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CdSe-Co₃O₄@TiO₂ nanoflower-based photoelectrochemical platform probing visible light-driven virus detection

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Abstract:

To develop effective photoelectrochemical (PEC) detection, the photoinduced charge carriers' generation, separation, and interfacial reaction mechanisms must be characterized. This study describes the design and construction of a visible-light-driven PEC device based on a CdSe-Co₃O₄@TiO₂ nanoflower (NF). Moreover, an application for the ultrasensitive detection of viruses, such as hepatitis E virus (HEV), HEV-like particles (HEV-LPs), and SARS-CoV-2 spike protein in complicated lysate solution is demonstrated. The photocurrent response output of a PEC device based on CdSe-Co₃O₄@TiO₂ is enhanced compared to the individual components, TiO₂ and CdSe-Co₃O₄. This can be attributed to the CdSe QDs sensitization effect and strong visible light absorption to improve overall system stability. A robust oxygen-evolving catalyst (Co₃O₄) coupled at the hole-trapping site (CdSe), extends the interfacial carrier lifetime, and energy conversion efficiency was improved. The effective hybridization between the antibody and virus resulted in a linear relationship between the change in photocurrent density and the HEV-LPs concentration ranging from 10 fg mL⁻¹ to 10 ng mL⁻¹, with a detection limit of 3.5 fg mL⁻¹. This CdSe-Co₃O₄@TiO₂-based PEC device achieved considerable sensitivity, good specificity, acceptable stability and demonstrated a significant ability to develop an upgraded device with affordable and portable biosensing capabilities.

Keywords: Photoelectrochemical, hepatitis E virus, coronavirus, CdSe-Co₃O₄, PEC device, catalyst.

44 **Introduction:**

45 Photoelectrochemical (PEC) biosensor has attracted considerable interest among other
46 analytical methods due to their promising and burgeoning working principles [1- 4]. Its benefit
47 stems from the combination of optical and electrochemical properties. PEC was based on the
48 light-harvesting electrical response, making it a crucial component to amplify the
49 corresponding signals in biosensing design [5]. This light-harvesting property is closely
50 associated with photoreceptors, charge separation, and the interfacial redox reaction. Recently,
51 most techniques rely significantly on the light-harvesting capability and charge-transfer
52 kinetics of the PEC system. They overlooked the redox reaction at the surface of the
53 photoelectrode/electrolyte interface.

54 Building a PEC biosensor requires a decent photoactive material with several ideal
55 properties, such as fast exciton generation, migration, increased signal intensity, and stability.
56 Consequently, as the photogenerated carriers accumulate on the interfacial surface, the
57 semiconductor undergoes metal ions leaching due to photo corrosion [6, 7]. This leads to a
58 significant photocurrent decay and hampers the quantitative analysis. Boosting the catalytic
59 kinetics of the reaction at the interface will increase the charge separation of photogenerated
60 electron-hole pairs [8].

61 Quantum dot (QD)-metal oxide junctions have been explored as a key component in
62 developing next-generation PEC devices, light-emitting diodes, and nanostructured electronic
63 arrays [9]. Semiconducting QDs have received much attention for their size-dependent
64 electronic structure [10]. It offers a broad-spectrum design on various devices and systems with
65 tailored electrical characteristics by altering the size and complexity of component elements
66 [10, 11]. QDs were integrated and functionalized into biomolecules for bioimaging and
67 monitoring devices [12]. Although the electronic interactions between QDs and organic

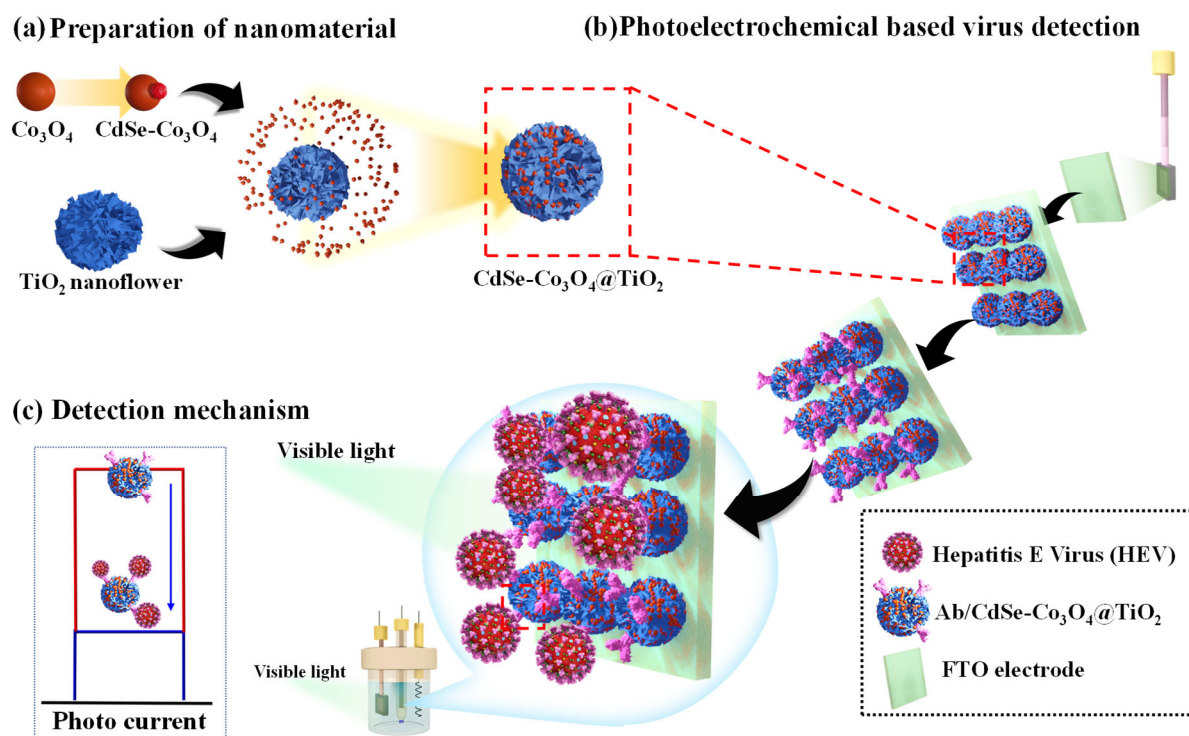
molecules have been well established, a recent trend showcased an interesting annotation on the coupling QDs to other inorganic species. Because inorganic materials have a continuum of electronic states rather than the discrete conditions seen in organic molecule receptors, the pairing is fundamentally different from QD-organic coupling. When QDs are coupled to an inorganic species, it acts either as an electron donor or both electron receptors and donor [13]. In the case of practical application, electron transfer processes are closely engaged in the QDs' adaptive function.

Titanium dioxide (TiO_2) is widely used in PEC biosensors due to its chemical stability, photocatalytic activity, and environmental friendliness [14, 15]. However, it suffers from the rapid interacting photogenerated charges, impeding the progress of TiO_2 -based PEC devices. Various strategies have been reported to suppress photogenerated electron-hole recombination: nanostructure engineering, doping enrichment, surface treatment, and heterostructure modification [16, 17]. Among the various strategies reported to suppress photogenerated electron-hole recombination, combining other semiconductors has been attempted to improve the overall photocatalytic performance. Designing a TiO_2 -based catalyst with a heterojunction structure on the surface would enable natural self-sustaining charge management, encouraging the separation of photogenerated carriers, and supplying electrons continuously for the catalysis reaction [18].

We have now elucidated interactions between unique nanostructured semiconducting metal oxides, TiO_2 and Co_3O_4 , focusing on a QD-implemented PEC device (**Scheme 1**). CdSe QDs semiconductors produce photo-introduced electron-hole pairs (e^-/h^+) under light irradiation [19, 20]. When combined with metal oxides like TiO_2 , the high efficiency of this heterostructure is compromised by its limited stability because of the trapping of oxidizing holes in chalcogenides nanocrystal subject to rapid photo corrosion of the material. More importantly, these catalysts cannot produce oxygen at their anodic site. Thus, coupling with a

robust oxygen-evolving catalyst (Co_3O_4) at the hole-trapping site (CdSe) will enhance the overall system's stability and benefit oxygen evolution [21, 22]. The excellent chemical stability and sensitive response of light irradiation make Co_3O_4 much attractive to researchers. The combination of CdSe , Co_3O_4 , and TiO_2 prevents electron-hole recombination and boosts photoinduced carriers' transfer. In addition, Co_3O_4 allows the loading of CdSe QDs and antibodies and acts as a hole collector in catalytic redox, preventing the rapid photo corrosion of the chalcogenide's nanocrystal by the oxidizing holes. The steric impedance of Co_3O_4 blocks the transfer of charge. As a result, $\text{CdSe-Co}_3\text{O}_4$ impacts steric impedance and competition, improving signal reduction from the photoactive matrix and enhancing the PEC sensor's sensitivity.

