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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Self-Powered Photoelectrochemical Device for Ultrasensitive Virus Detection using Multi-component Nanoheterostructure

研究課題名（英文）Self-Powered Photoelectrochemical Device for Ultrasensitive Virus Detection using Multi-component Nanoheterostructure

研究代表者

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研究者番号：９０９３７２５２

交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：私たちは、E型肝炎ウイルス（HEV）、HEV様粒子（HEV-LP）、およびSARS-CoV-2スパイクタンパク質をターゲットとした超高感度ウイルス検出用の自己発電型光電気化学（PEC）バイオセンサーを開発しました。これらのバイオセンサーは、CdSe-Co3O4@TiO2およびCo3O4-CdSe-CdS-Pt光電気触媒を使用し、可視光励起により光電流応答を生成します。これらのバイオセンサーは、10 fg/mLから10 ng/mLの範囲のウイルス濃度を検出できます。これらのデバイスは、手頃な価格でポータブルなバイオセンシングソリューションの可能性を示す高い感度と特異性を提供します。

研究成果の学術的意義や社会的意義
この研究は、ウイルス検出技術の大きな進歩を表しています。新しい光電気触媒を開発することで、感度と光電流応答が大幅に向上しました。このデバイスは、10 fg/mLという非常に低い濃度のE型肝炎ウイルスとSARS-CoV-2スパイクタンパク質を検出できます。手頃な価格で持ち運び可能な当社のバイオセンシングデバイスは、現場での迅速なウイルス検出を提供します。これは、特にリソースが限られた環境でのタイムリーな公衆衛生対応に不可欠です。この技術は、感染率を下げ、医療システムへの負担を軽減し、最終的には人命を救うのに役立ち、世界の健康に大きなプラスの影響をもたらします。

研究成果の概要（英文）：In the absence of therapeutic agents, early virus detection is crucial to prevent outbreaks and control pandemics. We developed a self-powered photoelectrochemical (PEC) biosensor for ultrasensitive detection of hepatitis E virus (HEV), HEV-like particles (HEV-LPs), and SARS-CoV-2 spike protein in complex lysate solutions. This biosensor uses novel electrocatalysts CdSe-Co3O4@TiO2 and Co3O4-CdSe-CdS-Pt, generating a photocurrent response under visible light. Encapsulation within a polymer shell amplifies the detection signal. Enhanced photocurrent response is due to CdSe quantum dots' sensitization and strong light absorption, coupled with Co3O4's stability. The biosensor shows a linear relationship between photocurrent output and virus concentration (10 fg/mL to 10 ng/mL), offering high sensitivity, specificity, and stability. This development shows significant potential for creating affordable, portable biosensing devices

研究分野：Nanomaterials and Bio Analytical chemistry

キーワード：Photoelecrtochemical　Biosensor　Nanomaterial　water oxidation　Encapsulation　Virus　Visible light　Self powered

１．研究開始当初の背景

Current virus diagnostic methods like RT-PCR and immunochromatography are essential but face challenges in speed and sensitivity, crucial for managing outbreaks and pandemics effectively. Developing on-site tools with high sensitivity and rapid results is critical. Recent advances focus on improving detection sensitivity through innovative approaches. Our research has introduced a norovirus detection method using metal oxide-based nanozymes in liposomes. This method utilizes colorimetry and electrochemical impedance, achieving sensitivity levels about 100 times higher than conventional methods, crucial for detecting low virus concentrations in complex samples.

For Hepatitis E virus (HEV), we employ dual-modality with engineered quantum dots in hollow iron oxide nanoparticles, capable of detecting HEV at very low copy numbers. Challenges arise in real-world samples due to contaminants affecting detection accuracy. To enhance sensitivity further, we developed Pt-Co3O4 HC, an electrocatalytic nanomaterial enabling rapid HEV detection via water oxidation, ensuring high sensitivity and specificity. However, biomolecule interference on the nanomaterial surface remains a concern.

Our ongoing research advances virus detection through a novel photoelectrochemical (PEC) approach. This method integrates visible light for excitation and photocurrent for detection, combining photo-illumination and electrochemistry advantages. PEC promises ultra-sensitivity and reduced background noise compared to traditional methods, aiming to overcome current detection limitations effectively for more reliable and rapid virus diagnostics in diverse settings.

２．研究の目的

In the absence of therapeutic agents, early detection of viruses and pathogens is crucial for preventing outbreaks and controlling pandemics. To address this need, we are developing a self-powered biosensor capable of quantitatively detecting individual viral particles. Our focus lies in pioneering a novel class of self-powered photoelectrochemical (PEC) biosensors, which have emerged as promising tools in healthcare for their potential in ultrasensitive virus and pathogen detection.

