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INNOVATION IN ACTION: TURNING IDEAS INTO REALITY

Chapter 25

Disposable 3D-Printed Flow Cell Biosensor for Pathogenic Escherichia Coli Bacteria Detection

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ABSTRACT

This research seeks to develop an innovative 3D-printed biosensor for the rapid and accurate detection of Escherichia coli (E. coli) bacteria in water samples. The proposed biosensor will incorporate a flow cell design, which allows continuous monitoring and efficient detection of E. coli in real-time. The sensor will be fabricated using conductive polymer-based materials integrated with specific biological recognition elements to ensure high sensitivity and specificity. The 3D printing technology will be utilized to create a precise and reproducible flow cell structure, optimizing the sensor's functionality and scalability. The research will proceed through several key phases: designing and simulating the flow cell structure, selecting and functionalizing the sensing materials, fabricating the biosensor using 3D printing techniques, and conducting extensive testing with water samples containing various concentrations of E. coli. This research will establish a foundation for future advancements in portable and effective biosensing devices for early detection of bacterial contamination.

Key Words: Escherichia Coli; Biosensor; Flow Cell; 3D printing.

1. INTRODUCTION

Escherichia coli (E. coli) is a diverse group of bacteria commonly found in the intestines of humans and warm-blooded animals. While most E. coli strains are harmless and play a crucial

role in maintaining intestinal health, some strains can cause severe food poisoning, urinary tract infections, and even life-threatening conditions such as hemolytic uremic syndrome (HUS). These pathogenic strains are a significant public health concern, particularly in environments where food and water contamination are prevalent.

2. LITERATURE REVIEW

Flow cell biosensors based on electrochemical structure have emerged as a superior design for detecting bacterial contamination. The flow cell structure offers several advantages that include continuous monitoring, enhanced sensitivity, improved response time, and reduced fouling (Stilman et al., 2022). The flow cell design allows for the continuous flow of samples through the sensor, enabling real-time monitoring and immediate detection of contaminants.

This research will leverage 3D printing technology in biosensor production. 3D printing allows for the precise and reproducible creation of complex sensor structures that would be challenging to produce using traditional manufacturing methods (Siller et al., 2020). Key benefits of using 3D printing for biosensor production include customization, scalability, precision, and material versatility. In this case, 3D printing enables the design and production of biosensors tailored to specific applications and requirements.

3. METHODOLOGY

3.1. Design and selection of sensing materials

The initial phase of the research involves the conceptualization and optimization of the 3D-printed flow cell biosensor's design using computer-aided design (CAD) software Figure 1. The second phase focuses on the selection and functionalization of conductive polymer-based materials to be used in the biosensor. The process begins by identifying and testing various conductive polymers suitable for 3D printing and biosensing applications.

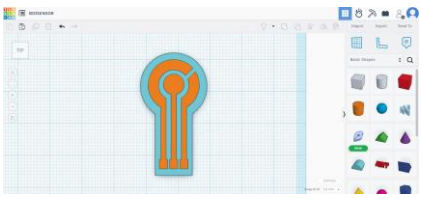

Figure 1: Schematic of the 3D-printed flow cell biosensor's design

3.2. Setup for the cyclic voltammetry (CV) testing

This phase began with the preparation of the cyclic voltammetry (CV) setup to evaluate its performance on the water samples Figure 2. The Analog Device ADALM1000 was used to demonstrate the relationship between current, voltage, and impedance (including resistance, inductance, and capacitance).

The CV setup included three different electrode materials: 1) platinum (Pt) as the counter electrode, which was connected to the GND of the ADALM1000, 2) graphite (Gr) as the working electrode, which was connected to the CHA of the ADALM1000, and 3) silver

chloride (AgCl) as the reference electrode, which was connected to the CHB of the ADALM1000. For the preparation of the water sample used to systematically evaluate the CV's performance, the experiment utilized two solutions consisting of: 1) 40 mL of 3M KCl combined with 3 mL of 0.1M KCl + 0.5 mg/mL of NiTsPc, and 2) 40 mL of 3M KCl combined with 6 mL of 0.1M KCl + 0.5 mg/mL of NiTsPc.

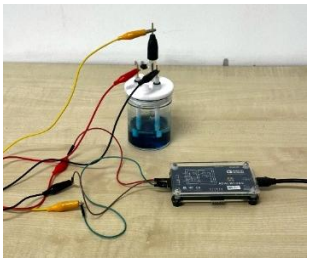

Figure 2: Setup for the cyclic voltammetry (CV) testing

3.3. Setup for the 3D-printed flow cell biosensor testing

The experiment continued with the preparation of the 3D-printed flow cell biosensor setup to evaluate its performance on the water samples Figure 3. In this phase, only the CV component was replaced with the biosensor, while the other devices remained the same for data evaluation.