Further, observing the desired need for virus sensing in recent times, a viable embodiment of sensitive and accurate strategies, especially for detection in the early stage of infection rapidly and giving a reliable signal, is in demand. In addition, based on our previous experience with virus-like particles (VLP) and viruses [23-25], we have developed a PEC device based on $\text{CdSe-Co}_3\text{O}_4@\text{TiO}_2$ heterojunction structure and applied it for HEV-LPs detection. As shown in **Scheme 1b**, the target virus could be captured by antibody-functionalized $\text{Ab/CdSe-Co}_3\text{O}_4@\text{TiO}_2||\text{FTO}$ electrode. After washing with PBS, virus conjugated virus/ $\text{Ab/CdSe-Co}_3\text{O}_4@\text{TiO}_2||\text{FTO}$ electrode is introduced into the PEC cell and utilized for measuring photocurrent response (**Scheme 1c**) based on a PEC principle. Optimizing conditions for sensor formulation and its sensing parameters are investigated thoroughly. The designed PEC device based on $\text{CdSe-Co}_3\text{O}_4@\text{TiO}_2$ was used to quantitatively detect HEV-LPs, HEV, and spike (S) protein of SARS-CoV-2. This PEC device enabled the development of a realistic embodiment of highly sensitive analytical equipment and reliable detection for the suppression of emerging infectious viral diseases.



Scheme 1. Schematic representation illustrating the (a) fabrication process of $\text{CdSe-Co}_3\text{O}_4@\text{TiO}_2$ NF heterostructure and (b) application of virus detection, and (c) change in photocurrent response based on virus conjugation.

Materials and methods

Synthesis of cobalt oxide:

To synthesize spherical cobalt oxide nanoparticles (Co_3O_4 NPs), 80 mg cobalt acetate and 3.5 mL benzylamine were mixed under constant stirring for 2 h at room temperature. Further, 3.5 mL ammonium hydroxide solution was added to the above solution under vigorous stirring, and the temperature was increased to 165°C and maintained for 2 h under continuous stirring. Diethyl ether was added to the above solution, and thus formed black precipitate was collected by centrifugation and washed several times with ethanol.

Synthesis of cobalt oxide-CdSe complex:

To synthesize Cobalt oxide-CdSe complex, initially in a separate flask, 85 mg of Co_3O_4 NPs, 5 mg Se, and 5 mL of dichlorobenzene were mixed in a flask and heated to 180°C in an Ar atmosphere. Further, cadmium precursor solution, prepared by dissolving 25.6 mg CdO in 0.9 mL oleic acid and 4.5 mL dichlorobenzene at 160°C under an Ar atmosphere, was injected into the above solution and continued to heat for 5 min. This changed the color of the solution from black to reddish-black. The temperature was reduced to 150°C , and a solution of 12.5 mg propylphosphonic acid in 0.5 mL dichlorobenzene was added to the above solution. The resulting nanoparticles were allowed to grow for 5 min, and the solution was allowed to cool down to room temperature. After reaching room temperature, the final product as precipitate was isolated and washed with ethanol.

Surface modification of CdSe- Co_3O_4 NPs by ligand exchange

The obtained nanoparticles were dissolved in a mixture of methanol, mercaptoundecanoic acid, and tetramethylammonium hydroxide. Each concentration was adjusted to ensure sufficient ligand exchange. The nanoparticles were precipitated using toluene as a non-solvent, followed by centrifugation, then dissolved in water.

Synthesis of TiO_2 NFs

TiO_2 NFs were prepared using the previously reported method [26]. A detailed description of the synthesis method is described in detail in the Electronic Supplementary Material (ESM).

Preparation of CdSe- Co_3O_4 @ TiO_2 nanocomposite

For CdSe-Co₃O₄@TiO₂ nanocomposite preparation, 10 mg of TiO₂ powder and 30 mg of CdSe- Co₃O₄ powder were dispersed in DI water and mixed overnight, followed by lyophilization for 24 h to obtain CdSe-Co₃O₄@TiO₂ nanohybridization.

Biological sample: These materials are described in detail in the ESM and also in our earlier research works [27 - 28].

Fabrication and application of the PEC-based device for virus detection.

The anode substrate was fluorine-doped tin oxide (FTO) conductive glass, treated with acetone and 95% ethanol, followed by sonication for 30 min, and dried with pure N₂ gas before use. The processed FTO was spin-coated with 20 μ L of CdSe-Co₃O₄@TiO₂ (20 mg mL⁻¹) and dried. Further, the CdSe-Co₃O₄@TiO₂-coated electrode was incubated with 10 μ L of antibody (16 μ g μ L⁻¹) and activated by N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)/1-Hydroxy-2,5-pyrrolidinedione (NHS) for 6 h at 4°C [29]. The electrode was further cleaned with DI water and used for detection. 0.2 mol L⁻¹ of AA in free-oxygen Phosphate-buffered saline (PBS, pH 7.0) was used as the electrolyte solution for detection.

A series of diluted virus concentrations were incubated for 20 min at 4°C with the functionalized electrode for virus detection. The electrode was washed three times using PBS solution to eliminate the uncaptured targets. Finally, the electrode was replaced in the chamber, and the signal was checked based on its photocurrent density from the PEC. In running the PEC for the detection of viruses, the irradiation source wavelength for PEC experiments was 430 nm, with an exposure period of 20 sec and a bias voltage of 0.1 V. The scan frequency

range was 100 mHz – 100 kHz for the EIS tests, and an AC sine wave with 5 mV amplitude was utilized. The anode's functional surface area was 1 cm².

For detection in the complex media, the sample should be 10X-diluted, and 0.5 mL of the diluted sample needs to be dropped onto the prepared Ab/CdSe-Co₃O₄@TiO₂||FTO electrode and incubated for 20 min (in room temperature around 25°C) for the conjugation of target virus to the antibodies on the modified electrode. Further, the electrode should be washed three times using PBS solution to remove the uncaptured targets. Finally, the electrode must be placed in the PEC cell, and the photocurrent should be measured based on the same PEC parameter mentioned above. After measuring the photocurrent density, the relationship between the PEC response and the amount of HEV in the sample is plotted and analyzed to obtain the calibration curve. The calculated linear equation would be used further as the standard calibration of the HEV concentration in the sample analysis.

Results and discussion

Characterization of Photoelectrode Materials

The sequential formation of nano heterostructure with increasing complexity comprises three stages, as illustrated in **Scheme 1a**. **Fig. 1a–d** shows characteristic TEM images of hetero nanostructure corresponding to consecutive stages of the growth protocol. The synthesis steps begin with forming cobalt oxide nanoparticles (Co₃O₄ NPs) by decomposition of cobalt nitrate with benzylamine in the presence of ammonium hydroxide. **Fig. 1a** shows the TEM image of Co₃O₄ NPs. The synthesized Co₃O₄ NPs are circular, with particle sizes ranging from 5 to 13 nm and an average particle diameter of 9 nm (**Fig. 1e**). Then, the CdSe QD was grown on the surface of Co₃O₄ NPs. The TEM image in **Fig. 1b** clearly demonstrates the CdSe-decorated Co₃O₄ NPs. The contrast in the TEM image confirmed the subsequent growth of CdSe QD

onto Co₃O₄ NP. The growth of CdSe QD on Co₃O₄ NP was accompanied by the onset of
 absorption and emission peak at $\lambda=576$ nm, which is shifted from the 2.4 nm size bare CdSe
 peak as shown in the UV-Vis spectra for different stages of growth in **Fig. 1f**. **Fig. 1c** clearly
 shows the SEM image of prepared TiO₂ NF. The arrangement of cross-linked nanosheets in
 TiO₂ NF increases the number of active sites for photocatalytic reactions [30]. The TiO₂ NFs
 are used as a photoconductive nanomaterial to capture excitation energy and generate photo
 response [31]. The TEM image shown in **Fig. S1** reveals that TiO₂ inherits the “flower-like”
 architecture. In addition, the specific surface area of TiO₂ NFs will significantly affect the
 contact area with the light source and the CdSe-Co₃O₄ NPs. CdSe@Co₃O₄ NPs are adequately
 spread over the surfaces of TiO₂ NFs, as shown in **Fig. 1d**, and their flower-like apparent
 morphologies of TiO₂ NFs remain unaltered. The surface of TiO₂ NF is negatively charged,
 while the Co₃O₄ is positively charged; thus, the two were readily adsorbed together. As shown
 in **Fig. S2a**, the lattice fringes observed on TiO₂ is 0.352 nm, which agrees with the in-plane
 lattice spacing pattern of TiO₂ (101 facet). In addition, two types of the fringes for TiO₂ and
 Co₃O₄ are also deciphered, and the distances of 0.352 nm and 0.243 nm clearly match the
 characteristic lattice plane of TiO₂ (101) and Co₃O₄ (311) (**Fig. S2b**) respectively, indicating
 the presence of CdSe-Co₃O₄ on the surface of TiO₂ forming CdSe-Co₃O₄@TiO₂. CdSe-Co₃O₄
 nanoparticles are precipitated uniformly on the surface of TiO₂ NF. This can be attributed to
 the positively charged Co₃O₄ being adsorbed on the negatively charged TiO₂ NF by
 electrostatic interaction.

