

A novel DNA-based electrochemical sensor for the detection of *Candida* spp.

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INTRODUCTION & AIM

Invasive fungal infections are increasingly common, especially in hospitalized or immunocompromised patients. Early diagnosis and antifungal treatment are crucial for survival, but routine lab tests often yield unclear results. Diagnostic methods like histopathology, serology, and cultures can be time-consuming and unreliable. Blood culture, the current gold standard for *Candida* detection, is slow and often insensitive. These challenges emphasize the need for faster, more accurate fungal detection methods in clinical laboratories. The main of this work is presented Figure 1 below.

The main objectives of this effort are described below:

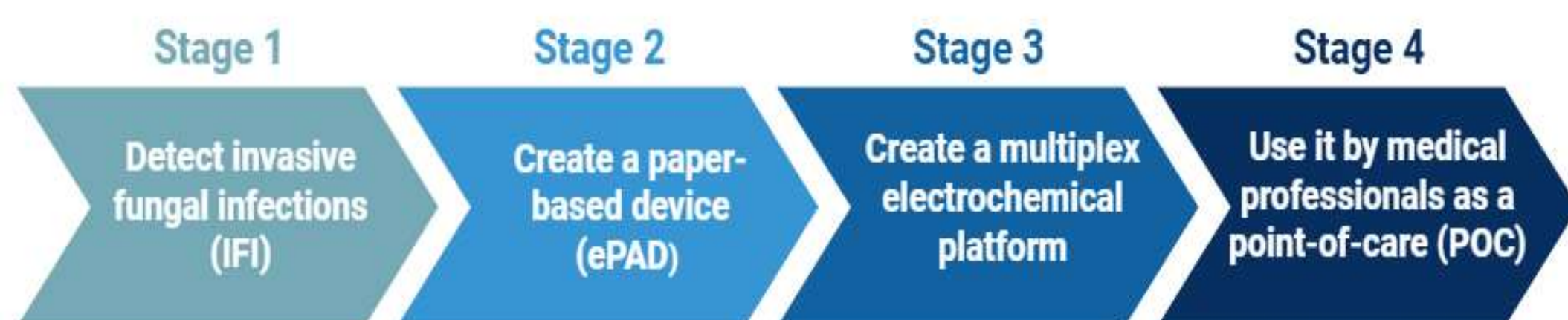


Figure 1. The main objectives described in four stages to be developed.

METHODS

A *C. albicans*-specific DNA oligonucleotide sequence was necessary for the genosensor's development. To identify this species, a 90 bp synthetic DNA fragment was selected. A 25 bp DNA-capture probe and a 65 bp DNA-signaling probe were used to cut the sequence into two pieces. Screen-printed gold electrodes (SPGE) served as the electrochemical transducer. Pretreatment, sensing, sandwich hybridisation, and electrochemical detection were all incorporated into the sensor design. Ethanol and ultrapure water were used as pretreatments for SPGE. After bonding to the target DNA in the sandwich experiment, the DNA-capture probe was left immobilised on the working electrode overnight. To ensure probe orientation, a SAM interface was utilised in conjunction with capture probes and 6-mercapto-1-hexanol (MCH). Through the binding of a fluorescein-labeled probe to the target, sandwich hybridisation increased selectivity. The anti-fluorescein antibody was coupled with horseradish peroxidase, and the oxidized product was measured by chronoamperometry (Figure 2).

CONCLUSION & FUTURE WORK

The new biosensor, with its great sensitivity and selectivity, could serve as a portable, user-friendly, and low-cost instrument for monitoring fungal infections in HSCT users. Preliminary studies showed that utilizing these nanogenosensors, *Candida* spp. could be detected in synthetic fungal samples. Despite these findings, the nanogenosensor is being optimized for the quantification of *Candida albicans*, which will be verified in future investigations.

In future advances, we will explore the applicability in the hospital environment in terms of sensitivity, accuracy, response time, obstacles, and potential. As disposable analytical instruments, affordable paper-based transducers (ePAD) are built with glossy cellulose paper and conductive inks (under optimization).

