Covalent immobilization of thiolated oligonucleotide onto glassy carbon: a cost-effective alternative for genosensor fabrication

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ABSTRACT:

Thiol-functionalised molecules and biomolecules have been extensively demonstrated to spontaneously self-assemble onto noble metal surfaces. However, the produced monolayer is known to suffer from thermal and chemical instabilities due to the non-covalent nature of the S-Au bond. In this work we report on the development of a novel approach for the covalent immobilisation of thiolated oligonucleotides onto glassy carbon surfaces. A glassy carbon sheet is first activated by sequential electrochemical hydrogenation followed by electrochemical chlorination. The activated glassy carbon electrode was then incubated at room temperature with a solution of the thiolated molecule. A preliminary evaluation and optimisation of the proposed approach was performed using ferrocene-hexanethiol and thioctic acid functionalised oligonucleotide probes. The approach was demonstrated to be highly effective, resulting in highly stable monolayers that show remarkable thermal stability. Moreover it was also shown via hybridization assay, that the immobilised biomolecules retained their activity demonstrating the suitability of the proposed approach for preparation of electrodes for solid phase electrochemical real-time polymerase chain reaction and high-resolution melting curve analysis. Characterisation of the prepared surface was performed electrochemically and using Kelvin probe force microscopy, FT-IR and Raman spectroscopy.