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OPTIMIZATION AND DEVELOPMENT OF DNA BASED BIOSENSOR FOR THE DETECTION OF PATHOGENS

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Abstract

The COVID-19 outbreak caused by SARS-CoV-2 disrupted global life and economies, but also spurred innovation in rapid diagnostics. DNA/RNA-based tools such as CRISPR and LAMP, along with AI-driven methods, have improved pathogen detection speed and accuracy. Among these, DNA biosensors are emerging as low-cost, efficient, and reliable diagnostic tools.

In this study, a DNA-based biosensor was developed and tested using carbon and gold electrodes to determine optimal biomolecule adherence, analyzed via TEM. Electrochemical impedance data were obtained using PCR-amplified positive and negative COVID-19 samples on a locally designed impedometer. Results indicated that gold electrodes exhibited superior signal performance compared to carbon electrodes. These findings provide a basis for further optimization and indigenization of DNA biosensors for rapid and cost-effective pathogen detection.

Keywords: Aptamer, DNA biosensors, DNA oligos, Electrodes, Probes, TEM, Thiolation

INTRODUCTION

The Outbreak and pandemic causative agent SARS-CoV-2 not only wedged life severely at global level it also caused serious losses to the economy around the world but at the same time it also opened a window for innovation in global health sector specially in the field of diagnostics specially the point of care devices. Many molecular DNA and RNA based techniques have been used for the detection of pathogens but developing a cost effective and robust diagnostic tool is always a challenge. Techniques like RT LAMP, CRISPR and now even AI tools are offering numbers of diagnostics techniques but biosensor is also making progress in the field of POC diagnosis (1).

Biosensors are based on the basic principal of converting biological signals/data in to electrical/electronic readable signal/data. They consist of four parts an analyte, bio-receptor, transducer and a substrate. These are also being differentiated on the basis of their output like; electrochemical output, Physical output or Optical output. On the basis of bio-receptors biosensors are categorize as DNA/RNA based biosensors, Abs based biosensors, Ags based biosensors, Enzyme based biosensors, Aptamers based biosensors, Tissue based biosensors, Cells based biosensors. One of the most promising and emerging Biosensor type is DNA based Biosensors as they are robust and gives highly specific diagnostic results (2).

Another important part of any biosensor is electrodes upon which bio-molecules along with their substrate are attached. A basic electrode frame consist of three conductive separate linings; a Counter electrode to complete circuit, Reference electrode which has a constant electrochemical potential, a working electrode upon which bio analytes are attached. Commonly electrodes are made up of gold, carbon, platinum or silicon compounds. When working with DNA based biosensors gold and carbon based electrode materials are preferred due to their adherence with DNA molecules (3)

This initial study has been conducted on both types of electrodes i.e. carbon and gold. Gold electrodes were found to be a better option because it provides better surface attachment for DNA oligos. And preliminary few positive and few negative samples of COVID-19 were checked that gave promising impedance results which will help in developing ingenious DNA based sensors.



MATERIALS AND METHODS

SELECTION OF ELECTRODE SURFACE

For this study a screen printed electrode (SPE) model TE100 was used in both carbon and gold material (Fig. 1) (4). Both materials had three electrodes (Working, Reference and Counter). The size of its nano structured working electrode was 4mm, reference electrode was made of Ag/AgCl and the total length of screen printed electrode was 29mm. This type of electrode can be used for rapid electrochemical detection, and it offers a plug and play feature that allows transducer to generate results at a very fast pace.

