Talanta 220 (2020) 121375

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Lectin-based impedimetric biosensor for differentiation of pathogenic *candida* species

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ARTICLE INFO

Keywords:
Candida spp.
Electrochemical impedance spectroscopy
Gold nanoparticles
Lectins
Atomic force microscopy

ABSTRACT

Fungi stand out as primary pathogens present in healthcare-acquired infections, presenting an increased number of cases even using appropriate antifungal therapy. Candida spp. is a predominant microorganism among several fungal pathogens present in the healthcare setting. Candidemia and candidiasis are fungal infections responsible for high morbidity and mortality among ill patients in hospitals. It is noticeable that prolonged hospital stays lead to a higher economic impact and increased risk for developing secondary fungal or even bacterial infections. New fast and sensitive approaches for the detection of *Candida* species is highly required. Electrochemical biosensors are an excellent alternative to conventional techniques by combining fast analyte detection, low cost, and the possibility of miniaturization. Lectins are carbohydrate-binding proteins with the capability to reach out to the microorganism cell wall. In this work, we proposed the development of an impedimetric biosensor for *Candida* spp. based on Concanavalin A (ConA) and wheat germ agglutinin (WGA) as recognition agents of the yeast cells. Atomic force microscopy images indicate changes in the biosensor surface after assembly of the molecules and exposure to fungal samples. Electrochemical impedance spectroscopy results revealed a proportional increase of charge transfer resistance (R_{CT}) as fungal CFU increased, where four *Candida* species were evaluated (*Candida krusei*, *Candida tropicalis*, *Candida parapsilosis* and *Candida albicans*). The biosensor is useful to differentiate *Candida* spp. with a detection limit between 10^2 to 10^6 CFU mL^{-1}. The obtained biosensor appears as an innovative candidate for the detection and differentiation of pathogenic *Candida* spp.

1. Introduction

The fungal kingdom comprises several different kinds of species (~1.5 million) on Earth [1]. Yeasts, molds, and mushrooms display an essential contribution to human life in baking, production of antibiotics, and chemicals. Previous studies estimate that ~400 species of these eukaryotic organisms presents pathogenicity in humans [2]. Yeasts fungi are commensal symbiont mainly found in human skin and mucous membranes. Also, *Candida sp*. is potentially harmful microorganism [3].

Pathogenic fungal species (e.g., *Candida* spp.) triggers most fungal infections on a worldwide level [4]. The infective episodes are commonly related to preterm neonates, immunocompromised, and ill patients in broad-spectrum antibiotic treatment or with invasive devices (e.g., catheters) [5]. *Candida* contains up to 150 different species, and seven *Candida* species (*C. albicans, C. krusei, C. parapsilosis, C. tropicalis, C. glabrata, C. lusitaneae,* and *C. guilliermondii*) are involved in 95% of human infections. It is estimated that more than 13% of the population suffers from microorganism infections that affect the skin and mucosal. The systemic infecting form (candidiasis and candidemia) affects about 300 million people [6].

The candida cell wall is composed of about 90% carbohydrate and 10% protein. Of note, the molecular organization on the cell wall contributes to the immune recognition and interaction with the cell host surface. The carbohydrate portion comprises O-/N-linked mannose polymers, β-glucans, and chitin [7]. Besides, their structural organization is intrinsically related to the pathogenicity of each species, and these differences in their structure should be explored, aiming for their identification and differentiation.

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https://doi.org/10.1016/j.talanta.2020.121375
Received 18 March 2020; Received in revised form 1 July 2020; Accepted 2 July 2020
Available online 11 July 2020

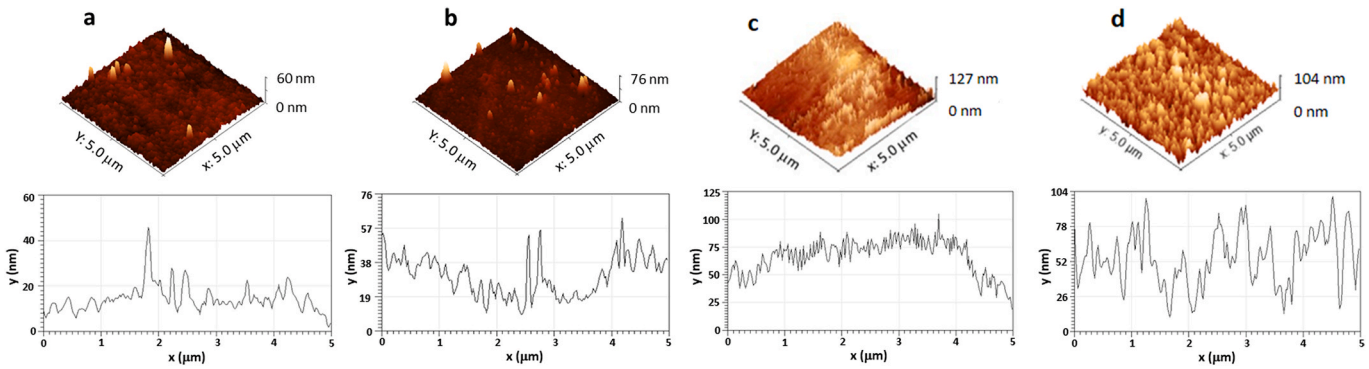

Fig. 1. AFM height images and surface roughness profiles of the modification process: (a) Cys monolayer, (b) Cys-MBA-AuNPs, (c) Cys-MBA-AuNPs-ConA, and (d) Cys-MBA-AuNPs-WGA.