Our biosensor utilizes advanced electrocatalysts such as Co_3O_4-CdSe-CdS-Pt and CdSe-Co_3O_4@TiO_2 nanoflowers, activated by visible light to generate a photocurrent response. Encapsulation of these materials within a polymer shell enhances the detection signal, significantly improving sensitivity compared to individual components. Specifically, CdSe quantum dots in CdSe-Co_3O_4@TiO_2 enhance visible light absorption, prolong interfacial carrier lifetime, and enhance energy conversion efficiency when coupled with the oxygen-evolving catalyst Co_3O_4 at the hole trapping site (CdSe), thus stabilizing the system overall. Our biosensor demonstrates exceptional sensitivity, specificity, and stability in detecting viruses such as hepatitis E virus (HEV), HEV-like particles (HEV-LPs), and SARS-CoV-2 spike protein in complex lysate solutions. The photocurrent response shows a linear correlation with HEV-LPs concentration ranging from 10 fg/mL to 10 ng/mL, with a detection limit of 3.5 fg/mL. These capabilities underscore its potential for developing affordable and portable biosensing devices crucial for early disease detection.

Moving forward, our research aims to synthesize heterostructure nanomaterials (Co_3O_4-CdSe-CdS-Pt and CdSe-Co_3O_4@TiO_2 nanoflowers) optimized for optical and catalytic properties, facilitating advanced virus detection strategies. Characterization and optimization of these materials will precede the development of highly sensitive sensing methods. Ultimately, our goal is to apply these methodologies to real sample analysis, ensuring practical and effective virus detection in diverse settings. This integrated approach promises to significantly advance virus detection technology, addressing current limitations and contributing to robust public health measures against infectious diseases.

３．研究の方法

(1) Visible Light-Driven Photoelectrochemical Platform for Sensitive Virus Detection

①　Synthesis of Cobalt Oxide:

Spherical cobalt oxide nanoparticles (Co_3O_4 NPs) were synthesized by mixing 80 mg cobalt acetate with 3.5 mL benzylamine under constant stirring for 2 hours at room temperature. Ammonium hydroxide was then added, and the mixture was heated to 165°C for 2 hours. The resulting black precipitate was collected by centrifugation and washed with ethanol.

②　Synthesis of Cobalt Oxide-CdSe Complex:

To prepare the Cobalt oxide-CdSe complex, 85 mg of Co_3O_4 NPs, 5 mg of selenium, and 5 mL of dichlorobenzene were mixed and heated to 180°C under an argon atmosphere. A cadmium precursor solution was added, changing the solution color from black to reddish-black. The temperature was adjusted to 150°C, and propylphosphonic acid was introduced. The nanoparticles were allowed to grow, cooled to room temperature, isolated as precipitate, and washed with ethanol.

③ Surface Modification of CdSe-Co_3O4 NPs by Ligand Exchange:

The synthesized nanoparticles underwent surface modification using methanol, mercaptoundecanoic acid, and tetramethylammonium hydroxide to facilitate ligand exchange. Precipitation with toluene and subsequent centrifugation followed by dissolution in water completed the process.

④ Synthesis of TiO_2 Nanoflowers (TiO_2 NFs):

TiO_2 NFs were prepared by adding titanium isopropoxide to concentrated hydrochloric acid and a cetyltrimethylammonium bromide (CTAB) solution. The mixture was stirred, then transferred to an autoclave and heated to 150°C for 20 hours. The resulting product was washed and dried.

⑤ Preparation of CdSe-Co_3O_4@TiO= Nanocomposite:

For the CdSe-Co_3O_4@TiO_2 nanocomposite, TiO_2 powder and CdSe-cobalt oxide powder were mixed in DI water, frozen for 24 hours, and lyophilized for 24 hours to achieve nanohybridization.

⑥ Biological Sample:

Biological samples included anti-SARS-CoV-2 S protein chimeric antibody and anti-HEV IgG antibody obtained from rabbit immunization with purified G3 HEV-LPs. Genotype 7 HEV (G7 HEV) RNA copies used were at 5.0×10^8 RNA copies/mL.

⑦ Fabrication and Application of PEC-Based Device for Virus Detection:

An FTO conductive glass substrate was coated with CdSe-Co_3O_4@TiO_2 and incubated with antibodies activated by EDC/NHS at 4°C. After cleaning, the electrode was used for virus detection using PBS and 0.2 mol L^{-1} AA as electrolyte. Various virus concentrations were incubated and washed before signal detection.