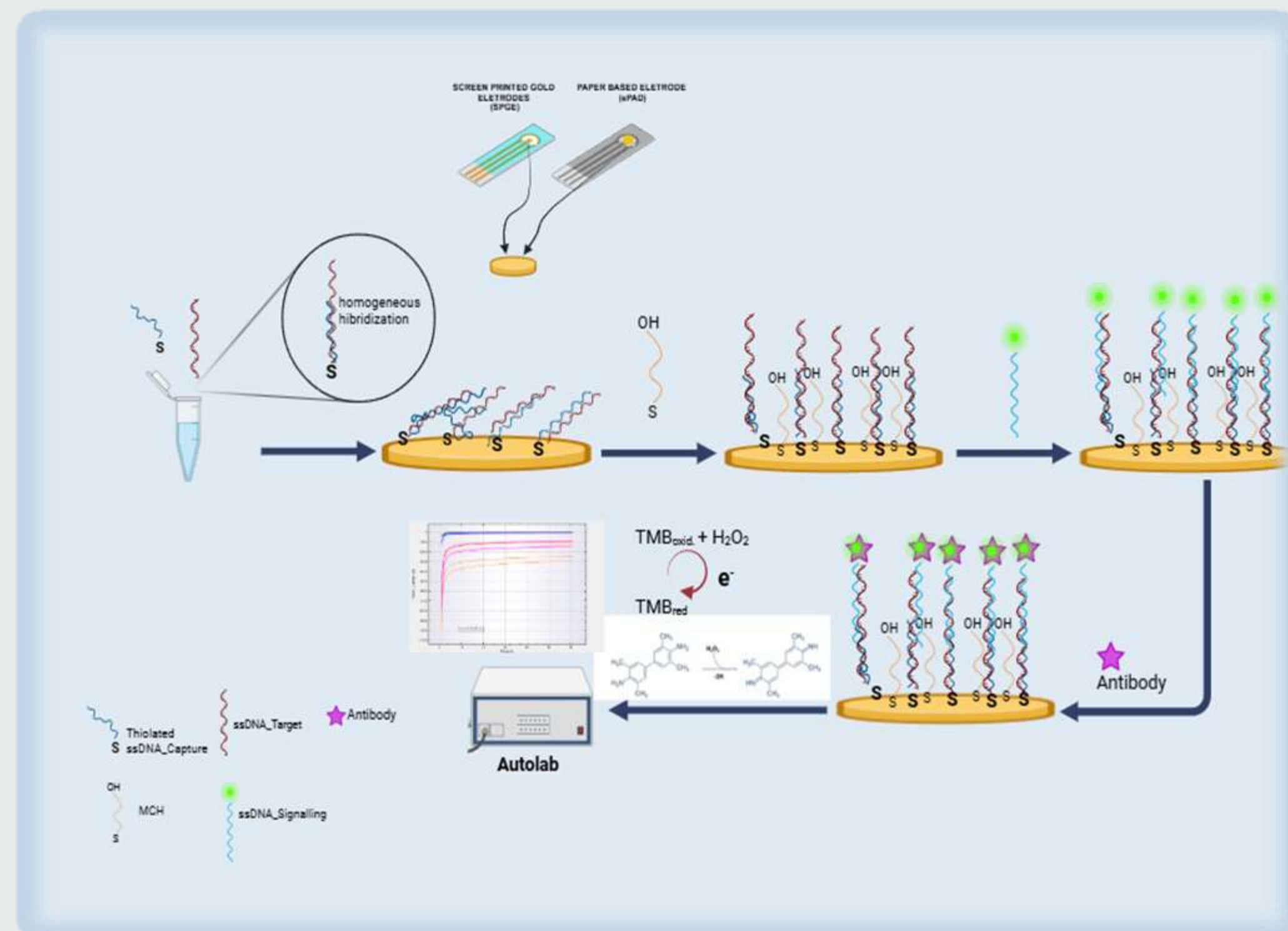


Figure 2. Develop of genosensors designed to evaluate *Candida* spp.

RESULTS & DISCUSSION

To develop a genus-specific electrochemical genosensor, a synthetic 90-bp DNA sequence specific to *Candida albicans* was selected. This sequence was divided into two fragments: a 25-bp capture probe and a 65-bp signaling probe. Chronoamperometric signals at -0.1 V were used to optimize sensor performance with 0 and 5 nM of target DNA. Gold SPGE electrodes were chosen due to their excellent conductivity, biocompatibility, and ability to form self-assembled monolayers. Electrodes A, B, and C were tested with 0.25, 0.5, and 1.0 μM of capture probe, respectively. Figure 3 shows that the highest current was obtained with 1.0 μM (assay C). Preliminary results demonstrate high sensitivity and selectivity for detecting *C. albicans* synthetic DNA.

