Development of a Label-Free Electrochemical Biosensor for Detection of Infectious Hepatitis A Virus

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Introduction

Hepatitis A virus (HAV) is a major cause of foodborne viral illness worldwide and has been associated with outbreaks linked to fresh produce and shellfish (Shieh et al., 2009). Recent produce-associated outbreaks have highlighted the need for improved surveillance tools capable of rapidly identifying infectious viral contamination before products reach consumers (Jacobsen, 2018). Current detection methods, such as RT-qPCR, provide sensitive detection of viral RNA but cannot distinguish infectious from non-infectious viruses (ISO 15216-1, 2017). Traditional infectivity assays require several days to weeks to generate results, limiting their utility for rapid decision-making (Migueres., et al., 2021). The objective of this study was to develop a label-free electrochemical biosensor capable of rapidly detecting infectious HAV by exploiting interactions between infectious viral particles and permissive host cells, thereby supporting improved food safety monitoring and precision agriculture applications.

Materials and Methods

Carbon screen-printed electrodes were modified with 20 nm gold nanoparticles (AuNPs) to enhance conductivity and promote cell attachment. FRhK-4 cells were immobilized on the AuNP-modified electrodes following an 8 h adhesion period. A cell culture-adapted HAV strain (HM175/18f) was propagated in FRhK-4 cells and quantified using the tissue culture infectious dose ($TCID_{50}$) assay. Electrochemical impedance spectroscopy (EIS) was used to measure changes in charge transfer resistance (R_{CT}) following HAV exposure. Biosensor sensitivity was evaluated using HAV concentrations ranging from 10^0–10^6 $TCID_{50}$/mL, while specificity was assessed using non-target viruses. Stability was evaluated under elevated temperature conditions (37 °C).

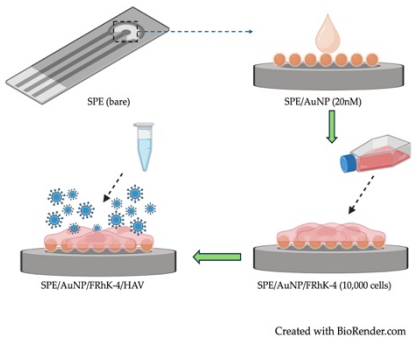

Figure 1. Electrode preparation steps, including drop-cast deposition of the gold nanoparticles (AuNP) on the bare screen-printed electrodes (SPE) followed by the immobilization of FRhk-4 cells and subsequent testing with hepatitis A virus (HAV).

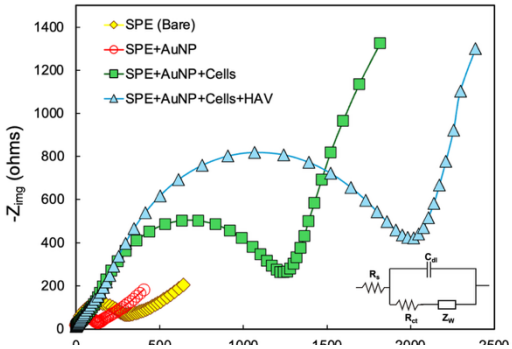

Figure 2. Nyquist plot of the electrode after each step of the electrode preparation process and the response of the biosensor to incubation with HAV for 6 hours.

Results and Discussion

Nyquist plots confirmed successful biosensor fabrication, with increasing charge transfer resistance following AuNP deposition, FRhK-4 cell immobilization, and HAV binding. Optimization studies

identified 6 h as the optimal incubation period, representing a substantial reduction in detection time compared with conventional infectivity assays for HAV. A significant linear relationship was observed between ΔR_{CT} and HAV concentration over a broad dynamic range ($R^2 = 0.9889$). The biosensor exhibited a sensitivity of 2198.6Ω·cm^{-2}/log$_{10}$(TCID$_{50}$/mL), with a limit of detection of approximately 5 TCID$_{50}$/mL and a limit of quantification of 100 TCID$_{50}$/mL. These findings demonstrate the capability of the biosensor to detect low concentrations of infectious HAV relevant to food safety applications. Specificity testing showed negligible responses toward FCV and HCoV-229E, whereas HAV produced a pronounced impedance signal. The biosensor retained approximately 90% and 60% of its original signal after 3 and 7 days, respectively, at 37 °C. Infectivity-based biosensing could complement molecular surveillance approaches by improving risk assessment and supporting targeted interventions in fresh produce systems (Li et al., 2023).

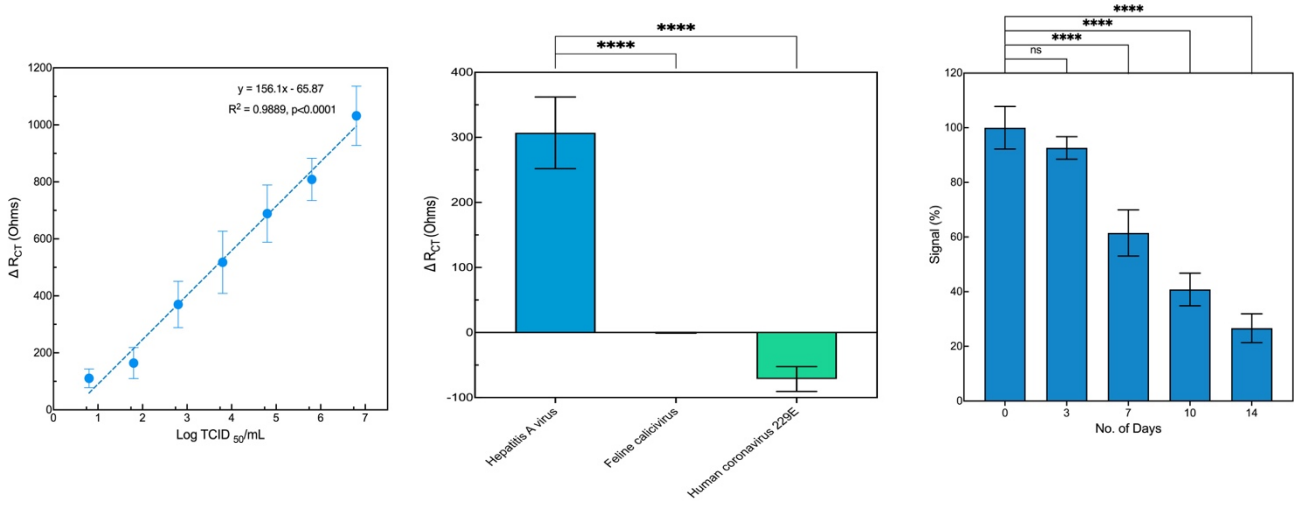

Figure 3. Analytical performance of the HAV biosensor showing (a) calibration curve, (b) specificity assessment against FCV and HCoV-229E, and (c) stability under elevated temperature conditions. The calibration curve demonstrated a linear relationship between ΔRCT and HAV concentration (y = 156.1x − 65.87; $R^2 = 0.9889$).

Conclusions

The proposed biosensor enabled rapid (6 h) and specific detection of infectious HAV with a detection limit of 5 TCID$_{50}$/mL. Ongoing studies aim to validate the platform in berry samples and improve stability for routine food safety monitoring.

References

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