The early detection of *Candida* species has become essential to a better prognosis of the patients, timely administration of antifungal treatment, and a decrease of the inpatient costs due to hospital length of stay [8]. Conventional culture-based methods remain the gold standard for the diagnosis of yeasts. However, these methods lack sensitivity and are time-consuming, requiring 2–4 days to identify the infecting microorganism. Also, serological tests such as enzyme-linked immunosorbent assays (ELISA) are used for the identification of *Candida*. However, despite presenting good sensitivity, ELISA requires the use of labels and can cross-react to pathogenic and commensal microorganisms displaying false-positive results [9]. Polymerase chain reaction (PCR) tests and other molecular approaches present reliable and sensitivity results, but are time-consuming, require specialized technicians, and high cost of reagents and equipment [10].

Biosensors are innovative approaches to detecting several analytes of clinical interest, especially microorganisms. Biosensors present excellent sensitivity, specificity, and low-cost. A biosensor comprises a bioactive sensing element able to identify the target analyte and a transducer (e.g., electrochemical, optical, piezoelectric, etc.) ready to convert the interaction between analyte and bioreceptor in a detectable electric signal [11]. In this sense, electrochemical biosensors became more suitable due to its simplicity, rapid response, and easiness for miniaturization [12].

Nanomaterials exhibit outstanding physicochemical properties such as biocompatibility, chemical stability, electroactive properties, and high surface-to-volume ratio. Also, nanomaterials present reinforced sensitivity and capability of interactions with biological analytes.

