembly facilitates a precise and sensitive fluorescence resonance energy transfer (FRET)-based detection assay, demonstrating a linear detection range from 1.0 to 50.0 nM and an impressively low limit of 0.2 nM.[132]

Doping and Alloying of QDs

Doped QDs in which foreign atoms or ions are introduced into the lattice structure with new optical and electrical properties, are commonly used to improve their luminescence efficiency, adjust the wavelength of light emission, or enhance their stability. Nie et al[133] developed an ECL sensor that capitalizes on the synergistic enhancement strategy of Zn-doped MoS_2 QDs and reductive Cu(I) particles. This biosensor achieved sensitive detection of HPV16 DNA over a range from 0.1 nmol/L to 200 nmol/L, with a LOD as low as 0.03 nmol/L. Notably, the ECL signal, captured by a smartphone, can be transformed into high-resolution images through software processing. This innovation presents a significant potential for point-of-care HPV16 DNA testing in the future (Figure 5).

Composite Structure of QDs and HPV Detection

QDs can be further expanded by combining with magnetic materials, carbon and graphene, and metal-organic frameworks (MOFs), presenting improved performance, or new functionalities in probe or biosensor platform for HPV detection (Figure 2C).

Combining with Magnetic Materials

A novel hybridization assay for the detection of HPV16 infections has been developed, leveraging the combined advantages of QDs and superparamagnetic NPs. This assay offers a rapid and straightforward method that is significantly faster and more convenient than the conventional type-specific PCR method.[136] Furthermore, researchers have developed a magnetic glass particle (MGP) with a DNA probe attached to its surface to hybridize with the target DNA. Eventually, the MGP probe DNA

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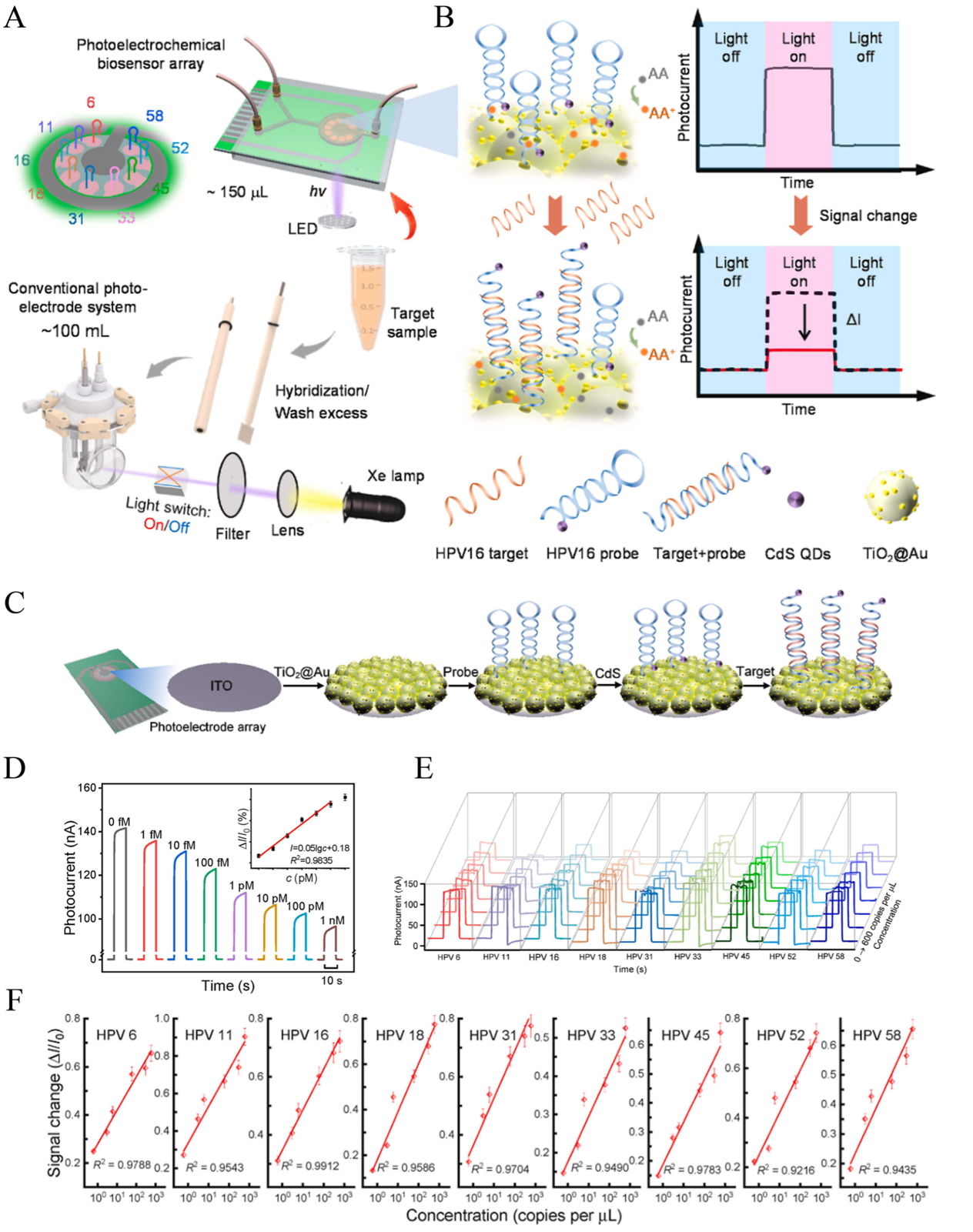