⑧ Experimental Conditions:

The PEC experiments used a 430 nm wavelength source for 20-second exposure at 0.1 V bias voltage on a 1 cm2 anode. EIS tests scanned from 100 mHz to 100 kHz with a 5 mV amplitude AC sine wave. This platform integrates advanced nanomaterial synthesis, surface modification, and specific antibody interaction for precise virus detection, highlighting its potential in sensitive and rapid virus diagnostics

4．研究成果

(1) Visible Light-Driven Photoelectrochemical Platform Probing Sensitive Virus Detection

Observing the desired need for virus sensing in recent times, a viable embodiment of sensitive and accurate strategies, especially for the early stage of infection, is in demand, rapidly giving a reliable and accurate signal. In response to the critical need for sensitive and accurate virus detection, we developed a PEC device based on CdSe-Co_3O_4@TiO_2 heterojunction structure for detecting hepatitis-E virus like particles (HEV-LPs). The fabrication of this device involved three sequential stages (Figure 1).

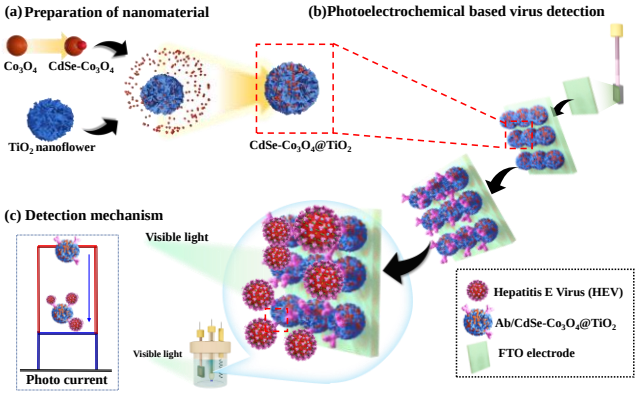

Figure 1. Schematic representation illustrating the **(a)** fabrication process of CdSe-Co_3O_4@TiO_2 NF heterostructure and **(b)** application of virus detection, and **(c)** change in photocurrent response based on virus conjugation.

① Synthesis of Co_3O_4-CdSe-TiO_2 nanoflower

Figure 2a–d depicts TEM images illustrating the sequential stages of hetero nanostructure growth. Initially, cobalt oxide nanoparticles (Co_3O_4 NPs) were synthesized by decomposing cobalt nitrate with benzylamine in the presence of ammonium hydroxide (Figure 2a). The TEM image shows Co_3O_4 NPs with a particle size ranging from 5 to 13 nm and an average diameter of 9 nm (Figure 2e). Subsequently, CdSe quantum dots (QDs) were grown on the surface of Co_3O_4 NPs, as evidenced by TEM (Figure 2b). This growth introduced an absorption and emission peak at λ=576 nm, shifted from the 2.4 nm bare CdSe peak, confirmed by UV-Vis spectra (Figure 2f). SEM imaging (Figure 2c) revealed CdSe@Co_3O_4 NPs uniformly distributed on TiO_2 nanoflowers (NFs) (Figure 2d), maintaining their flower-like morphology. Sequential X-ray diffraction (XRD) patterns confirmed the formation of CdSe@Co_3O_4@TiO_2 NFs.

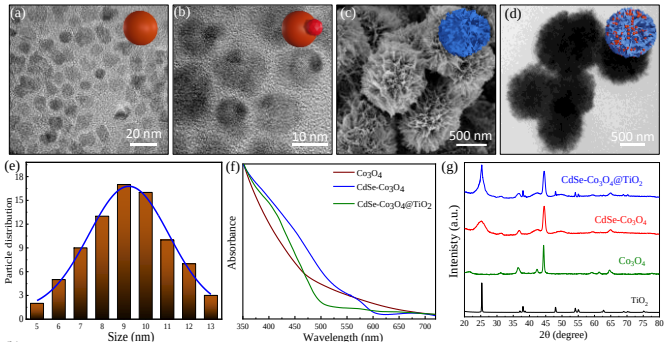

Figure 2. The **(a)**TEM images of Co_3O_4 NPs, **(b)** CdSe-Co_3O_4, and **(d)** CdSe-Co_3O_4@TiO_2 NF. **(c)** SEM image of TiO_2 NFs, **(e)** corresponding particle size distributions of Co_3O_4 NPs, **(f)** absorption spectra of Co_3O_4 and CdSe-Co_3O_4 and **(g)** XRD.