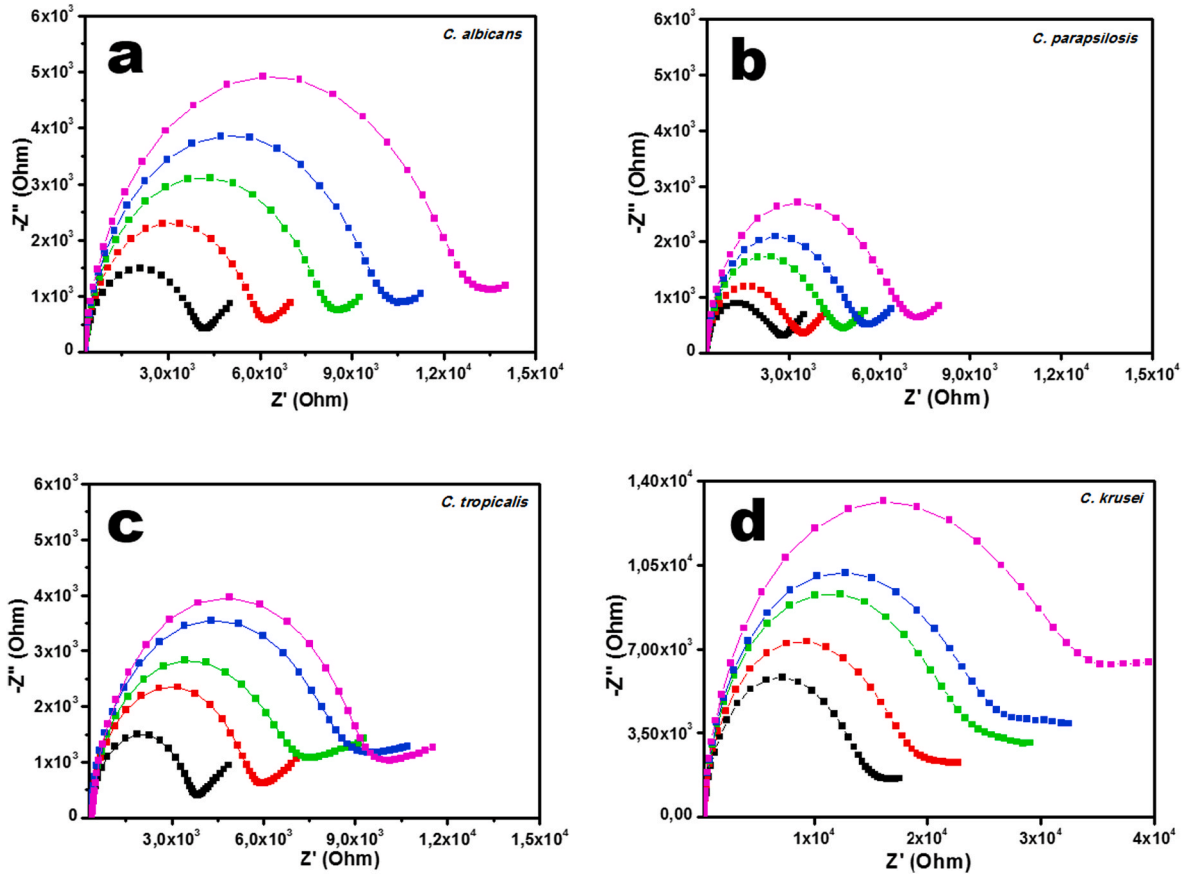

Fig. 2. Nyquist diagrams of the biosensors at different concentrations of the Candida species. ConA-biosensor: (a) *C. albicans*; (b) *C. parapsilosis*; (c) *C. krusei,* and (d) *C. tropicalis*. (■) 10^2 CFU mL^{-1}, (■) 10^3 CFU mL^{-1}, (■) 10^4 CFU mL^{-1}, (■) 10^5 CFU mL^{-1}, (■) 10^6 CFU mL^{-1}.

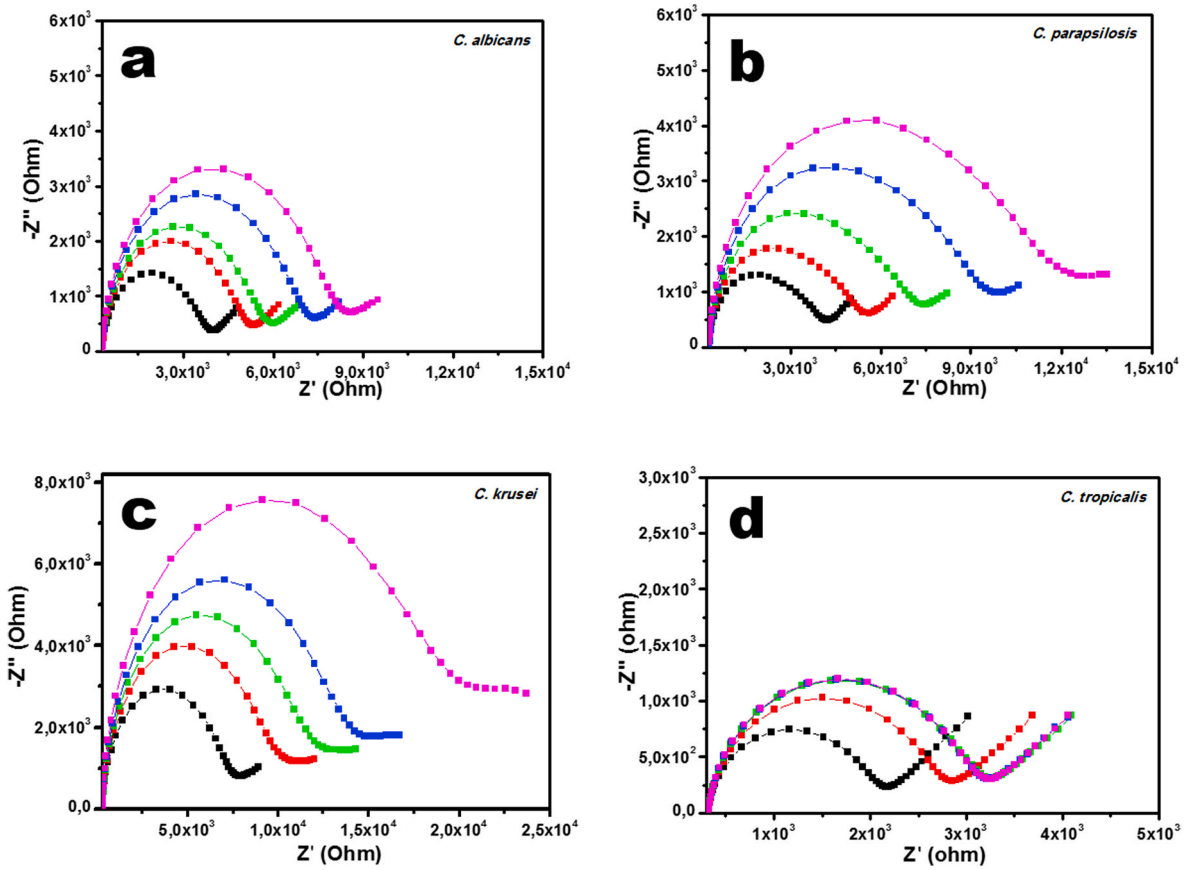

Fig. 3. Nyquist diagrams of the biosensors at different concentrations of the Candida species. WGA-biosensor: (a) *C. albicans*; (b) *C. parapsilosis*; (c) *C. krusei,* and (d) *C. tropicalis*. (■) 10^2 CFU mL^{-1}, (■) 10^3 CFU mL^{-1}, (■) 10^4 CFU mL^{-1}, (■) 10^5 CFU mL^{-1}, (■) 10^6 CFU mL^{-1}.