Figure 4 (**A**) Schematic illustration of the PEBA setup for HPV genotyping and a comparison between the proposed PEBA and conventional photoelectrochemical testing system. (**B**) Schematic depiction of the fabricated PEBA for detecting HPV-related genes. (**C**) Schematics of the PEBA assembly process. (**D**) Photocurrent responses of the PEBA toward different concentrations of synthetic HPV16 oligonucleotide standard with from 0 fM to 1 nM. Insert: the calibration curve of ΔI/I0 versus HPV16 target concentration. (**E**) Photocurrent responses of the PEBA to different concentrations of HPV DNA subtype plasmids (0, 0.6, 3, 6, 60, 300, 600 copies/μL). (**F**) The calibration curves of photocurrent responses versus the HPV DNA concentrations from 0.6 to 600 copies/μL. Error bars represented the standard deviations of three independent experiments. Reproduced from Sun Y, Liu J, Peng X, et al. A novel photoelectrochemical array platform for ultrasensitive multiplex detection and subtype identification of HPV genes. Biosens Bioelectron. 2023;224:115059. © 2023 Published by Elsevier B.V.[134]

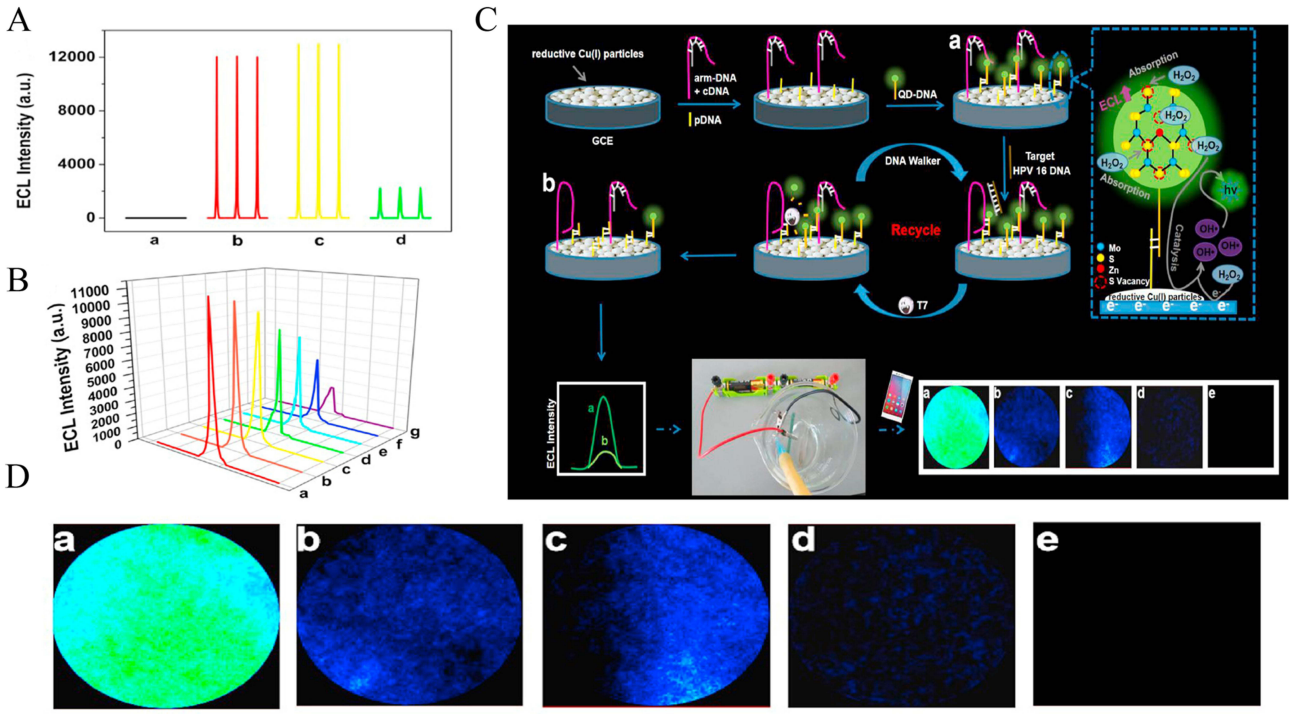

Figure 5 (**A**) The ECL performance. (**B**) ECL response of the biosensor with (a) 0.1 nM, (b) 1 nM, (c) 10 nM, (d) 20 nM, (e) 50 nM, (f) 100 nM and (g) 200 nM HPV 16 DNA. (**C**) Schematic illustration of reductive Cu(I) particles catalyzed Zn-doped MoS_2 QD-based ECL biosensor. (**D**) Visualized ECL images processed by the self-developed software with (a) 0.1 nM, (b) 10 nM, (c) 50 nM, (d) 100 nM and (e) 200 nM HPV 16 DNA. Reproduced from Nie Y, Zhang X, Zhang Q, et al. A novel high efficient electrochemiluminescence sensor based on reductive Cu(I) particles catalyzed Zn-doped MoS2 QDs for HPV 16 DNA determination. Biosens Bioelectron. 2020;160:112217. © 2020 Elsevier B.V. All rights reserved.[133]