The formation of CdSe-Co₃O₄@TiO₂ NF was evidenced by sequential X-ray diffraction
 (XRD) pattern. The diffraction pattern of the TiO₂ NFs (black in **Fig. 1g**) shows multiple
 prominent diffraction peaks at $2\theta = 25.3^\circ, 37.7^\circ, 38.5^\circ, 48.0^\circ, 53.8^\circ, 55.0^\circ, 62.7^\circ, 68.7^\circ, 70.2^\circ,$
 and 75.0° attributing to (101), (004), (112), (200), (105), (211), (204), (116), (220) and (215)

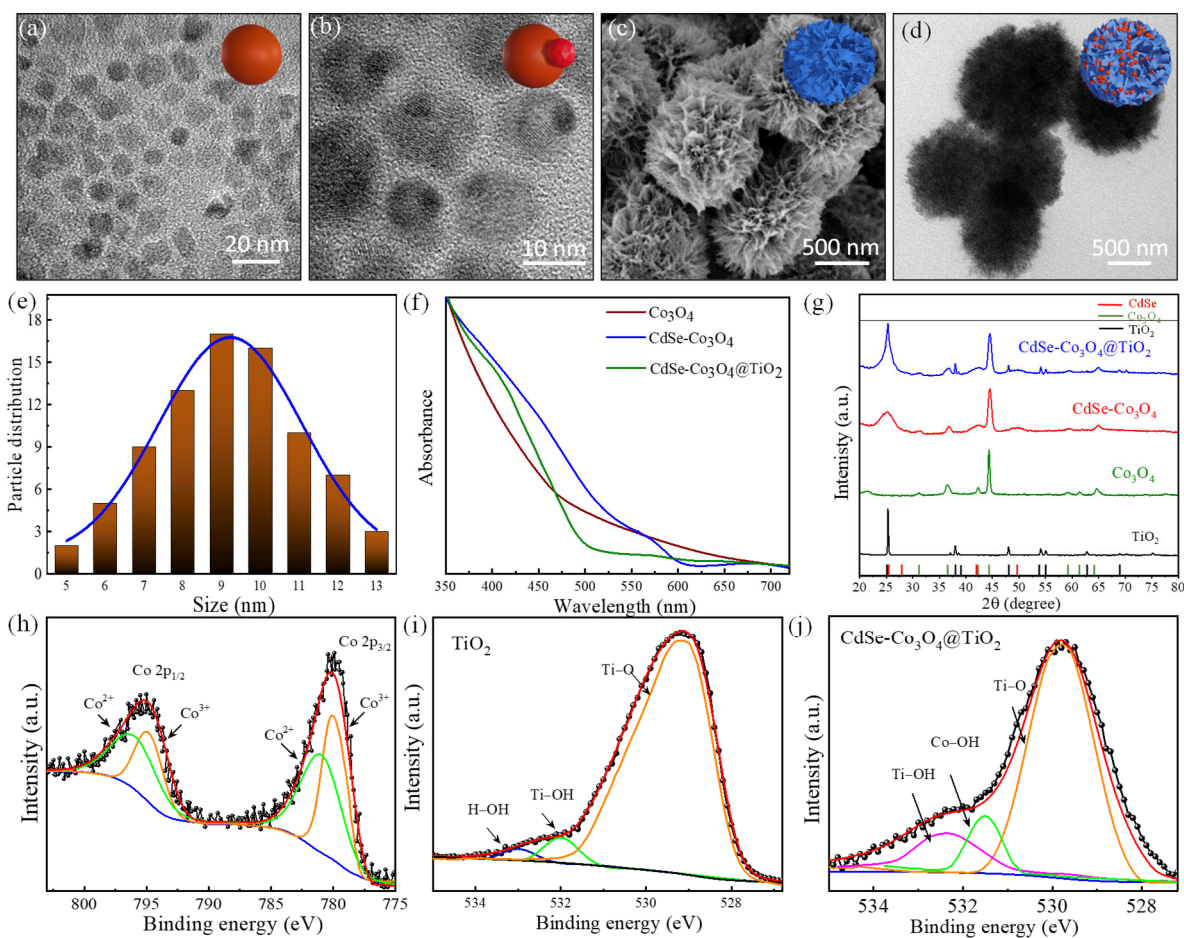


Fig. 1. Synthesis and characterizations of CdSe-Co₃O₄@TiO₂ NFs. The TEM images of Co₃O₄ NPs (a), CdSe-Co₃O₄ (b), and CdSe-Co₃O₄@TiO₂ NF (d). SEM image of TiO₂ NFs (c), corresponding particle size distributions of Co₃O₄ NPs (e), absorption spectra of Co₃O₄, CdSe-Co₃O₄ and CdSe-Co₃O₄@TiO₂ (f), XRD pattern (g), deconvoluted XPS spectra of Co 2p of Co₃O₄ (h), O 1s of TiO₂ (i) and CdSe-Co₃O₄@TiO₂ NFs (j).

planes, respectively. The diffraction peaks are correlated with the JCPDS card no. 84-1285, indicating the successful formation of the anatase phase of TiO₂ NF [32]. **Fig. 1g** (green) shows the XRD patterns of Co₃O₄ NPs with diffraction peaks at 31.27°, 36.85°, 44.81°, 59.35°, and 64.61° are attributing to the (220), (311), (400), (511), and (440) crystal planes of cubic spinel Co₃O₄ NPs respectively which are correlated to the JCPDS no. 43-1003 [33]. In addition, peaks

appearing at (2 theta) of 25.36°, 42.02°, and 49.72° in the XRD patterns of CdSe-Co₃O₄ (red in **Fig. 1g**) can be attributed to (111), (220), and (311) crystal planes of CdSe (JCPDS no. 88-2346) [34]. In the XRD pattern of CdSe-Co₃O₄@TiO₂, all peaks of TiO₂ and CdSe@Co₃O₄ can be noticed. The peak positions remain unaltered after the formation of the CdSe-Co₃O₄@TiO₂ NF heterostructure. This indicates the preservation of the nature of the CdSe-Co₃O₄ and the successful construction of the heterostructure.

The detailed bonding state of the material was further characterized by XPS measurement. **Fig. 1h** shows the Co 2p spectra of Co₃O₄. The Co 2p spectra exhibit a doublet with a low energy band (Co 2p_{3/2}) at 780.1 and a high energy band (Co 2p_{1/2}) at 795.5 eV. The energy difference between the Co 2p_{3/2} – 2p_{1/2} peaks is approximately 15.4 eV which is in corroboration with the reported values of Co (II, III) [35]. The absence of the prominent shake-up satellite peak in the Co 2p spectra suggests a dominant Co₃O₄ phase. The peak deconvolution of O 1s spectra of TiO₂ and CdSe-Co₃O₄@TiO₂ is further performed (**Fig. 1i and j**). The deconvolution spectra of O 1s of synthesized TiO₂ NF (**Fig. 1i**) shows three peaks at 531.9, 533.1, and 529.3eV, which belong to H–O–H, Ti–OH, and Ti–O–Ti, respectively. Furthermore, the weak shoulder peak at 531.2 eV of O 1s in the CdSe-Co₃O₄@TiO₂ is attributed to the Co–OH bonds.