Table 1
Analytical comparison between different biosensors platforms.

Analyte	Sensing element	Technique	LOD	Reference
C. albicans	–	Surface-enhanced Raman spectroscopy (SERS)	10^8 CFU mL^{-1}	[38]
C. albicans	Antibody	Electrochemical impedance spectroscopy (EIS)	10^1 CFU mL^{-1}	[39]
C. albicans	–	Multiple cross displacement amplification assay (MCDA)	200 fg of genomic DNA	[40]
C. albicans	Lectin (Con A)	UV resonance Raman spectroscopy	32 CFU mL^{-1}	[41]
C. albicans	Antibody	Surface Plasmon Resonance (SPR)	10^6 CFU mL^{-1}	[42]
C. albicans	Antibody	Field-effect Transistor	50 CFU mL^{-1}	[9]
C. krusei C. guilliermondii	–	PCR + Electrospray ionization-mass spectrometry (ESI-MS)	500 CFU mL^{-1}	[43]
C. albicans, C. krusei, C. tropicalis C. parapsilosis	Lectin (ConA/ WGA)	Electrochemical Impedance Spectroscopy (EIS)	10^2 CFU mL^{-1}	This work

Features in single-walled carbon nanotubes (SWCNTs) in conducting the electron transfer process were explored in the development of an immunosensor for the detection of *C. albicans* [9]. T2 magnetic resonance genosensor was obtained through iron oxide magnetic nanoparticles placed on a probe-decorating process to capture the amplified DNA of different *Candida* species [13].

Colloidal gold nanoparticles (AuNPs) stand out among other nanostructured material options due to its remarkable electronic, optical, and chemical properties [14]. The surface modification of AuNPs with functional groups allows the production of materials able to interact with clinically relevant analytes. Recently, Silva Junior and coworkers [15] reported an electrochemical sensor composed of chemically-modified AuNPs and antimicrobial peptides for microorganism detection. Also, an innovative PCR assay with AuNPs to amplify ITS-rDNA and β-tubulin gene of *Candida* species was developed [16].

Also, nanostructure-based sensor platforms to detect several types of analytes have been extensively studied. Recently, Yola and coworkers [17] developed a biosensor based on quantum dots for the detection of cardiac troponin-I in plasma samples. The nanostructures enhanced the sensitivity of the biosensor presenting a limit of detection (LOD) of 0.0005 ng mL^{-1}. Electrochemical techniques can be used to detect β-agonists (salbutamol and phenylethanolamine) in urine samples [18]. A new method for fast detection of methyl parathion using nanocomposites was previously reported [19]. Nanostructure-based sensor platforms can be used to detect diverse targets, such as organophosphate pesticides [20,21], arboviruses [22] and bacteria [23].

Among diverse types of lectins, those isolated from plants stand out for their hydrophilic behavior, easiness isolating, and biotechnological activity. The main characteristic of the lectins is the capability to connect with specific carbohydrates, which composes the cell wall surface, cytoplasm, and nuclear structures of several organisms [24]. In this sense, lectins have been widely used for biomedical applications, such as in biosensors area with the development of highly sensitive and selective lectin-based biosensors, exploring lectins as recognition elements to detect and differentiate pathogenic *Candida* species [25]. Con A and