hybrid combines with CdTe/ZnSe core/shell QDs to detect HPV infection using quantum dot-nucleotide specific interactions (Figure 6D).[144] These studies have successfully substantiated the clinical efficacy of these innovative assays. The marked reduction in detection time, coupled with the simplicity of the process, endows the approach with immense potential for clinical applications, particularly in the realm of large-scale epidemiological screening. Notably, aside from detecting HPV16, QDs-based biosensors can also help identify different types of HPV. A novel biobarcoded analytical method was developed to detect multiple DNA sequences simultaneously based on the CdSe/ZnS QDs and magnetic microparticle, offering enhanced sensitivity, rapid preparation, and simplified analysis compared to traditional biobarcode assays.[146]

Combining with Carbon and Graphene

Carbon quantum dots (CQDs) and graphene quantum dots (GQDs) are nanomaterials with excellent biocompatibility and non-toxicity, featuring high charge transfer capabilities and useful for analytical detection and quantification in electrochemical applications.[131,147] Utilizing a novel CQDs/(3-aminopropyl)triethoxysilane (APTES) composite nanofilm to enhance sensitivity, researchers have developed a highly sensitive electrochemical biosensor for the detection of HPV16, with remarkable linear range and LOD of 100 fM-100 nM and 0.73 fM, respectively (Figure 6A–C).[143] Furthermore, Léony S Oliveira et al[148] introduced an innovative electrochemical genosensor for the detection of HPV oncogenes E6 and E7. The sensor utilized a nanostructure based on cysteine and GQDs, which not only provided a rich environment for functional groups and surface area but also enhanced electrochemical properties. The sensor demonstrated high sensitivity and selectivity, with a detection limit of 26 fM and a quantification limit of 79.6 fM. Researchers have developed a highly sensitive ECL biosensor for DNA detection by harnessing the ECL properties of GQDs and a multiple cycling amplification technique, achieving good selectivity and high sensitivity for HPV16 DNA and showcasing potential for point-of-care screening (Figure 6E).[145]

Combining with Metal-Organic Frameworks (MOFs)

Metal-organic frameworks (MOFs) exhibit great potential in applications as fluorescent sensors and luminescent probes.[149,150] Functionalizing MOFs with QDs that possess ECL activity results in a synergistic combination that