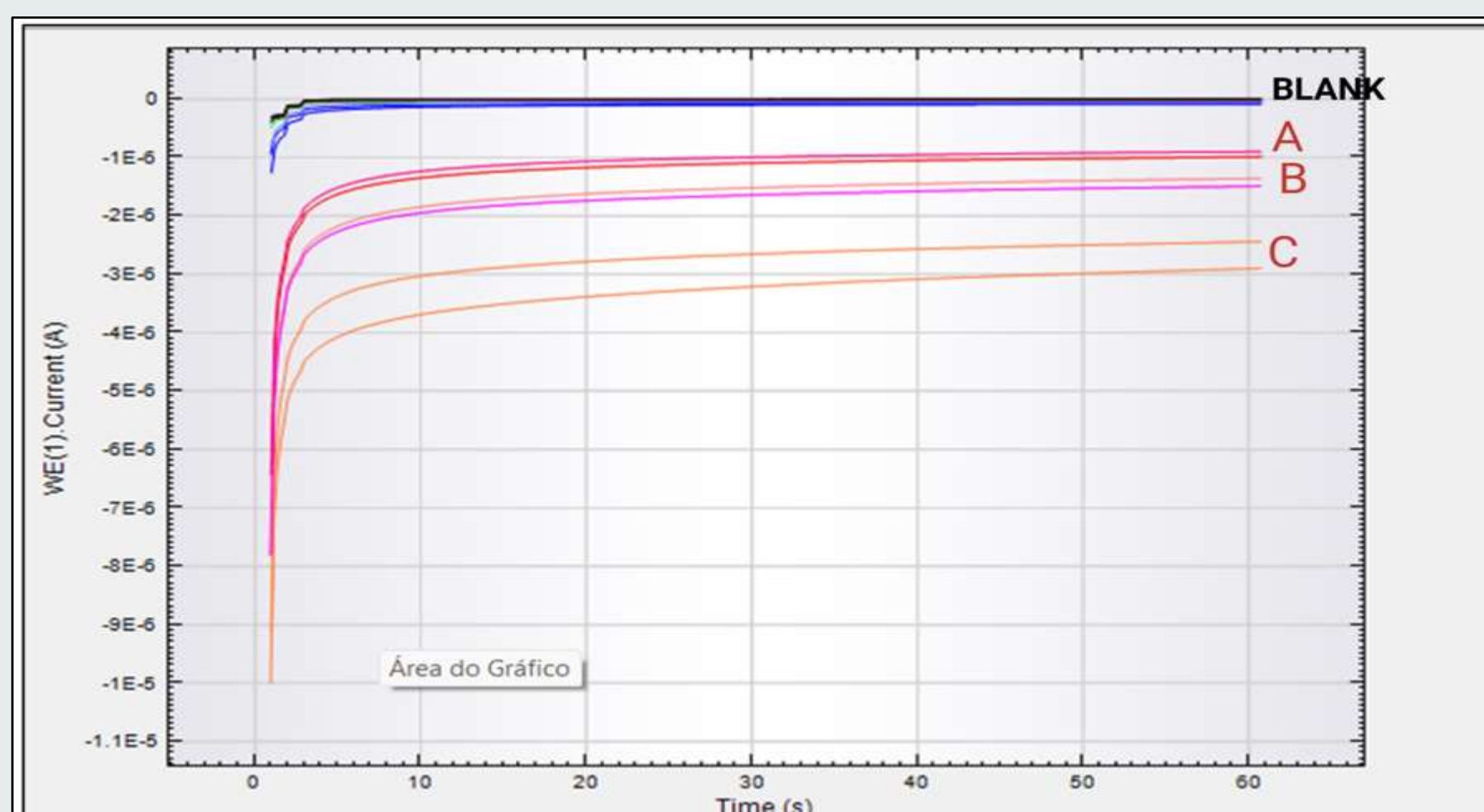


Figure 3 - A representative chronoamperogram of an optimization assay of DNACapture concentrations using a concentration of DNATarget 5nM.

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