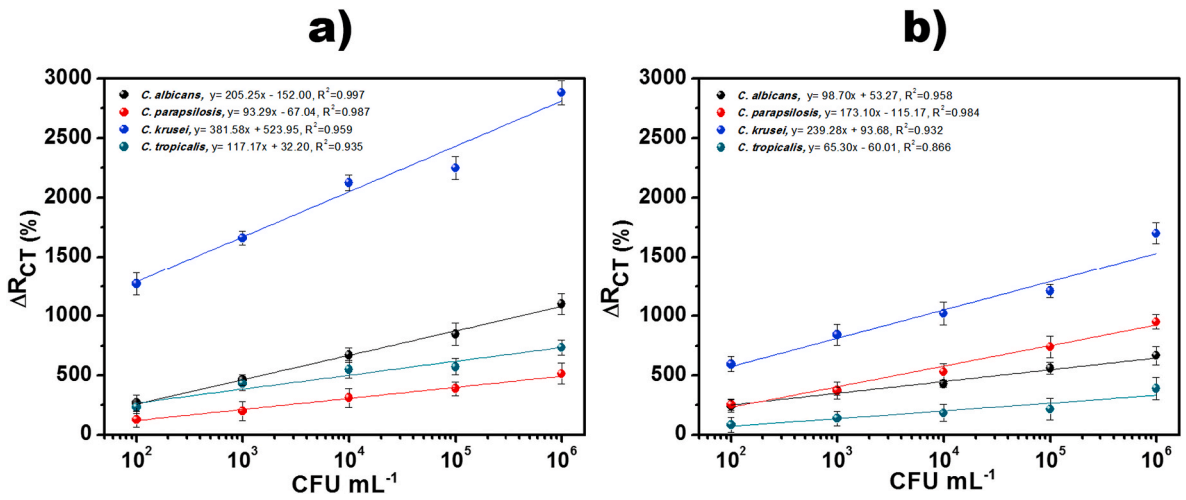

Fig. 4. ΔR_{CT} (%) of the biosensor platform after exposure to different fungal dilutions: (a) ConA biosensor, and (b) WGA biosensor.

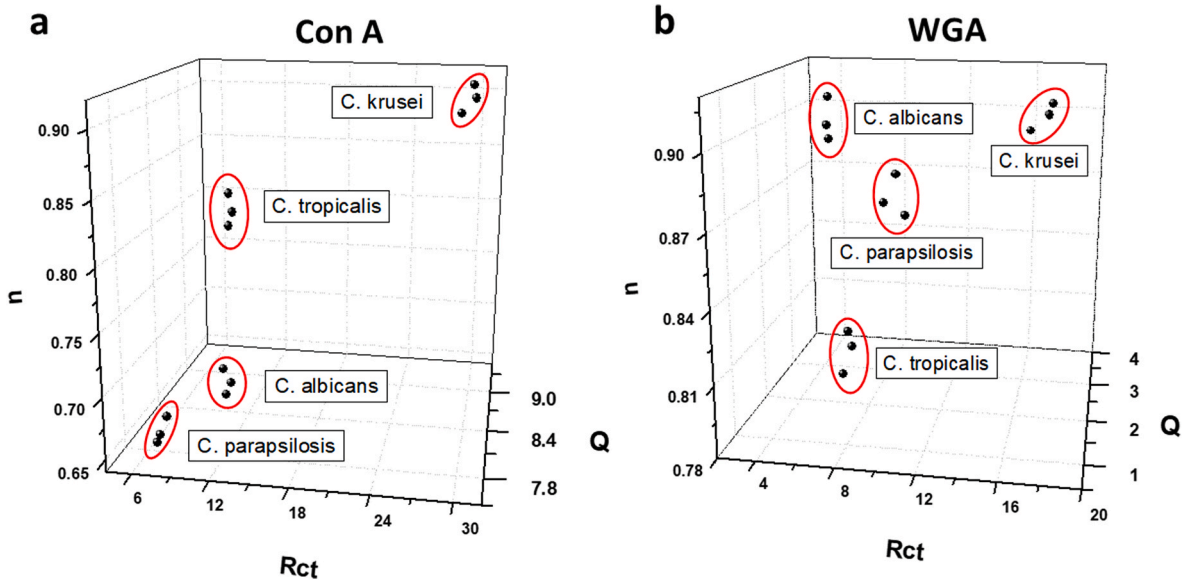

Fig. 5. Three-dimensional plot for Rct, Q, and n values given in Table S1 for the recognition pattern of the ConA (a) and WGA (b).

wheat germ lectin (WGA) lectins have been widely used in electrochemical biosensors as recognition molecules [26]. Con A and CramoLL lectins can effectively bind to glycan and mannan units present in the structure of yeasts and lipopolysaccharides of bacteria. Thus, ConA and CramoLL can be used to detect and differentiate clinically relevant microorganism species.

Herein, we reported the development of a novel electrochemical biosensor platform based on cysteine monolayers and lectin-modified AuNPs to detect *Candida* species. Atomic force microscopy (AFM) and electrochemical impedance spectroscopy (EIS) were used to characterize the assembly process of the biosensor and to evaluate the fungal detection through changes in charge transfer resistance (R_{CT}) responses. The infectious agents tested were *C. albicans, C. parapsilosis, C. krusei,* and *C. tropicalis*.

2. Experimental

2.1. Materials

Cysteine (Cys), 4-mercaptobenzoic acid (MBA), sodium citrate, Gold (III) chloride trihydrate ($HAuCl_4.3H_2O$), Concanavalin A (ConA) Type IV and wheat germ agglutinin (WGA) were obtained from Sigma-Aldrich (USA). The fungal inoculation process used in this study followed the procedures explored in a previous work developed by our group [27]. All materials were previously sterilized. Ultrapure water was obtained from a purification system (Billerica, USA). All reagents were of analytical grade and used as received.