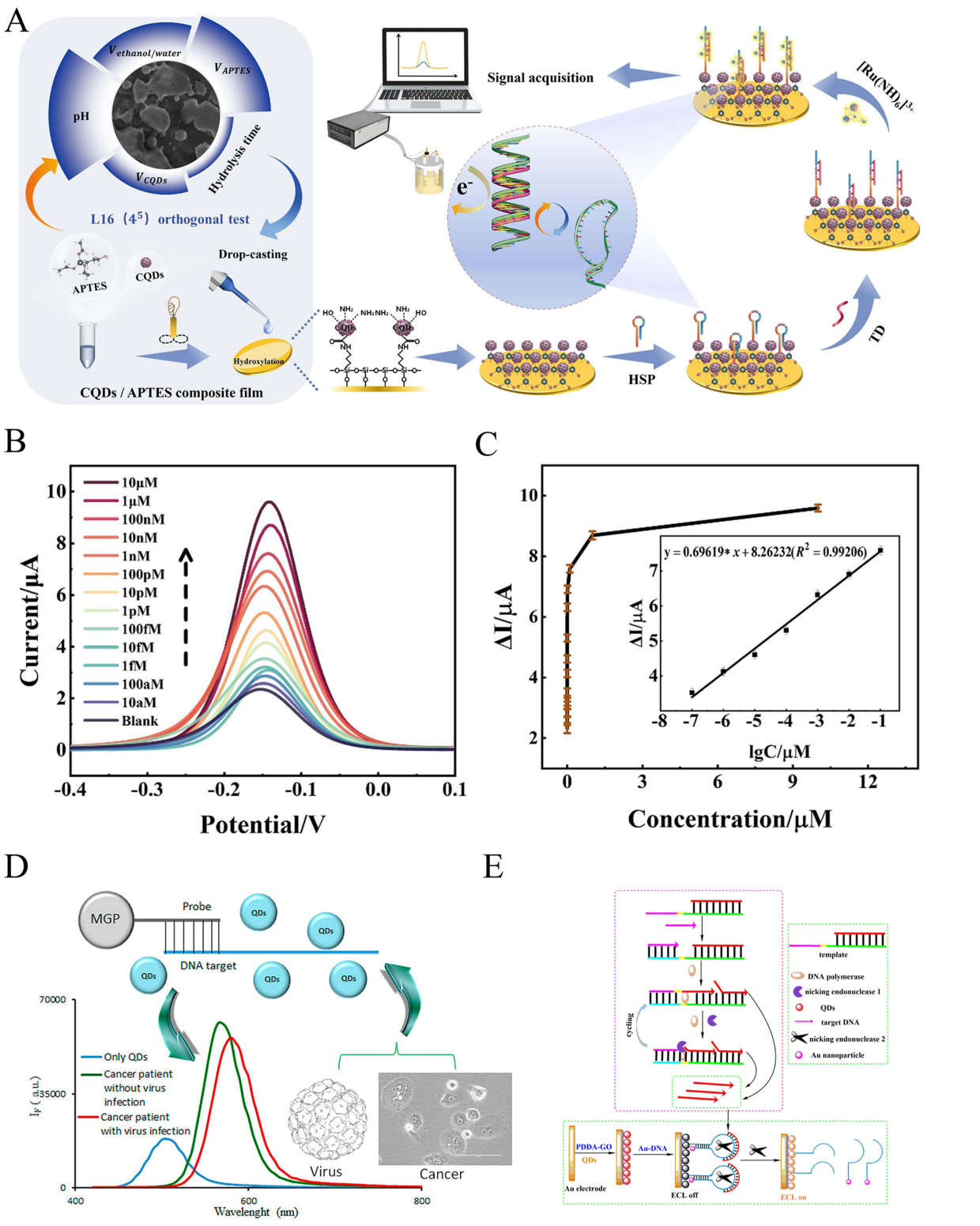

Figure 6 (**A**) Schematic representation of the proposed electrochemical DNA biosensor. (**B**) DPV responses of the proposed biosensor with various TD concentrations for HPV-16 detection. (**C**) the correlation between DPV peak current ΔI and TD concentrations, obtained by an inserted calibration with the logarithm of the TD concentrations, error bars = SD (n = 3). Reproduced from Yu J, Dong C, Yang Y, et al. Electrochemical DNA biosensor for HPV-16 detection based on novel carbon quantum dots/APTES composite nanofilm. Microchem J. 2024;204:110949. © 2024 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.[143] (**D**) Optical biosensing system utilizing MGPs and CdTe/ZnSe QDs coupled with nucleic acid probes. Reproduced from Jimenez Jimenez AM, Moulick A, Bhowmick S, et al. One-step detection of human papilloma viral infection using quantum dot-nucleotide interaction specificity. Talanta. 2019;205:120111. © 2019 Elsevier B.V. All rights reserved.[144] (**E**) Schematic representation for principle of sensitive DNA detection based on GQDs ECL coupled with cycling amplification technique. Reproduced from Jie G, Zhou Q, Jie G. Graphene quantum dots-based electrochemiluminescence detection of DNA using multiple cycling amplification strategy. Talanta. 2019;194:658–663. © 2018 Elsevier B.V. All rights reserved.[145]