② Detection of virus

The PEC device for HEV-LPs detection utilizes anti-HEV antibody-conjugated CdSe-Co_3O_4/TiO_2 nanoflowers (NFs) on an FTO electrode, demonstrating sensitivity through photocurrent changes in response to varying HEV-LPs concentrations (Figure 3a). As HEV-LPs concentration increases, the photocurrent decreases consistently, highlighting the sensor's sensitivity to HEV-LPs. Figure 3b displays a linear calibration plot of I/I0 against the logarithm of HEV-LPs concentrations from 10 fg mL^{-1} to 100 ng mL^{-1}, achieving a high correlation coefficient of 0.990. The limit of detection (LOD) calculated using 3σ/S, where σ is the standard deviation of the lowest signal and S is the slope of the calibration plot, is 3.5 fg mL^{-1}. Assessment in 10% human serum (Figure 3c) shows a comparable detection pattern to DI water, albeit with a slightly flattened slope due to complex serum components. The LOD in serum is 8.5 fg mL^{-1}, indicating robust sensitivity suitable for practical applications despite the matrix interference.

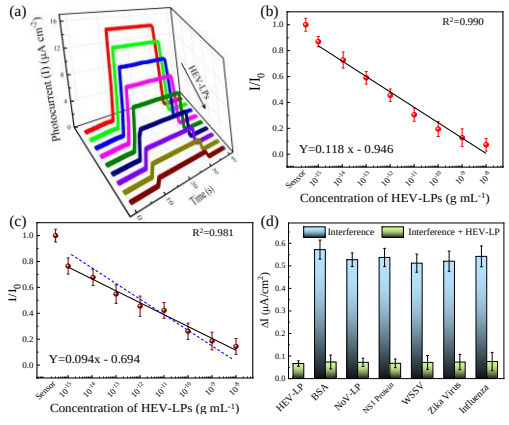

Figure 3. (a) Photocurrent response to varying HEV-LPs concentrations, (b) Calibration curve (I/I0 vs. HEV-LPs concentration), (c) Calibration curve in 10% human serum, and (d) Photocurrent response to HEV-LPs and interferents in 10% human serum, demonstrating high specificity for HEV-LPs over other analytes.

In summary, the CdSe-Co_3O_4/TiO_2 NF-based PEC sensor demonstrates high sensitivity (LOD of 3.5 fg mL^{-1} in DI water), specificity, and robust performance for detecting HEV-LPs, including in complex biological matrices like 10% human serum, promising applications in clinical diagnostics.

(2) Self-powered Device for Ultrasensitive Virus Detection using bimetallic Co-catalyst with Pt Tips

Using Co_3O_4-CdSe-CdS-Pt as an electrocatalyst activated by visible light, this system achieves ultrasensitive virus sensing. The architecture, including hollow spheres, rods, and quantum dots, optimizes hole-electron recombination rates. Co_3O_4-CdSe-CdS-Pt is encapsulated in a polymer shell with aptamers for signal amplification. This setup allows for varied analytical responses to virus concentrations through conjugation and magnetic separation techniques (Figure 4). The synthesis of Co_3O_4-CdSe-CdS-Pt nanorods begins with a cobalt oxide-CdSe seed, initiating CdS rod growth. CdSe dimers nucleate the rods exclusively on their CdSe portion, with cobalt oxide remaining at one end. Pt nanoparticles are subsequently added to the rods' other end. TEM images (Figures 5b-e) and high-angle annular dark-field micrographs (Figures 5f-k) confirm elemental presence.

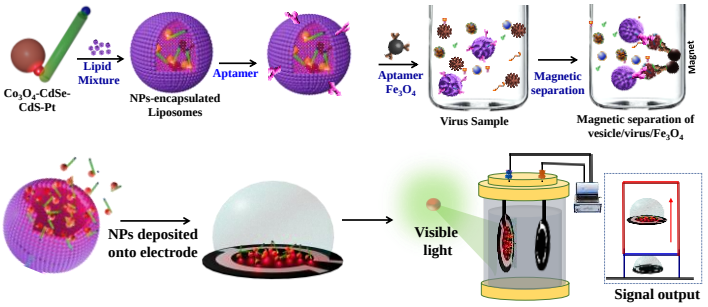

Figure 4. Schematic illustration of magnetic separation of Co_3O_4-CdSeCdS-Pt NPs-LPs/spike protein/Ab-MNP from impurities, release of Co_3O_4-CdSe-CdS-Pt NPs from liposomes and application of the biosensing device.