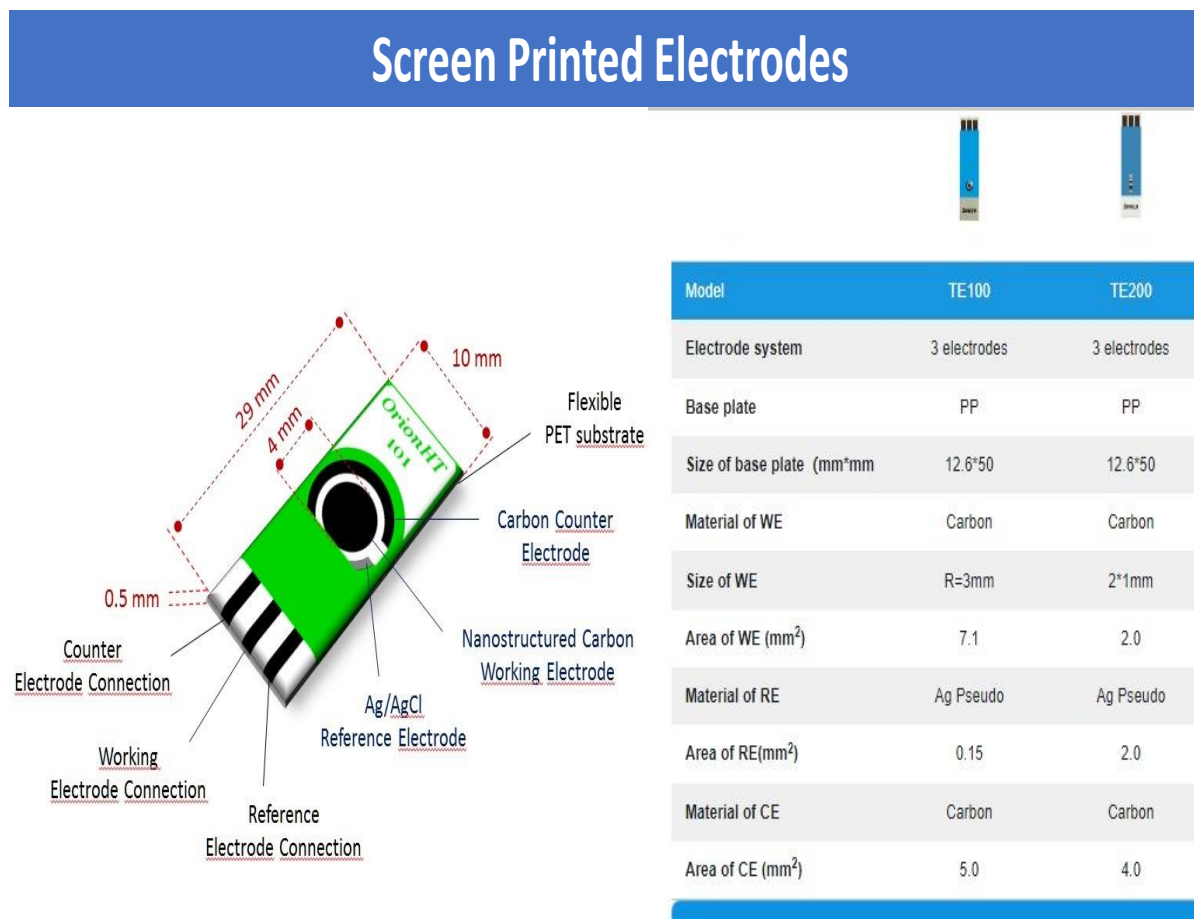


Fig. 1. <https://www.nanochemazone.com/product/nano-structured-carbon-screen-printed-electrodes/>

<https://www.zensord.com/PrintedElectrodes.html>

Screen printed electrode, model TE100, used in the study

PREPARATION OF DNA OLIGO MIX

DNA oligo sequences of COVID-19 were prepared in Nuclease free Water. The surface of working electrode of both carbon and gold electrodes were cleaned with alcohol swab before applying DNA oligos afterward incubation at room temperature was given for 30 min. Later the same electrodes were observed for DNA oligo attachment under Electron microscope (TEM). Images were taken before and after applying DNA oligos at working electrode. (Fig. 2 & 3).