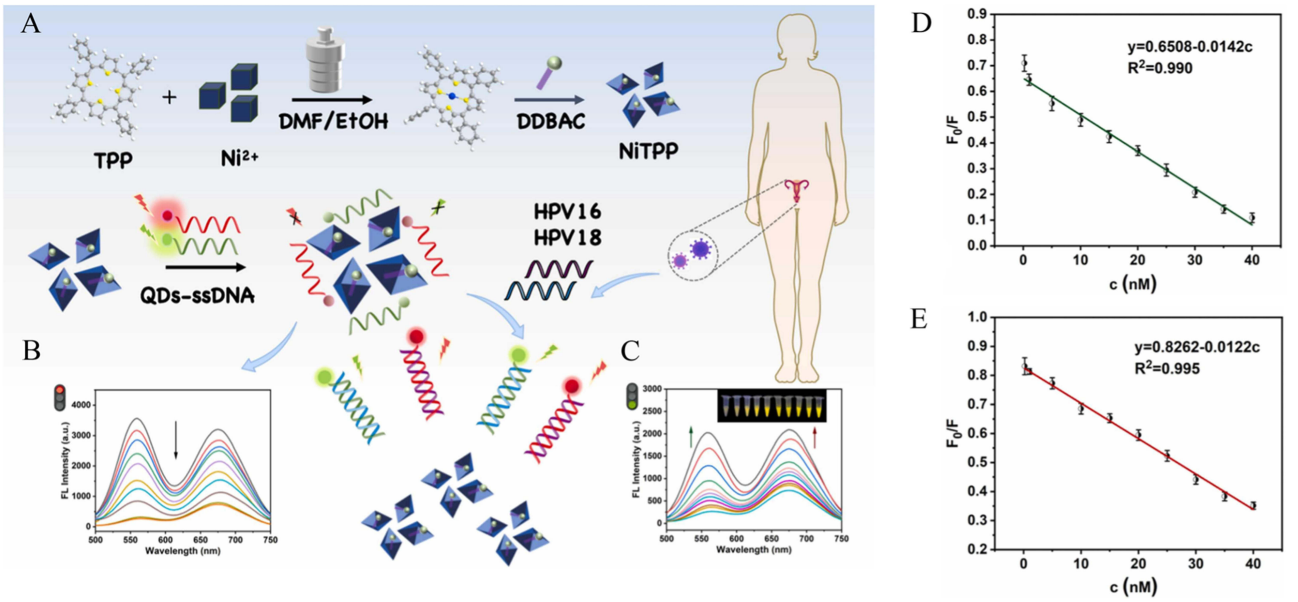

Figure 7 (**A**) Schematic outlining the method for analyzing HPV16 and HPV18. (**B**) Fluorescence spectra of QDs-ssDNA reacting with NiTPP solutions of different concentrations (Ex=300 nm, Em_{gQDs}=567 nm, Em_{rQDs}=678 nm). (**C**) Fluorescence spectra of QDs-ssDNA reacting with different concentrations of HPV16 and HPV18 (Inset: fluorescence changes under ultraviolet lamp). (**D**) Linear relationship between gQDs-gDNA and HPV16. (**E**) Linear relationship between rQDs-rDNA and HPV18. reproduced from Jiang X, Yin C, Wu M, et al. Fluorescent switch based on QDs modified DNA probe and NiTPP for simultaneous dual color sensitive sensing of HPV16 and HPV18. Sensors and Actuat B Chem. 2024;403:135128. © 2023 Elsevier B.V. All rights reserved.[153]

leverages the high accumulation and catalytic capabilities of MOFs with the luminescent properties of QDs, thereby significantly boosting the ECL emission.[151,152] Yang et al's study demonstrated a marked improvement in ECL efficiency by integrating CdTe QDs into isoreticular metal organic framework-3 (IRMOF-3), through both internal encapsulation and external surface decoration.[152] In 2024, researchers have successfully developed an innovative "off-on" fluorescent sensor, leveraging the properties of water-soluble dual-color emitting CdTe QDs conjugated with ssDNA probes. The sensor utilizes porphyrin derivatives (NiTPP) to quench the fluorescence, enabling a highly sensitive response. In vitro detection of HPV16 and HPV18 demonstrated excellent linearity within the ranges of 0.1–40 nM and 0.05–40 nM, respectively, with detection limits reaching as low as 62 pM and 40 pM. This method has been successfully developed and validated using spiked urine and cervical swab samples, showcasing its capability for the quantitative and sensitive detection of HPV16 and HPV18 concurrently (Figure 7).[153]