Performance of PEC-based biosensing device.

Furthermore, the binding ability and the detection performance of the anti-HEV antibodies conjugated to CdSe-Co₃O₄@TiO₂ towards HEV-LPs are investigated (**Fig. 2a, b**). A time-based *i*–*t* approach was used to understand the performance of the PEC device following the assembly of Co₃O₄, CdSe-Co₃O₄, and CdSe-Co₃O₄@TiO₂ NFs, respectively. When CdSe-Co₃O₄ or TiO₂ NFs alone, the photocurrent density response remained at 2.21 or

4.9 $\mu\text{A cm}^{-2}$, respectively. However, after the CdSe-Co₃O₄@TiO₂ NFs nanocomposite formation, the current density improved drastically to 15.5 $\mu\text{A cm}^{-2}$, owing to the synergistic effect among CdSe-Co₃O₄ and TiO₂ NFs. The heterostructure formation boosted the interfacial electron-transfer efficiency while suppressing e^-/h^+ recombination in converting light to electrical energy. The photocurrent was reduced to 14.1 $\mu\text{A cm}^{-2}$ after the conjugating of the anti-HEV antibody, probably due to the antibody acting as an insulator. After incubation with HEV-LPs (100 pg mL⁻¹), the current density further decreased to 7.1 $\mu\text{A cm}^{-2}$, indicating that the antibody successfully captured HEV-LPs on the CdSe-Co₃O₄@TiO₂ NFs electrode. Modifying the nanocomposite with an anti-HEV antibody helped in the specific binding of the HEV-LPs when incubated with samples containing HEV-LPs. These HEV-LPs bound on the surface of the nanocomposite acted as insulating agents and hindered the light source, preventing the excitation of the CdSe-Co₃O₄@TiO₂. This resulted in a reduced photocurrent response of the CdSe-Co₃O₄@TiO₂ electrode hybridized with HEV-LPs compared with the CdSe-Co₃O₄@TiO₂ electrode without HEV-LPs.

As shown in **Fig. 2c**, electrochemical impedance spectroscopy (EIS) analyzes the electrochemical activity, the antibody-modified electrode's capturing property, and the antibody's detection ability toward HEV-LPs. Randle's equivalent circuit model is used for fitting the EIS curves as shown in the inset in **Fig. 2c**. It consists of the circuit components like charge-transfer resistance (R_{ct}), solution resistance (R_s), double-layer capacitance (CPE), and the Warburg impedance (W). R_{ct} was majorly affected during the construction of the biosensor and HEV-LPs detection. Therefore, changes in R_{ct} can be monitored to analyze the interfacial electron transfer during the fabrication of biosensing devices with individual components and the detection process. The R_{ct} of the electrode upon deposition of CdSe-Co₃O₄@TiO₂ NFs is 447 Ω , and further conjugation of the anti-HEV antibody increased the R_{ct} to 772 Ω . The R_{ct} value further increased to 1125 Ω upon incubation of anti-HEV antibody conjugated electrode

was HEV-LPs (10 pg mL^{-1}). The changes in R_{ct} values evidenced the successful formation of the biosensing device with the apparent detection ability of HEV-LPs. Further, modified ELISA was performed to confirm the conjugation of anti-HEV Abs onto anti-HEV-Ab/CdSe- $\text{Co}_3\text{O}_4@\text{TiO}_2||\text{FTO}$ electrode. As shown in **Fig. S4**, only the anti-HEV-Ab conjugated anti-HEV-Ab/CdSe- $\text{Co}_3\text{O}_4@\text{TiO}_2||\text{FTO}$ electrode generated high absorbance compared with bare FTO and CdSe- $\text{Co}_3\text{O}_4@\text{TiO}_2||\text{FTO}$ electrodes. Because anti-rabbit IgG-conjugated HRP could only bind with the anti-HEV-Abs present on anti-HEV-Ab/CdSe- $\text{Co}_3\text{O}_4@\text{TiO}_2||\text{FTO}$ electrode, this indicated the conjugation of anti-HEV-Ab on the anti-HEV-Ab/CdSe- $\text{Co}_3\text{O}_4@\text{TiO}_2||\text{FTO}$ electrode.

Photovoltaic working mechanism of the device.

The possible mechanism of photocurrent response for the developed PEC device is shown in **Fig. 2d**. The CdSe- $\text{Co}_3\text{O}_4@\text{TiO}_2$ -modified FTO showed good photocurrent intensity, attributed to the excellent matching cascade band-edge levels of CdSe- $\text{Co}_3\text{O}_4@\text{TiO}_2$ and its increased photocurrent conversion efficiency by minimizing electron-hole recombination. TiO_2 NFs have a conduction band (CB) edge potential of about -0.44 V and a wide bandgap of 3.2 eV [36]. Using the equation $E_g = V_{VB} - V_{CB}$, the valance band (VB) edge potential of TiO_2 NFs is calculated to be about 2.76 V . In addition, previous reports claim that CdSe QDs possess a band gap of $1.8\text{--}1.9 \text{ eV}$ [13, 37]. **Fig. 2d** depicts the energy level diagram with well-matched energy levels of TiO_2 NFs and CdSe QDs forming a heterojunction alignment structure.