The biosensor also contained three different electrodes: 1) the counter electrode was connected to the GND of the ADALM1000, 2) the working electrode was connected to the CHA of the ADALM1000, and 3) the reference electrode was connected to the CHB of the ADALM1000. The biosensor was tested by before and after applying a solution of 0.1M KCl + 0.5 mg/mL of NiTsPc onto its conductive surface to obtain the readings.

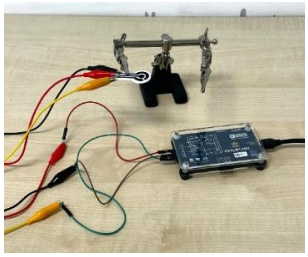

Figure 3: Setup for the 3D-printed flow cell biosensor testing

4. RESULTS & DISCUSSION

4.1. Fabrication of the biosensor using 3D printing

The research is centered on the actual fabrication of the 3D-printed flow cell biosensor using advanced 3D printing techniques Figure 4. High-resolution Bambu Lab A1 mini 3D printers will be employed to fabricate the optimized flow cell structure, ensuring precise replication of the design developed in the first phase.

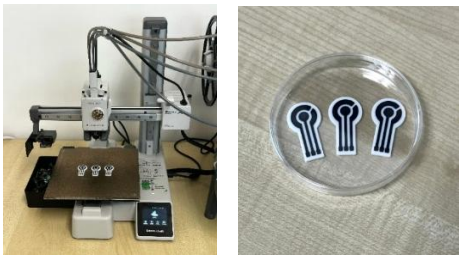

Figure 4: (a) The advanced 3D printing techniques, and (b) The actual fabrication of the 3D-printed flow cell biosensor

4.2. Testing on the cyclic voltammetry (CV)

The CV graph illustrated the response of how the current changed as the voltage was scanned in both the forward and reverse directions Figure 5. The solution for this experiment was prepared using 40 mL of 3M KCl combined with 3 mL of 0.1M KCl + 0.5 mg/mL of NiTsPc. It showed that there were no clear peaks of oxidation and reduction available because the electrolyte solution might be insufficient at low concentration. The current (A) ranged from approximately -0.035 to 0.04 A, while the voltage (V) varied between 0 and 3 V.

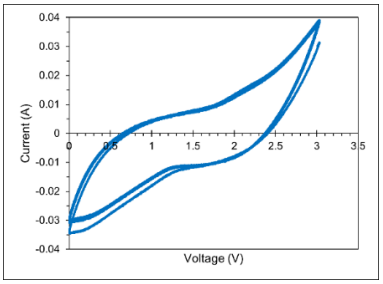

Figure 5: CV's graph of 40 mL of 3M KCl with 3 mL of 0.1M KCl + 0.5 mg/mL of NiTsPc

This part showed the typical shape with an oxidation (anodic) and reduction (cathodic) peak illustrating the electroactivity of the system Figure 6. Red lines showed the slope of different regions with response to the change in the current, and green arrows showed the voltage readings for oxidation was 2.2 V and reduction was 0.9 V. The solution for this experiment was prepared with 40 mL of 3M KCl with 6 mL of 0.1M KCl + 0.5 mg/mL of NiTsPc. The current (A) ranged from approximately -0.027 to 0.03 A, while the voltage (V) varied between 0 and 3 V.

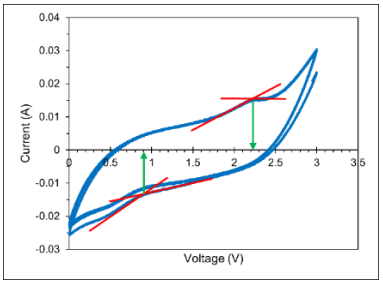

Figure 6: CV's graph of 40 mL of 3M KCl with 6 mL of 0.1M KCl + 0.5 mg/mL of NiTsPc

4.3. Testing on the 3D-printed flow cell biosensor

As stated in the method, the 3D-printed flow cell biosensor was tested before and after the electrolyte solution was applied. Figure 7 displayed the graph obtained when the biosensor was tested without any solution. The result showed that the voltage (V) ranged from 0 to 0.5 V, remaining nearly constant at Channel B across each cycle.

Figure 7: Biosensor's graph without any solution

Next, the solution for this experiment was prepared using 0.1M KCl + 0.5 mg/mL of NiTsPc, then applied to the biosensor to obtain the graph Figure 8. The results showed that the voltage (V) ranged from 0 to 1.4 V. A clear change was detected as the peaks increased, indicating that the biosensor exhibited conductive capability in detecting the solution.

Figure 8: Biosensor's graph with the solution of 0.1M KCl + 0.5 mg/mL of NiTsPc

5. CONCLUSION & RECOMMENDATION

It could be concluded that the fabrication of the 3D-printed flow cell biosensor using advanced 3D printing techniques achieved early success, as it was a low-cost, disposable, biodegradable, and mass-producible alternative to conventional methods.

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