The detection mechanism utilizes photo current response from Co_3O_4-CdSe-CdS-Pt under visible light. Its architecture includes hollow spheres, rods, and quantum dots, optimizing Pt and Co_3O_4 positions for enhanced catalytic performance. Co_3O_4-CdSe-CdS-Pt NPs-LPs, synthesized with DOPC, DOPG, DSPE, show 100 nm spherical structure via TEM (Fig. 5b-c), validating potential for varied virus detection via conjugation and separation techniques.

① Synthesis of Co_3O_4-CdSe-CdS-Pt nanoparticles

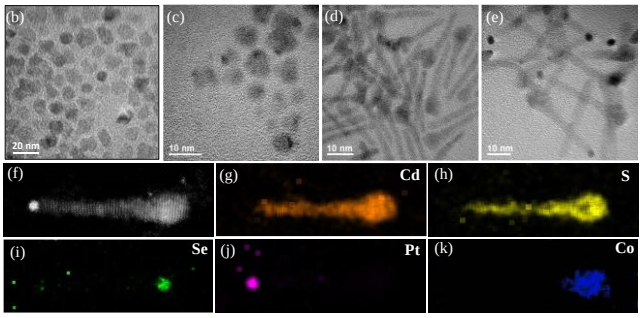

Figure 5. (a)TEM images of **(b)** Co_3O_4, **(c)** Co_3O_4-CdSe, **(d)** Co_3O_4-CdSe-CdS and **(e)** Co_3O_4-CdSe-CdS-Pt. **(f)** HAADF-STEM image of Co_3O_4-CdSe-CdS-Pt, elemental mapping images of **(g)** Cd, **(h)** S **(i)** Se, **(j)** Pt and **(k)** Co.

② Sensor preparation and its detection measurements:

Antibody-conjugated Co_3O_4-CdSe-CdS-Pt NPs embedded in liposomes and Ab-MNPs were mixed with varying SARS-CoV spike protein concentrations, forming sandwich immunocomplexes. After magnetic separation, surfactant broke the liposomes to release Co_3O_4-CdSe-CdS-Pt NPs as photocatalysts on an electrode, where photocurrent was measured (Fig. 6a). The linear correlation (Fig. 6b) spanned 10 fg mL^{-1} to 10^7 fg mL^{-1} with a high coefficient of 0.995. Evaluation in SARS-CoV-2 cell lysate (Fig. 6c) confirmed clinical suitability despite a slightly reduced slope in the calibration curve due to matrix complexities.

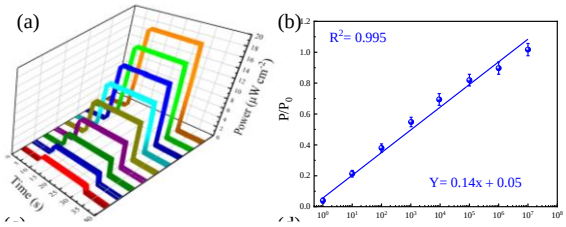

Figure 6. (a) Photocurrent response after incubation with spike proteins and (b) calibration curve of power density vs. spike protein concentration

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　0件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Ganganboina Akhilesh Babu、Khoris Indra Memdi、Konno Akinori、Li Tian-Cheng、Okamoto Akihiro、Park Enoch Y.	４．巻 190
２．論文標題 CdSe-Co3O4@TiO2 nanoflower based photoelectrochemical platform probing visible light driven virus detection	５．発行年 2023年
３．雑誌名 Microchimica Acta	６．最初と最後の頁 46
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/s00604-022-05623-9	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Dega Naresh Kumar、Ganganboina Akhilesh Babu、Venkatesan Prashanth、Roy Deblina、Tran Hai Linh、Doong Ruey-an	４．巻 390
２．論文標題 AHMT functionalized g-C3N4 quantum dots/Au based localized surface plasmon resonance sensors for ultrasensitive and rapid detection of adrenaline	５．発行年 2023年
３．雑誌名 Sensors and Actuators B: Chemical	６．最初と最後の頁 133906～133906
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.snb.2023.133906	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Akhilesh Babu Ganganboina
２．発表標題 Visible Light-Driven Photoelectrochemical Platform Based Biosensor
３．学会等名 242nd ECS Meeting（国際学会）
４．発表年 2022年

〔図書〕　計2件

１．著者名 Ganganboina, Akhilesh Babu., Park, Enoch.Y.	４．発行年 2024年
２．出版社 Springer	５．総ページ数 442
３．書名 Trends in Biosensing Research. Advances in Biochemical Engineering/Biotechnology	

１．著者名 Ganganboina, Akhilesh Babu., Indra Memdi Khoris, and Kenshin Takemura	４．発行年 2024年
２．出版社 CRC Press	５．総ページ数 364
３．書名 MXenes : From Research to Emerging Applications	

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関