Conclusions and Perspectives

Currently, nanotechnology has brought revolutionary changes to various fields. Among them, QDs have emerged as a transformative tool in the detection of HPV. Their unique optical properties, such as broad absorption spectra, size-tunable emission, and exceptional photostability, have made them a powerful tool for improving the accuracy and sensitivity of HPV detection regarding the limitations of traditional methods. The development of biosensors based on QDs has opened up new possibilities for the diagnosis of cervical cancer based on photoelectric analysis, fluorescence-based detection, and electrochemical genosensors. These biosensors can detect multiple genotypes of HPV simultaneously, providing more comprehensive information for screening. In addition, the combination of QDs with other nanomaterials, such as AuNPs, heavy-metal-free carbon and graphene, has further enhanced the detection efficiency and reduced the complexity of the detection process. The transition from laboratory-based testing to point-of-care testing is gaining momentum, with the development of portable devices and simplified protocols.

However, the trajectory of HPV detection technology is poised for further innovation and refinement. The pursuit of point-of-care testing solutions will continue, with a focus on miniaturization, user-friendliness, and cost-effectiveness. Long-term use could lead to the release of toxic ions, damaging cells and organs. To address this, researchers are

exploring the development of biocompatible and non-toxic QDs.[154] Protocols for QD green synthesis and surface modification are being optimized. For instance, coating QDs with protective layers can reduce ion release. Also, the development of heavy-metal-free QDs is a promising direction. These approaches can ensure the reproducibility and reliability of QDs-based diagnostic tools for broader application. Green synthesis methods, such as using plant extracts or microorganisms, can produce QDs with lower toxicity. Surface modification can introduce functional groups to enhance stability and reduce immunogenicity, minimizing potential adverse reactions.[155,156] For instance, PEGylation of QDs has been shown to significantly reduce their cytotoxicity and improve their biocompatibility.[157] Adverse effects, such as inflammatory responses or oxidative stress, should be closely monitored. Long-term animal experiments and clinical trials are needed to assess potential toxicity. Studies have indicated that QDs with ZnS shell and PEG coating are more beneficial to cell proliferation compared to naked QDs, demonstrating the effectiveness of surface modification in enhancing QDs' biocompatibility and reducing toxicity.[154,157]

Looking ahead, several key development directions should be prioritized to further advance the field: (1) Enhanced novel materials and designs for QDs: The development of biocompatible and non-toxic QDs is a priority. Protocols for QD green synthesis and surface modification are being optimized to ensure the reproducibility and reliability of QDs-based diagnostic tools for the broader application. (2) Improvement in detection efficiency: By coupling various high-performance materials, the detection efficiency of biosensors will see continuous improvement. This will address the current limitations of insufficient LODs, poor stability, and meet the urgent need for efficient on-site detection and diagnosis. (3) Advances in multifunctional sensing: The combination of magnetic separation, microfluidic systems, and dielectrophoresis technologies with existing sensors is expected to yield highly sensitive sensors with multiple detection functions. (4) Suitability for promotion: The successful translation of QD technology from the laboratory to clinical settings will necessitate the ability to process a diverse range of specimens, including blood, urine, paraffin-embedded tissue, frozen tissue, and cytological samples. Additionally, it is essential to conduct further metabolism studies to ensure the safety of these technologies in humans. (5) Convergence with advanced technologies: The integration with mobile health technologies, artificial intelligence, and machine learning algorithms will enable promising real-time monitoring and data analysis, potentially transforming the way HPV and other diseases are detected and managed.

Significantly, to achieve these improvements, the role of collaborative efforts in driving research forward cannot be overstated. International partnerships, interdisciplinary research, and knowledge sharing will be vital in overcoming the remaining challenges and in translating these innovative technologies into routine clinical practice in the forthcoming years.

Data Sharing Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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