TESTING ELECTROCHEMICAL IMPEDANCE

After the topological differences observed on the surfaces of both carbon and gold electrodes the electrochemical impedance (Z , is a measure of the opposition to electrical flow) was measured after applying 25 μ L of known few positive and few negative COVID-19 samples on to working electrodes. The electrochemical impedance range was set between 1 KHz to 11 KHz and impedance (Z) range was 10,000 to 22,000 z value (Fig. 4).

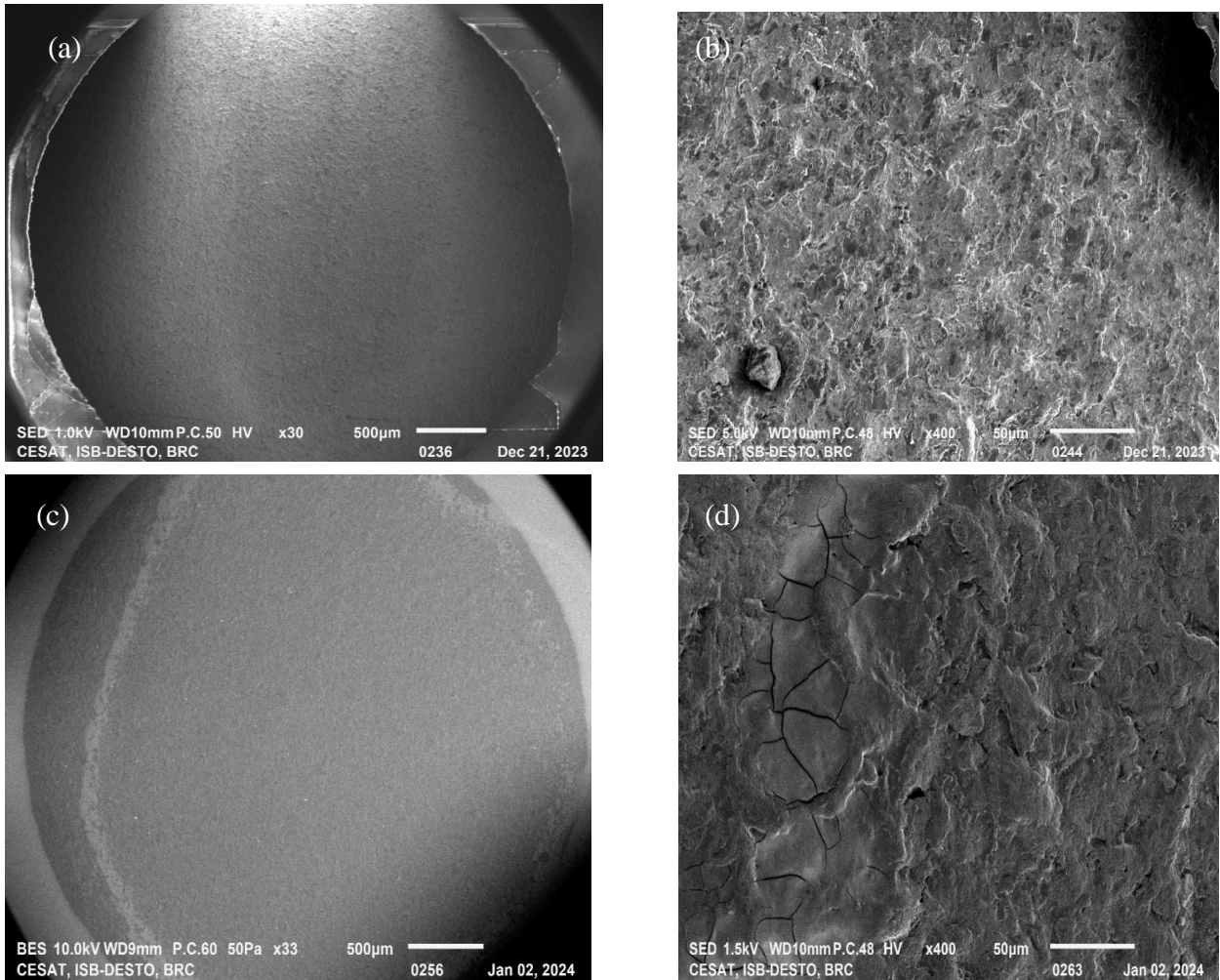


Fig. 2. Carbon electrodes, (a & b) before DNA oligos attachment; (c & d) after DNA oligos attachment