2.2. Synthesis of MBA-modified AuNPs

Citrate-reduced gold nanoparticles were synthesized as described elsewhere. Briefly, 1 mL of $HAuCl_4$ was diluted in 99 mL of water and

homogenized in magnetic stirring. Then, 4 mL of 1% sodium citrate was placed into the flask, heated to 60°C and stirred for 30 min. A purplish-red solution was obtained. The preparation of MBA-AuNPs was performed diluting the previously synthesized AuNPs in 1:4 (v/v) water and 10^{-3} M MBA (v = 0.2 mL) and kept under magnetic stirring (2 h, 24°C ± 1°C). UV–vis spectrophotometer (Labomed Inc., USA) was used to evaluate the colloidal AuNPs synthesis and its chemical modification.

2.3. Preparation of the sensor system

Before use, Al_2O_3 paste (0.05 μm) was used to clean the bare gold working electrode (BGE) through polishing and then rinsed in deionized water. Subsequently, the gold electrode was functionalized with 2 μL of 30 mM Cys for 20 min and then rinsed with water to remove unbound molecules. A self-assembled monolayer was achieved at the electrode surface, reaction promoted by gold-thiol interaction. After that, coupling agents 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 0.1 M hydroxysuccinimide (NHS) were added for 10 min activating the Cys hydroxyl group. Then, 2 μL of MBA-AuNPs was incubated on the electrode for 1 h. Finally, 2 μL of the lectins was added for 30 min, completing the assembly of the sensor platform. Fig. S1 shows the manufacturing process of the biosensor assembly.

2.4. Recognition experiments

Candida sp. cells were grown in Sabouraud-dextrose agar with chloramphenicol for 24 h at 37 °C. Subsequently, a purified saline solution (0.9%) was used to wash and suspend the harvested cells. Candida clinical isolates obtained from the Medical Mycology Laboratory (MML) from the Federal University of Pernambuco were analyzed. The following fungal species were evaluated: *Candida albicans* (ATCC 90028), *Candida krusei* (URM 4263), *Candida tropicalis* (ATCC 13803), and *Candida parapsilosis* (ATCC 22019). The sensor response was assessed with 2 μL of yeast solutions (10^2–10^6 CFU mL^{-1}) incubated for 20 min.

Yeast identification was performed by mass spectrometry. Spectra of clinical strains were obtained according to Lima-Neto and coworkers [28] with modifications. The MALDI TOF Autoflex III Mass Spectrometer (Bruker Daltonics Inc., USA/Germany) set up with a 1064 nm laser of neodymium crystal (Nd:Y3Al5O12) to 66% power was used. The linear mode with a 104 ns pulsed laser and an acceleration voltage of +20 kV was used to register the mass range between 2000 and 20,000 Da. The peak lists obtained were exported to the software package MALDI Biotyper™ 3.1 (Bruker Daltonics, Bremem, Germany) where identifications were achieved (Fig. S2).

2.5. Electrochemical measurements

The electrochemical analyses were performed on an Autolab PGSTAT 128 N potentiostat/galvanostat (Ecochemie, The Netherlands) associated with an electrochemical cell with a three-electrode system. Ag/AgCl 3 M KCl and platinum wire were used as a reference and auxiliary electrodes, respectively. BGE was used as the working electrode. All electrochemical measurements were performed at 24°C ± 1°C using a solution of 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (1:1) in PBS pH 7.4 as a redox probe. Impedimetric analyses were carried out in a frequency range of 100 mHz to 100 kHz with an AC potential of 10 mV. Electrochemical impedance results were evaluated through NOVA 1.8 software.

2.6. Atomic force microscopy analysis

Topographical measurements of the biosensor assembly and after interactions with *Candida sp*. cells were performed using an atomic force microscope (SPM-9700; Shimadzu, Japan). The images were collected using cantilevers with a silicon AFM tip with a resonant frequency = 300 kHz and nominal spring constant = 42 N m^{-1} (Nanoworld, Japan). The lateral resolution was set as 512 pixels per line (scan area 5 × 5 μm). The images were obtained and analyzed using AFM Gwyddion software.

3. Results and discussion

3.1. Morphological characterization

Optical absorption of the synthesized nanoparticles (AuNPs and MBA-AuNPs) was performed through UV–Vis spectrophotometry, where the absorption band of λ = 524 nm and λ = 533 nm) was assessed, followed by a hypochromic shift. The absorption peaks correspond to the surface plasmon resonance of colloidal gold. The thiol group present in the MBA structure bind to AuNPs surface with high affinity, resulting in –COOH functional group free for posterior linkages [29].

AFM technique was used to evaluate the manufacturing process of the biosensor and interaction against *Candida* spp. AFM height images and surface roughness analysis are shown in Fig. 1. Fig. 1a shows a self-assembled Cys monolayer homogeneously covering the gold in the working electrode, with an average height of 17 nm. Subsequently, MBA-AuNPs were covalently linked to Cys, presenting a uniform shape distribution with an average height of 76 ± 2 nm (Fig. 1b). In sequence, lectins were added to the modified surface leading to an increment of the surface roughness of 127 ± 0.5 nm and 104 ± 0.2 nm for ConA and WGA (Fig. 1c–d), respectively.