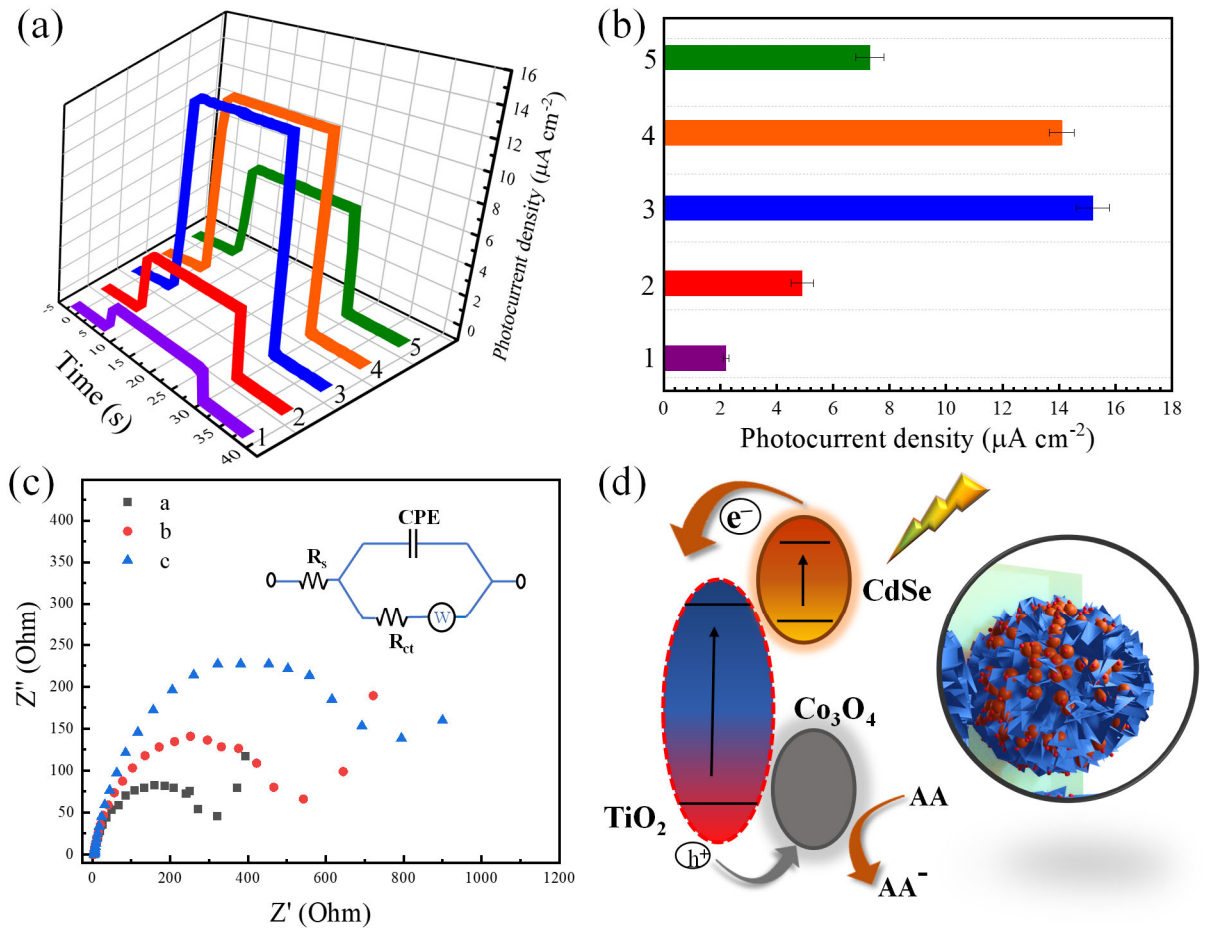
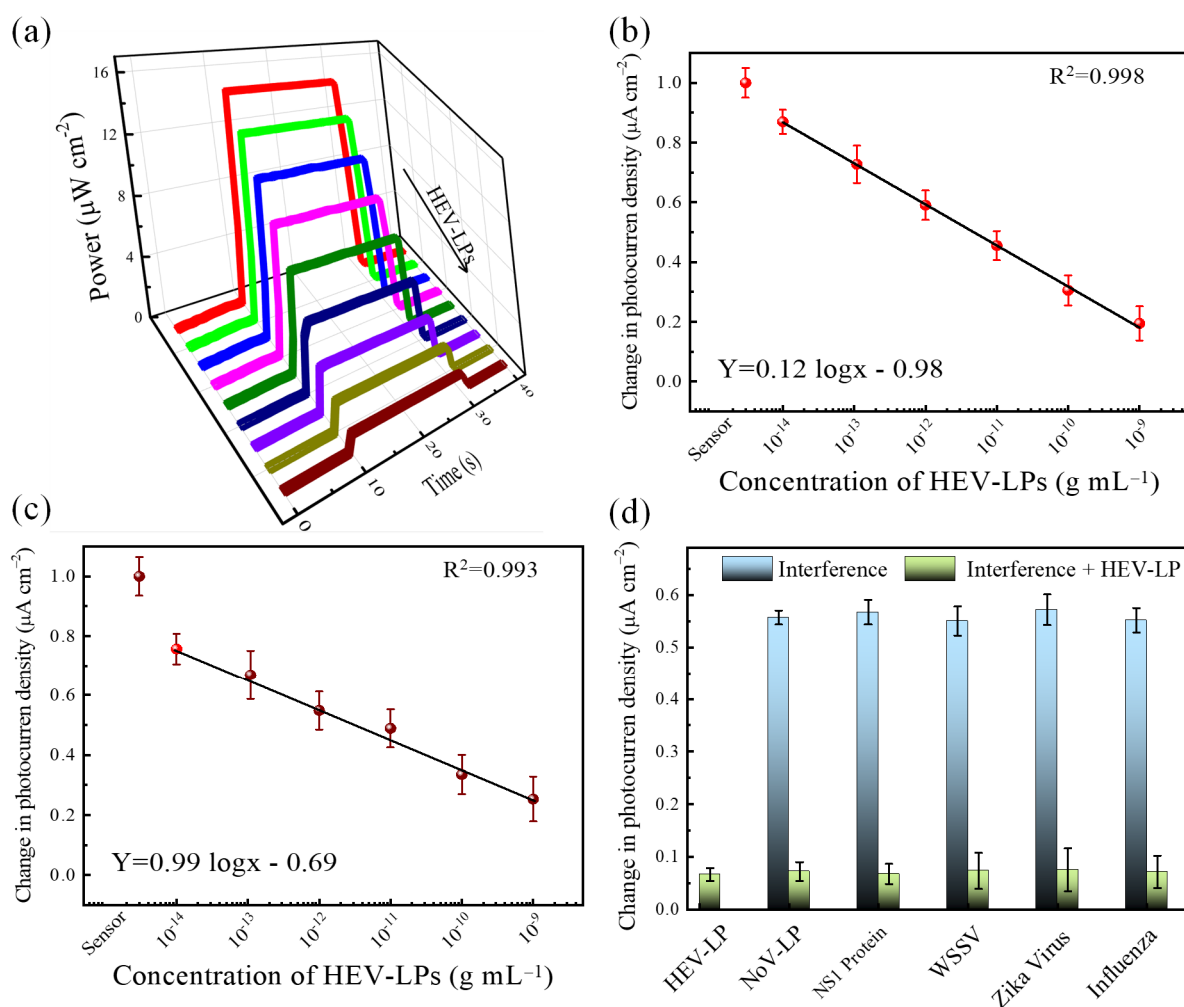


Fig. 2. Time-based photocurrent (a), histogram (b), of (1) CdSe- Co_3O_4 ||FTO, (2) TiO_2 /FTO, (3) CdSe- Co_3O_4 @ TiO_2 ||FTO, (4) Anti-HEV-Ab/CdSe- Co_3O_4 @ TiO_2 ||FTO, and (5) HEV-LPs/Anti-HEV-Ab/CdSe- Co_3O_4 @ TiO_2 ||FTO (irradiation source wavelength of 430 nm and a bias voltage of 0.1 V). Nyquist plots (c) of CdSe- Co_3O_4 @ TiO_2 ||FTO (1), Anti-HEV-Ab/CdSe- Co_3O_4 @ TiO_2 ||FTO (2), HEV-LPs/Anti-HEV-Ab/CdSe- Co_3O_4 @ TiO_2 ||FTO (3). Inset in C is Randles equivalent circuit. Schematic illustration of the energy band structure and the proposed charge-transfer mechanism (d). Error bars in b denote data as average $\pm$ SD ($n = 3$).

The developed PEC device generated an electrical signal under visible light excitation in this report. Because of the external energy provided by visible light, the carriers in the VB of CdSe QDs were excited and injected into the CB of CdSe QDs. In most semiconductors, the occupied VB and the vacant CB existed simultaneously [38]. Electrons were injected from the VB to the CB upon excitation by the external energy. This result generates holes in the VB

because of the bandgap. However, the excited electrons and the holes could quickly recombine because this course was very fast [39]. The recombination occurred quickly when TiO₂ NFs were alone. However, by forming the CdSe-Co₃O₄@TiO₂, the recombination of excited electrons and holes was inhibited.

Further, these excited carriers were injected instantly into the CB of TiO₂ NFs. CdSe QDs served as electron donors. The absorbed light energy was transformed into an electric current and quickly delivered to the external circuit. The carriers in the VB of TiO₂ NFs and CdSe QDs were reduced by Co₃O₄ and ascorbic acid (AA), which improved the energy conversion efficiency and reduced carrier residence time across the energy band. The UV-Vis spectra (**Fig. 1f**) indicated that the hybridization between CdSe-Co₃O₄ and TiO₂ facilitated carrier transmission efficiency and energy conversion efficiency enhancement. In addition, photocurrent response was enhanced several folds in CdSe-Co₃O₄@TiO₂ nanocomposite, thereby providing evidence for the working mechanism mentioned above. Prolonged irradiation of the CdSe-based system caused photo-corrosion in the presence of oxygen on the surface of the photocatalyst by the photogenerated hole [40]. In our system, the photogenerated hole transfer to the Co₃O₄; thus, an oxidation reaction occurs at the Co₃O₄ surface, increasing the system's stability. Thus, the CdSe-Co₃O₄@TiO₂ carrier-transfer nanohybridization boosts external energy and photocatalytic capability. As explained below, the photocurrent response from the CdSe-Co₃O₄@TiO₂-based device was successfully exported for virus detection.



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Fig. 3. The photocurrent response of the electrode after incubation with various concentrations of HEV-LPs ranging from 10 fg mL^{-1} to 10 ng mL^{-1} (irradiation source wavelength of 430 nm and a bias voltage of 0.1 V) (a), calibration curve obtained using change in photocurrent density vs. concentration of HEV-LPs (b), calibration curve for HEV-LPs ranging from 10 fg mL^{-1} to 10 ng mL^{-1} in 10% human serum as a sensing medium (c), and the change in photocurrent density of anti-HEV-Ab/ $\text{CdSe-Co}_3\text{O}_4@\text{TiO}_2\|\text{FTO}$ electrode when incubated with 1 pg mL^{-1} HEV-LPs and other common interferences at a concentration of 10 ng mL^{-1} using 10% human serum as a sensing medium (d). Error bars in b, c, and d denote data as average $\pm$ SD ($n = 3$).

