ABSTRACT - TECHNOLOGICAL INNOVATION;

ELECTROCHEMICAL BIOSENSOR FOR LUNG CANCER MONITORING BASED ON POLYPYRROLE AND CARBON NANOTUBES FOR DETECTATION OF CYFRA-21-1.

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Abstract: Innovative strategies have been proposed for early diagnosis of diseases and to avoid the risks associated with already well-established methods. Tumor biomarkers like Cytokeratin-19 fragment (CYFRA-21-1) offer a new perspective in the early identification of lung cancer cells. In the present study, we report the development of an electrochemical immunosensor based on polypyrrole (Ppy) films and multi-walled carbon nanotubes (MWCNTs), functionalized with the CYFRA-21-1 antibody and tested using human lung cancer cell samples. For this purpose, gold working electrodes and a PGSTAT 128N potentiostat/galvanostat were used for cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) experiments. Gold electrodes

were polished with alumina slurry and rinsed with ultrapure water. Electrochemical characterization was performed using CV and EIS in a 5 mM potassium ferri/ferrocyanide ($[Fe(CN)_6]^{3-/4-}$, 1:1) solution prepared in 0.01 M phosphate buffer (pH ~7.4). Polypyrrole (PPy) films were electrodeposited by applying 4 CV cycles (−0.4 V to +1.0 V, 50 mV/s) in 0.5 M HCl containing 31 µL of pyrrole monomer. After deposition, CV and EIS were repeated. Next, multi-walled carbon nanotubes (MWCNTs) were drop-cast and incubated on the surface, followed by electrochemical analysis. Surface activation was carried out using EDC:NHS (0.4 M e 0.1 M, 1:1, v/v). Then, CYFRA-21-1 antibody (10 µg/mL) was applied and incubated to enable its covalent immobilization on the activated surface, allowing subsequent recognition of the CYFRA-21-1 biomarker. Blocking of nonspecific binding sites was performed by applying bovine serum albumin (BSA, 0.0001%). Finally, lung cancer cell samples were added to the modified surface, and electrochemical responses were recorded after 15 min of interaction. Each step of surface functionalization resulted in a progressive decrease in cathodic and anodic peak current and an increase in charge transfer resistance (Rct), confirming successful layer-by-layer assembly. CV analysis of lung cancer cell suspensions ranging from 10^0 to 10^6 dilutions, revealed a dilution-dependent decrease in anodic current, from 43.82 µA (10^0) to 22.27 µA (10^6), and cathodic current, from –38.52 µA (10^0) to –22.56 µA (10^6) indicating dilution-dependent signal reduction associated with decreased biomarker availability. EIS confirmed this behavior, showing a progressive increase in charge transfer resistance (Rct) as cell dilution increased, suggesting enhanced impedance due to antigen–antibody binding. Although some fluctuations were observed at intermediate concentrations, the overall pattern indicated concentration-dependent recognition and signal modulation, likely influenced by molecular rearrangements or steric hindrance effects at the sensing interface. These results confirm the biosensor’s ability to distinguish varying cell concentrations and detect CYFRA-21-1 with high sensitivity. Additionally, a control experiment using NSE (neuron-specific enolase) was performed, demonstrating no significant response, which reinforces the sensor's specificity to CYFRA-21-1. In conclusion, the proposed nanostructured biosensor demonstrates excellent electrochemical performance and sensitivity toward lung cancer cell biomarkers. Its effectiveness even at low analyte concentrations (10^0) supports its potential use in early-stage diagnosis. Further validation with well-defined cell lines and more complex clinical specimens is recommended to ensure practical application in pulmonary oncology.

Palavras-chave: cyfra-21-1; electrochemical biosensor; lung cancer; polypyrrole; multi-walled carbon nanotubes.