Both lectins chosen in the study have the feature of binding to specific carbohydrates present in the cell wall of microorganisms. ConA stands out in the selective binding of mannose and glucose present in *Candida* sp. structure [30]. WGA is a chitin-binding lectin, and its biological function is related to protection from fungal infection due to its ability to bind to chitin containing cell walls. WGA structure comprises two identical polypeptide subunits (MW = 18 kDa, each) that assemble at neutral pH to a dimer [31].

The interaction with *Candida* samples resulted in a significant increase in height and roughness. The obtained results reveal differences in the film roughness, demonstrating the specificity of the evaluated lectins for diverse pathogenic *Candida* species (Fig. S3 a-d for ConA and Fig. S4 a-d for WGA). The AFM images revealed differences in the rough profiles of the sensor recognition led by disagreements in yeast lengths and sugar composition. Each recognition profile of the lectins against candida species reflects the ability of ConA and WGA recognition to the sugars present in the cell wall of the species studied. Candida's superficial structure comprises diverse components (e.g., mannan and β-(1 → 3)-D-glucan). Of note, these molecules are considered circulating antigens, detected in the blood at an early stage of the illness, and before the onset of clinical symptoms [32].

3.2. Electrochemical characterization of the biosensor platform

EIS is an effective method widely explored for the evaluation of biosensor interfaces, enabling monitoring the electrochemical reply of the BGE after each layer assembly. EIS studies were performed in a frequency range between 100 mHz and 100 kHz. Figs. S5a-b shows the impedance responses of the manufacturing process of the lectin-based biosensor.

A previous study of Cys adsorption on the BGE was performed with different Cys concentrations (5, 10, 20, and 30 mM). Our results showed that after 30 mM of Cys (20 min) is obtained a saturation of the electrode surface and SAM formation (Fig. S5a-b). Similar responses were obtained in previous works [15,33]. The impedimetric response of the Cys monolayer shows a higher interfacial resistivity of the electrode (R_{CT} = 1.42 KΩ) as compared to BGE response (R_{CT} = 0.198 KΩ) (Figs. S5a-b).

Afterward, MBA-modified AuNPs were added to the Cys monolayer (Fig. S5a-b). Before use, EDC:NHS coupling agents were used to activate free amine groups of Cys, enabling a covalent binding to the free carboxyl group of the MBA. Due to the neutral-positive charges present on the generated NHS ester occurs a transfer of a negative redox probe to

the electrode, leading to a reduction in the resistivity of the system (R_{CT} = 0.862 KΩ). Finally, after the deposition of ConA and WGA (Fig. S5a-b) occurs a noticeable increase in the resistivity of the system (ConA R_{CT} = 2.14 KΩ, WGA R_{CT} = 1.71 KΩ), due to partial blockage of the electron transfer rate.

3.3. Detection of candida pathogenic species

Fig. S6 shows the Randles equivalent circuit model used to evaluate the sensor response against Candida pathogenic species. The circuit model comprises the resistance of the electrolyte solution (R_S), charge transfer resistance (R_{CT}), constant phase element (Q), and Warburg impedance (W). R_{CT} was used to represent the main electrochemical processes on the electrode interface since it is closely related to the recognition of the microorganisms by the nanoplatform.

As seen in Table S1, our results point out that ConA and WGA lectins interact effectively with different pathogenic Candida species. It was observed an increase in the interfacial resistivity of the electrode proportional to colony-forming units per milliliter (CFU mL^{-1}) concentration of Candida spp. for the ConA and WGA-based biosensor (Fig. 2 a-d and Fig. 3 a-d, respectively). Table 1 brings an analytical correlation between the performance of the developed biosensor with other platforms to detect Candida yeast cells. In this sense, through the relative variation of R_{CT} (ΔR_{CT}), we determine quantitative information related to the Candida-lectin sensor interaction. ΔR_{CT} is calculated according to equation (1), as follows:

$$\Delta \boldsymbol{R}_{\boldsymbol{CT}}(\%) = \frac{\boldsymbol{R}_{\boldsymbol{CT\ (Candida)}} - \boldsymbol{R}_{\boldsymbol{CT\ (Sensor)}}}{\boldsymbol{R}_{\boldsymbol{CT\ (Sensor)}}} \boldsymbol{x}\ 100 \quad (1)$$

where $R_{CT(Sensor)}$ and $R_{CT(Candida)}$ are the R_{CT} values of the biosensor before and after Candida recognition, respectively. The biosensor was tested against four different pathogenic Candida species, as follows: *Candida albicans*, *Candida parapsilosis, Candida krusei,* and *Candida tropicalis*. Different concentrations (10^2–10^6 CFU mL^{-1}) of each species were evaluated.