Detection of HEV-LPs

The application of the developed PEC device for HEV-LPs detection is based on the specific interactions of anti-HEV antibodies and HEV-LPs. Different concentrations of HEV-LPs are initially added to the anti-HEV antibody-conjugated CdSe-Co₃O₄@TiO₂ NF-modified FTO electrode. The photocurrent response at various concentration range of HEV-LP continuously decreased with the increase in HEV-LP concentration (**Fig. 3a**). The linear relationship between the photocurrent density and the HEV-LP concentration ranging from 10 fg mL⁻¹ to 10 ng mL⁻¹, shows a correlation coefficient of 0.998 (**Fig. 3b**). The limit of detection (LOD) was 3.5 fg mL⁻¹, as calculated by the $3\sigma/S$ where S is the slope of the linear calibration plot and σ is the standard deviation of the lowest signal [41–43]. The sensitivity of the developed PEC-based sensor for HEV-LPs detection is competitive with recently developed analytical sensing methods comprising optical methods, as shown in **Table S1** of ESM. These results indicate that the developed PEC sensor for virus detection exhibits benefits, including ultra-sensitivity of the electrochemical method and wide linear range detection, which can offer accurate and reliable quantitative results.

The applicability of the proposed sensor was evaluated in a complex biological matrix using 10% human serum as a sensing medium. The calibration curve obtained for HEV-LPs detection in 10% human serum (**Fig. 3c**) shows a comparable detection pattern, indicating the suitability of the proposed sensor for the clinical sample analysis. Compared with the calibration curve obtained in DI water, the slope of the calibration curve in 10% human serum is slightly flattened. The complex human serum matrix caused a modest decrease in the slope of the calibration curve, lowering the LOD value to 8.5 fg mL⁻¹. However, the sensitivity of the proposed sensor in the serum matrix is considerable compared to previous reports for its actual application.

Selectivity and stability of the PEC sensor

The selectivity of the biosensor is crucial for its practical application [44, 45]. To evaluate the selectivity of the developed sensor, several VLPs, and viruses, including norovirus-like particles (NoV-LPs), NS1 protein lysate, white spot syndrome virus (WSSV), influenza virus, zika virus at 10 ng mL^{-1} were spiked in 10% human serum. The interfering effect was evaluated by comparing the response with 1 pg mL^{-1} HEV-LPs. Photocurrent intensity of anti-HEV antibody-conjugated $\text{CdSe-Co}_3\text{O}_4@\text{TiO}_2$ electrode decreases only in the presence of target HEV-LP (**Fig. 3d**), demonstrating interfering materials cannot bind to anti-HEV antibody-conjugated $\text{CdSe-Co}_3\text{O}_4@\text{TiO}_2$ electrode.

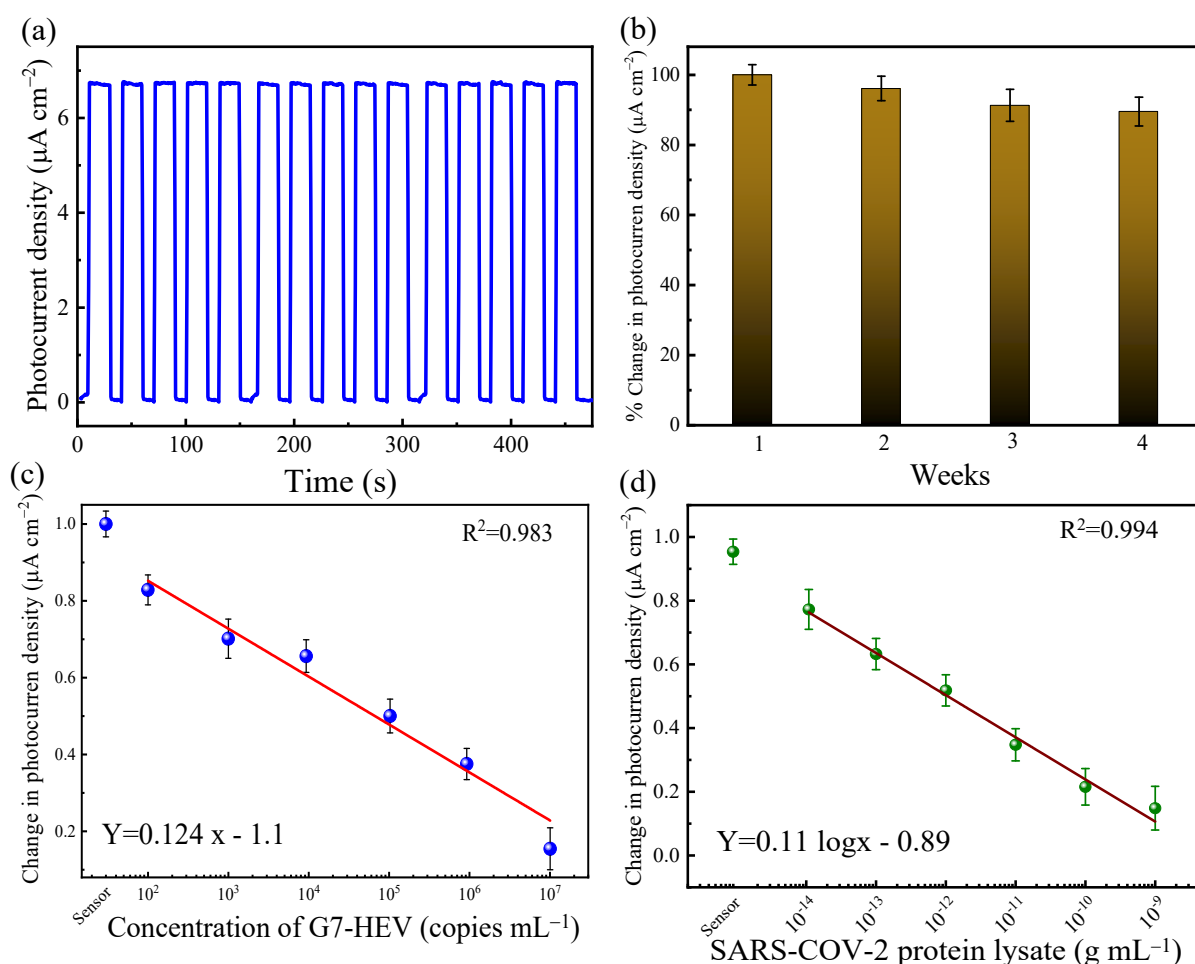


Fig. 4. Stability evaluation of the PEC sensor (a), long-time stability of PEC microfluidic sensor (irradiation source wavelength of 430 nm and a bias voltage of 0.1 V) (b), the

calibration curve for detection of G7 HEV ranging from 10^2 – 10^7 RNA copies mL^{-1} in cell culture supernatants using PEC sensor **(c)**, and detection of Spike protein of SARS-COV-2 ranging from 10 fg mL^{-1} to 10 ng mL^{-1} in cell lysate **(d)**. Error bars in b, c and d denote data as average $\pm$ SD ($n = 3$).

To examine the stability of the developed PEC sensor, photocurrent response and long-time stability are evaluated. As shown in **Fig. 4a**, there is no discernible fluctuation in the photocurrent response in the recurring 15 times on/off irradiation cycles for 475 s with incubating 10 pg mL^{-1} of HEV-LPs. This indicates exceptional photocurrent stability for HEV-LPs detection. In addition, the stability of the developed PEC sensor is also examined by storing the modified electrodes at 4°C and measuring the photocurrent response of the modified electrode every week. After storage in a refrigerator at 4°C for 2 weeks, the photocurrent response of the sensor electrode remained steady at 96.1% compared to its original photocurrent; at 91.3% and 89.5% for 3 and 4 week storage, respectively (**Fig. 4b**), suggesting good long-term stability.

Furthermore, the repeatability of the constructed PEC-based sensor is investigated by fabricating five separate electrodes to detect HEV-LPs at 10 pg mL^{-1} . The detected concentrations of HEV-LPs utilizing five different electrodes vary from 10.2 to 11.3 pg mL^{-1} , with an average of 10.7 pg mL^{-1} , demonstrating that the PEC sensor's accuracy is acceptable. Furthermore, the relative standard deviation (RSD) of the measurements is 6.7%, indicating that the PEC sensor has excellent repeatability.

PEC-based detection of HEVs and SARS-CoV-2 Spike protein

The photocurrent responses are recorded to evaluate the PEC sensor's performance for HEV detection from the cell culture supernatant. As shown in **Fig. 4c**, G7 HEV shows a correlation coefficient (R^2) of 0.983 with LOD of 69 RNA copies mL^{-1} , confirming the high

accuracy and sensitivity of the developed PEC sensor. The photocurrent response continuously decreased with the increase in HEV concentration from 10^2 – 10^7 RNA copies mL^{-1} .