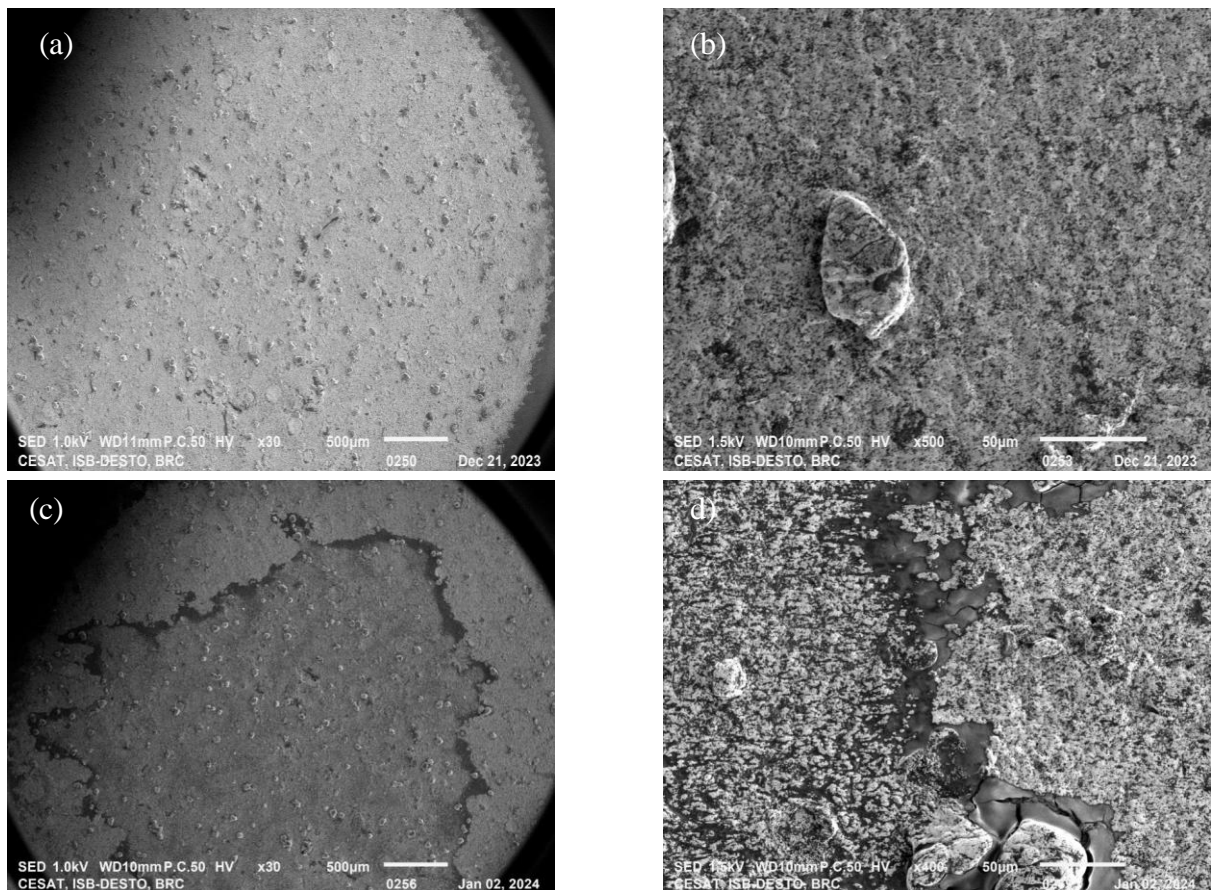


Fig. 3. Gold electrodes, (a & b) before DNA oligos attachment; (c & d) after DNA oligos attachment