MALDI-TOF was used to validate the *Candida sp*. identification. Besides, MALDI-TOF identified the yeasts like *Candida albicans*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*, all with scores above 2.0. The peaks on the spectra of each Candida strain are showed in Fig. S2. There was a significant difference in Candida concentration detection by both measurement methods. In both approaches, we can identify Candida species in low concentration.

As shown in Fig. 4a–b, we observe an increment in ΔR_{CT} values increase following the raise of *Candida* concentration, presenting a linear behavior. The biosensor showed different responses according to the type of lectin, as follows: ConA-based biosensor (*C. parapsilosis* < *C. tropicalis* < *C. albicans* < C. *krusei*) and WGA-based biosensor (*C. tropicalis* < *C. albicans* < *C. parapsilosis* < *C. krusei*).

ConA and WGA are hololectins and present binding sites to sugars. The main lectin receptors present in a fungal structure are cell wall glucans, chitin, arabinans, galactans, mannans, and sialic acid [34]. We can highlight some monosaccharides in *Candida* spp. structure, where D-glucose (Glc), N-acetyl-D-glucosamine (GlcNAc), D-mannose (Man), and sialic acid (SA) stand out among others. Of note, ConA presents high binding affinity to Glc and Man, while WGA shows binding specificity towards GlcNAc and SA residues [35]. Therefore, ConA and WGA present variable electrochemical responses after *Candida* spp. detection, mainly due to the specific molecular structures in the yeast cell wall.

As an additional parameter, we calculated the degree of coverage (θ) according to the following equation:

$$\boldsymbol{\theta} = 1 - \left(\frac{\boldsymbol{R}_{\boldsymbol{CT\ (Biosensor)}}}{\boldsymbol{R}_{\boldsymbol{CT\ (Candida)}}}\right) \quad (2)$$

where $R_{CT(Biosensor)}$ and $R_{CT(Candida)}$ are the charge transfer resistance values of the biosensor before and after *Candida* recognition, respectively. In Fig. S7a-b, we show the plot of the degree of coverage values as a function of *Candida* spp. concentration (10^2–10^6 CFU mL^{-1}). We observed the increase of θ values proportional to the microorganism concentration. The highest values were obtained for *C. krusei* at 10^2 CFU mL^{-1} with ~0.84 (84%) and ~0.81 (81%) for ConA and WGA-based biosensor, respectively.

Also, we evaluated three variables to assess the discrimination ability of the sensor, as follows: Rct, Q, and n. These parameters resulted in characteristic values for each serotype (*C. Krusei*, *C. tropicalis*, *C. albicans,* and *C. parapsilosis*) (Fig. 5). *C. parapsilosis* and *C. tropicalis* responses are located in the region of lower Rct, Q, and n values, which corresponds to a smaller blocking effect of the biosensor surface associated with smaller capacitive dispersion. Thus, a discrete agglutination of *Candida* on the electrode surface was obtained. Conversely, *C. krusei* recognition results in intermediate dispersion and blocking of the sensor surface, since higher values of Rct, n, and Q were obtained. Different pattern recognition of the lectins can be attributed to the various sugars on the *Candida* surface. Structural analysis of *C. krusei* revealed anomeric carbons (C1) of terminal non-reducing, 2-substituted, and 2,6-disubstituted mannose with α-linkage. On the other hand, *C. albicans*, *C. tropicalis*, and *C. glabrata* have phosphodiesterified 1,2-linked mannose residues [36]. Of note, the absence [presence] of phosphodiesterified mannose residue plays an essential role in lectin interaction, with a consequent increase [decrease] in the agglutination response [37].

4. Conclusions

ConA and WGA lectins were used as recognition elements in an electrochemical biosensor platform for the identification of pathogenic *Candida* species. The selectivity of the lectins to specific carbohydrates on *Candida* spp. surface was studied to determine characteristic patterns of the impedimetric response. The sensor platform was able to detect and differentiate viable cells of *C. albicans*, *C. krusei*, *C. tropicalis,* and *C. parapsilosis* in a sensitive manner. The limit detection ranging from 10^2 to 10^6 CFU mL^{-1} was obtained for all microorganisms tested in both lectin-based biosensors. Hence, we point out that the developed biosensor as a feasible alternative to the recognition of *Candida* spp in a fast and sensitive approach.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

Dr. Oliveira and Andrade are grateful for the financial support provided by CNPq. S. R. Sá would like to thank Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for the Ph.D. scholarship. Oliveira and C.A.S. Andrade would like to thank CNPq (grant 314894/2018-7, 314756/2018-3 and 406154/2018-0).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.121375.

Credit author statement

Sandra R. Sá: Methodology, Investigation, Formal analysis, Writing - Original Draft. **Alberto G. Silva Junior:** Investigation, Formal analysis. **Reginaldo G. Lima-Neto:** Validation, Formal analysis, Funding acquisition. **César A.S. Andrade:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition. **Maria D.L. Oliveira:** Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition.

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