In addition to the HEV detection, they substitute the corresponding antibodies from anti-HEV Ab into anti-SARS-CoV-2 Ab, targeting S protein of SARS-CoV-2 (**Fig. 4d**). The developed Ab-SARS-CoV-2-CdSe- Co_3O_4 @ TiO_2 -based PEC sensor demonstrated a good response of SARS-CoV-2 protein biomarker in cell lysate with a detection limit of 7.8 fg mL^{-1} with an R^2 value of 0.991. These results strongly showcased how the developed PEC sensor is reliable for detecting the target viruses and their biomarker protein, exhibiting the potential application in detecting HEVs and other targets.

Conclusions

A photoelectrochemical (PEC) device based on CdSe- Co_3O_4 @ TiO_2 is developed, and ultrasensitive virus detection is demonstrated. The fabricated device exhibited a stable and continuous photocurrent response up to $15.5 \mu\text{A cm}^{-2}$ with a longer exciton lifetime. Based on the specific strong recognition ability between the anti-HEV antibody and HEV-LPs, the device demonstrated a wide detection range, a low detection limit of 3.5 fg mL^{-1} , and ultrahigh selectivity and sensitivity. In addition, it could detect HEVs in the complex cell culture supernatant and sub-femtogram level of SARS-CoV-2 S protein in cell lysate solution in high precision. Therefore, the proposed device should aid in developing quantitative detection strategies for biomolecule detection by replacing the corresponding antibodies and broadening the applications of PEC-based biosensing devices. It is worth mentioning that although this method has achieved good results, however, there are still distances to the clinical application, such as the detection limit being affected by the substrate type, exploitation of corresponding kits, and the development of portable equipment.

438

439 **Notes**

440 The authors declare that they have no competing financial interests or personal
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442

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CdSe-Co₃O₄@TiO₂ nanoflower-based photoelectrochemical platform probing visible light-driven virus detection

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S1. Materials and methods:

S1-1. Synthesis of TiO₂ NFs

Briefly, 0.14 g of titanium isopropoxide was added to 6.7 g of concentrated hydrochloric acid (36.0–38.0 wt%) under continuous stirring to prepare the titanium isopropoxide solution. Cetyltrimethylammonium bromide (CTAB) solution was prepared in a separate flask by dissolving 0.11 g of CTAB in 13.65 mL of distilled water and vigorously stirred for 40 min at room temperature. Later, titanium isopropoxide solution was added to the prepared CTAB solution under vigorous stirring for 2 h at room temperature. In addition to the 9.5 mL of the preceding solution, 28 mL of ethylene glycol and 0.45 g of urea were added and stirred for 3 h. The solution was then transferred to a 50 mL stainless-steel autoclave with a Teflon liner. The sealed autoclave was heated in an oven to 150°C and held for an additional 20 h. The finished product was centrifuged, washed several times with water, and dried at 60°C.

S1-2. Biological sample:

Sandwich anti-SARS-CoV-2 S protein chimeric antibody (Ab) (AbSARS-CoV-2 targeting S protein) was purchased from Sino Biological Inc. (Beijing, China). The anti-HEV IgG antibody was obtained from a rabbit 136 immunized with purified G3 HEV-LPs and purified by protein G column. The G3 HEV-LPs were produced by a recombinant baculovirus expression system [27]. Genotype 7 HEV (G7 HEV) was obtained from the cell culture supernatant of a human hepatocarcinoma cell line of a human hepatocarcinoma, PLC/PRF/5 (JCRB0406, the Health Science Research Resources Bank, Osaka, Japan) [28]. The RNA copy numbers of the used HEVs were 5.0×10^8 RNA copies mL⁻¹.

S2. Result and Discussion

S2-1. Optimization of the PEC device for HEV-LPs detection

For the best sensing performances of the developed sensor, six major optimum experimental conditions were assessed, including the concentrations of TiO₂ NF, CdSe-Co₃O₄, CdSe-Co₃O₄@TiO₂, pH, AA, and antibody. The maximum photocurrent was 6.78 μA when the TiO₂ NFs concentration was 4 mg mL^{-1} (**Fig. S3a**) because the thicker TiO₂ NF film causing higher concentration obstructs the electron transfer, causing the photocurrent lower. A similar phenomenon was observed in the CdSe-Co₃O₄ concentration shown in **Fig. S3b**, and the optimal dosage of 12 mg mL^{-1} of CdSe-Co₃O₄ favors the heterojunction formation resulting in increased photocurrent response. As shown in **Fig. S3c**, 20 mg mL^{-1} is the optimal concentration of CdSe-Co₃O₄@TiO₂. The optimal pH value is 7.0, at which the photocurrent intensity is maximum (**Fig. S3d**). The electron donor of AA might inhibit the e^-/h^+ recombination. As shown in **Fig. S3e**, the photocurrent intensity reaches a plateau when the concentration of AA is 0.2 mol L^{-1} , attributing to the saturation of the electron donor. Considering the specific recognition between the antibody and HEV-LPs, experiments were performed to study the best immobilization effect of the antibody. As shown in **Fig. S3f**, the current intensity decreased with increasing antibody concentration because the biomaterial cannot realize the energy conversion. In addition, the decrease in current intensity suggested the conjugation of antibodies to CdSe-Co₃O₄@TiO. To achieve a good calibration curve with a big linear range and good energy conversion property, the optimal antibody concentration was 16 $\mu\text{g }\mu\text{L}^{-1}$.

Table S1. Comparison of the visible-light-driven PEC biosensor based on CdSe-Co₃O₄@TiO₂ nanoflower with recently reported methods in respect to linear range and detection limit.

Sensing method	Target virus	Linear range	LOD	Ref
Fluorescence	Norovirus	2^{-18} copies mL ⁻¹	1.2 copies mL ⁻¹	[3]
	Influenza virus	10 ng mL ⁻¹ – 100 ng mL ⁻¹	3×10^{-10} g mL ⁻¹	[4]
	Dengue RNA	5 – 500 ng mL ⁻¹	5.2×10^{-9} g mL ⁻¹	[5]
	HBV	264 ng mL ⁻¹	8.3×10^{-9} g mL ⁻¹	[6]
Colorimetric	NoV	10^2 – 10^6 copies mL ⁻¹	13.2 copies mL ⁻¹	[7]
	Influenza virus	5 fg mL ⁻¹ – 5 µg mL ⁻¹	44.2×10^{-15} g mL ⁻¹	[8]
Photo- Electrochemical	HIV	1 fM – 1 nM	0.65 fM	[9]
	Sars-Cov-2	0.5–32.0 nM	0.12 nM	[10]
	Norovirus	10 ng mL ⁻¹ – 100 µg mL ⁻¹	4.9 pM	[11]
	Sars-Cov-2	10 fg mL ⁻¹ – 100 ng mL ⁻¹	7.8 fg mL ⁻¹	This work
	HEV	10 fg mL ⁻¹ – 100 ng mL ⁻¹	3.5 fg mL ⁻¹	This Work

S2-2. Recovery of HEV-LPs in human serum samples using CdSe-Co₃O₄@TiO₂ nanoflower-based photoelectrochemical detection

We have performed the spike test by adding 500 and 5,000 pg mL⁻¹ of HEV-LPs in undiluted (100%) serum samples. As shown in **Table S2**, the recovery of the HEV-LPs in the undiluted serum is 88.9 and 90.1%, with a relative standard deviation of 7.9 and 8.6%, suggesting that the undiluted serum sample interferences with the performance of the proposed PEC based detection method. Further, we performed the spike test using the diluted human serum 2X (50%) and 10X (10%). The recovery of 5X and 10X pre-diluted serum samples spiked with 500 pg mL⁻¹ was 92.5% and 97.5%, with a relative standard deviation of 6.9 and 4.9%, respectively. Besides the number of interferences species, the viscosity of the 10X diluted human serum was significantly less compared with 2X dilution and undiluted serum samples. Using the 10X diluted serum samples results in the highest recovery of 95.5% with a relative standard deviation of 4.9%, suggesting the best suitable matrix to minimize the interference effect of the human serum.

Table S2. Recovery of various concentrations of HEV-LPs in human serum samples using CdSe-Co₃O₄@TiO₂ nanoflower-based photoelectrochemical platform.