RESULTS AND DISCUSSION

The electron microscopy (TEM) of both carbon and gold screen printed electrodes (SPE) showed promising results while showing a prominent surface structure changes after applying DNA oligos on to working electrode surface without any additional adhering molecules. The electrochemical impedance data was found such as positive samples showed impedance between 19,000 to 21,000 (z), negative samples showed impedance between 16,000 to 18,000 (z) and simple buffer/blank sample showed impedance between 12,000 to 14,000 (z). The results were promising and it was observed that gold electrode gave better results as compared to carbon electrode.

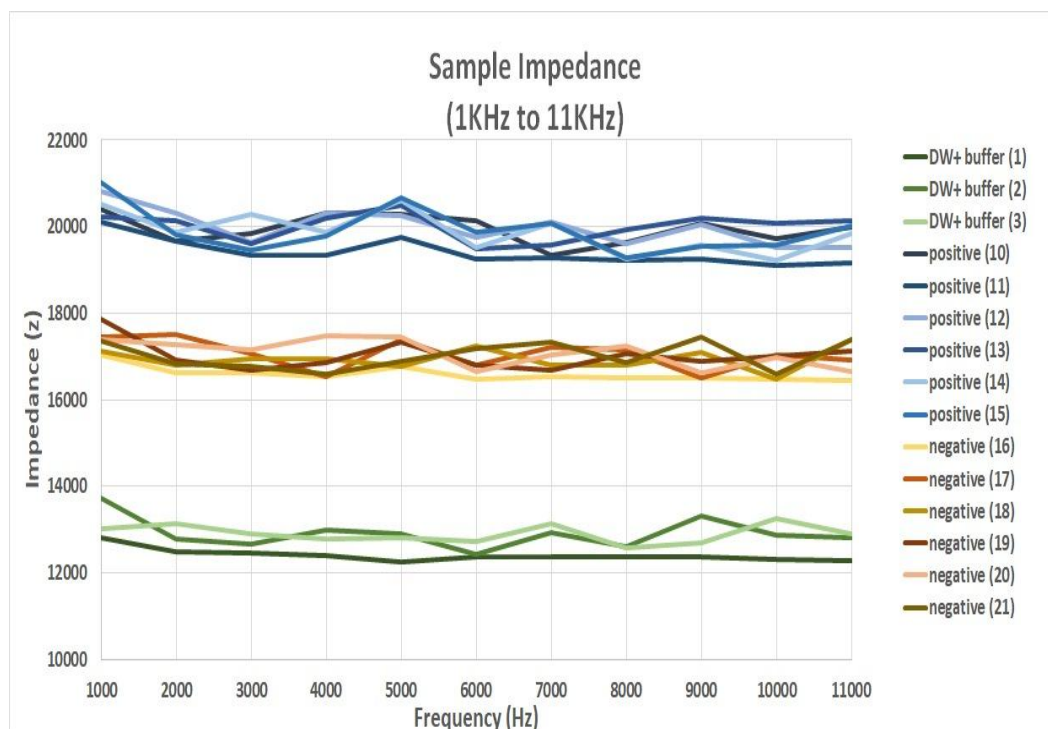


Fig. 4. Electrochemical impedance measurements

The field of diagnostics is growing at a very fast pace specially after the COVID-19 pandemic this field has revolutionized itself and numbers of new advance, cost effective tools and techniques have been introduces as a point-of-care solutions. Biosensors are cost effective and robust diagnostic tools that can be used on field and in lab both. DNA biosensors have few prominent features over others like: ruggedness of DNA molecules (oligos or aptamers), high specificity, and cost effectiveness (4).

Current study showed that Oligos attached without any linker molecules to carbon and gold surface can also give results after sample processing, this technique can further reduce the cost, however the ongoing experiments will undertake the Oligos attached to carbon / gold surface with the help of linker molecules and its comparison with directly attached Oligos.