Serum concentration	Spiked (pg mL ⁻¹)	Detected (pg mL ⁻¹)	Recovery	RSD (%) n=5
100 %	500	444.5	88.9%	7.9
	5000	4505.0	90.1 %	8.6
50 %	500	462.5	92.5%	6.9
	5000	4580.0	91.6 %	5.8
10 %	500	487.0	97.4%	4.9
	5000	5120.0	102.4 %	5.2

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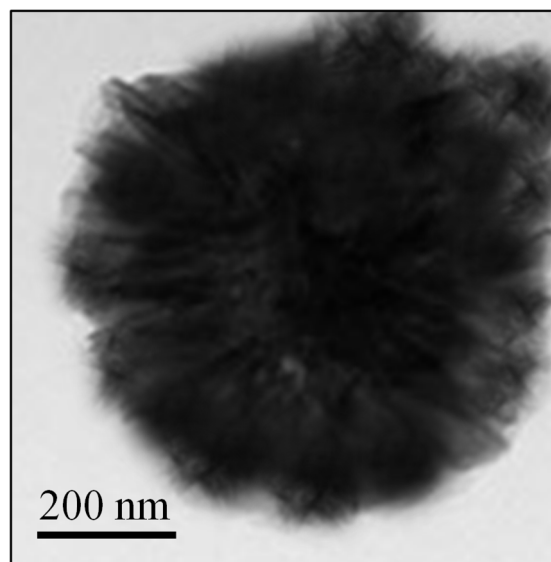


Fig. S1. TEM image of TiO₂ NF

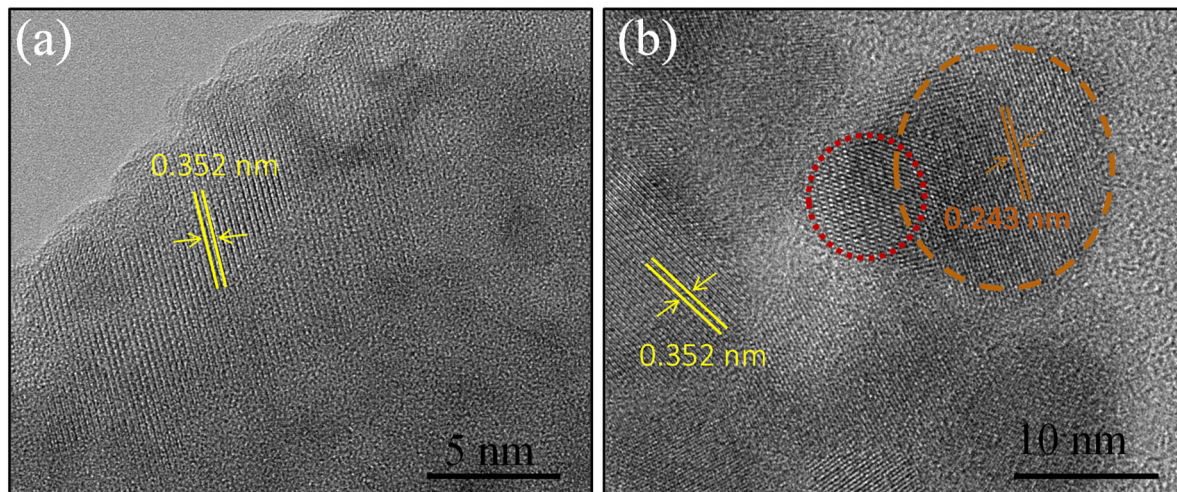


Fig. S2. The HR-TEM images of TiO₂ NF **(a)** and CdSe-Co₃O₄@TiO₂ NF **(b)**.

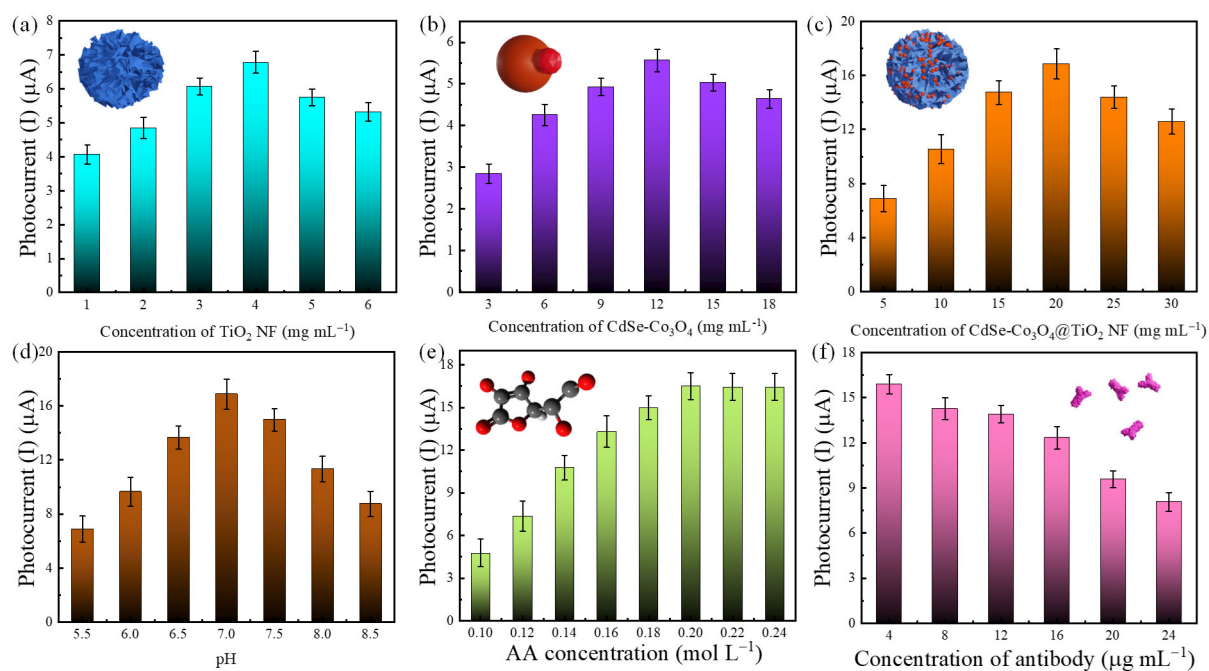


Fig. S3. Optimization of concentrations of the TiO_2 NFs (a), $\text{CdSe-Co}_3\text{O}_4$ (b), $\text{CdSe-Co}_3\text{O}_4@\text{TiO}_2$ (c), the pH after modifying $\text{CdSe-Co}_3\text{O}_4@\text{TiO}_2$ (d), AA (e), and anti-HEV antibody (f).

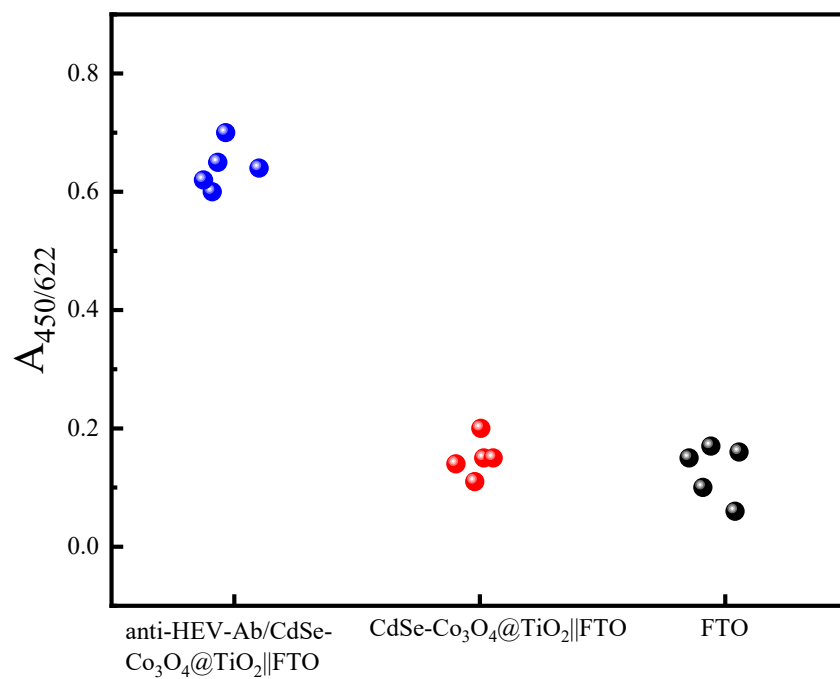


Fig. S4. Modified ELISA to confirm the conjugation of anti-HEV Abs onto anti-HEV-Abs/CdSe-Co₃O₄@TiO₂/FTO electrode.