The clear difference between impedance of the positive, negative and blank samples showed that this technique can be used for successfully detecting target genes / pathogens in a very small sample amount (5, 6). Furthermore the preliminary data showed that no sample processing was required. However the work is still in process of optimization for time duration and concentration of target and applied DNA molecules. Once optimized completely this technique has potential to be used in field and in lab both. Currently only 45-50 minutes are required for sample detection. In addition to that it was found that gold coated electrode gave better results as compared to carbon electrode, for DNA oligos surface attachment and monitoring the impedance, therefore further optimization work will be conducted on gold coated electrodes.

The COVID-19 pandemic served as a major catalyst for advancements in diagnostic science, accelerating the development of rapid, cost-effective, and reliable detection methods for infectious agents. Within this evolving landscape, biosensors have emerged as a promising class of diagnostic devices due to

their ability to provide accurate and real-time results with minimal sample processing (7). The present study focused on the optimization of a DNA-based biosensor utilizing both carbon and gold electrodes to evaluate their suitability for pathogen detection, specifically for SARS-CoV-2.

The comparative analysis revealed that gold electrodes exhibited superior performance in terms of DNA oligonucleotide attachment and electrochemical impedance response (8, 9). This can be attributed to the inherent physicochemical properties of gold, including its excellent electrical conductivity, chemical stability, and strong affinity for thiolated DNA molecules. The distinct impedance values recorded for positive, negative, and blank samples further confirmed the biosensor's ability to differentiate between target and non-target genetic materials, validating its diagnostic potential. These findings suggest that gold electrodes offer a more conducive platform for stable biomolecular immobilization and efficient signal transduction compared to carbon-based alternatives.

A significant observation from this study was that DNA oligonucleotides could be effectively attached to both carbon and gold surfaces without the use of linker molecules. This simplified approach not only reduces fabrication costs but also streamlines the overall biosensor development process, making it more adaptable for large-scale production and deployment in resource-limited settings (10, 11). Moreover, the biosensor demonstrated rapid detection capability, requiring approximately 45–50 minutes from sample application to result acquisition, which underscores its potential as a practical point-of-care diagnostic tool.

Despite these promising results, further optimization is required to enhance the device's performance and reproducibility. Future research should focus on refining parameters such as the incubation time, DNA concentration, and electrode surface modification techniques to achieve greater sensitivity and specificity.

The COVID-19 pandemic accelerated advancements in diagnostic technologies, leading to the development of rapid, cost-effective, and field-deployable tools. Among these, DNA biosensors stand out for their robustness, specificity, and affordability (12). This study demonstrated that oligonucleotides (oligos) can attach directly to carbon and gold electrodes without linker molecules while still producing measurable electrochemical signals. This approach simplifies fabrication and reduces cost. Further experiments will compare direct vs. linker-assisted oligo attachment to enhance performance.

A clear difference in impedance signals among positive, negative, and blank samples confirmed the biosensor's ability to detect target genes in small sample volumes, with minimal sample processing required. The detection process currently requires 45–50 minutes, and gold electrodes showed superior signal response and biomolecule adherence compared to carbon electrodes. Ongoing optimization aims to refine detection time and probe concentrations for reliable lab and field applications.

CONCLUSION

In conclusion, the findings of this study underscore the potential of DNA-based biosensors as an efficient, robust, and cost-effective diagnostic platform. The observed performance of gold electrodes establishes a strong foundation for subsequent optimization and indigenization efforts aimed at developing localized, high-performance biosensing technologies capable of contributing meaningfully to global infectious disease diagnostics.

Conflict of interest:

There is no conflict of interest in this study.

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Authors' contribution:

NMG Performed the experiments; AK Provided technical outputs and wrote the manuscript; SAS Provided relevant literature; SND Provided some agars; AKh Proof reading.



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