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Magnetic Nanobead Paper-Based Biosensors for Colorimetric Detection of *Candida albicans*

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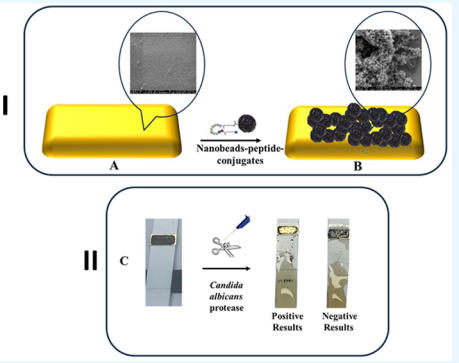
ABSTRACT: *Candida albicans* (*C. albicans*) infections pose significant challenges in clinical settings due to their high morbidity and mortality rates in addition to their role in tumor progression. Current diagnostic methods, while effective, often suffer from limitations that hinder a timely intervention. Therefore, there is an urgent need for a simple, sensitive, specific, and low-cost colorimetric biosensor for the rapid detection of *C. albicans*. This new biosensing platform comprises a gold platform carrying a specific *C. albicans* peptide substrate conjugated with magnetic nanobeads. Hence, the sensing platform was black, and the operation was based on the proteolytic activity of *C. albicans*, offering a visual color change to yellow upon cleavage of the conjugated peptide substrates on the magnetic nanobeads. Specificity testing demonstrated the biosensor's ability to distinguish *C. albicans* from other *Candida* species and microorganisms, while stability testing confirmed its long-term performance. Clinical testing revealed the biosensor's high sensitivity in detecting *C. albicans* in both standard cultures and clinically isolated samples with a lower limit of detestation of 3.5×10^3 CFU/mL. Although further validation against conventional and molecular methods is warranted, our colorimetric biosensor holds promise as a rapid (5 min) and cheap (Less than 2 $) point-of-care solution for the early detection of *C. albicans* infections, facilitating a timely intervention and improving patient outcomes in clinical practice.



1. INTRODUCTION

The fungal kingdom encompasses around one million species.[1,2] Fungi can have positive effects on human existence, yet certain species can pose risks to human health.[1] According to the Global Action for Fungal Infections factsheet, more than 300 million people worldwide suffer from serious fungal infections and 25 million are at high risk of death.[3] In 2022, the World Health Organization (WHO) published the fungal priority pathogens list, identifying *Candida albicans* (*C. albicans*) as one of the four critically important fungal pathogens that pose a threat to public health.[4]

C. albicans is the predominant etiological agent responsible for both mucosal and systemic infections,[5] accounting for over 70% of the fungal infections worldwide.[6] This fungus can colonize almost all regions of the gastrointestinal tract, including the oral cavity, perianal tissues, epidermis, and vulvovaginal region.[7]

C. albicans is responsible for 75% of women's acute vulvovaginal candidiasis (VVC), making it the second most common vaginal infection after bacterial vaginosis.[6,8] Approximately, 9% of women aged 25−34 years suffer from recurrent vulvovaginal candidiasis (RVVC).[9] Although RVVC is less common, it is more serious. However, both have a significant negative effect on the patient's quality of life.[10] In addition, VVC and RVVC are associated with preterm labor, late miscarriage, infertility, and pelvic inflammatory disease.[11] This fungus can also cause candidiasis, a condition that can harm the esophagus and potentially spread throughout the body, resulting in a more severe condition known as candidemia,[12] which particularly impacts people with impaired immune systems.[13] Candidemia treatment requires prolonged hospitalization and high healthcare costs.[14] Furthermore, other infections with *Candida* may result in multiple organ failure and septic shock.[15]

The association between *C. albicans* and cancer has been recognized for many years.[16] Infection with *C. albicans* can be both a consequence of and a contributing factor to the development of cancer. This fungus is highly prevalent in various types of cancer, such as oral cancer,[17] gastric cancer,[18] and esophageal cancer.[19]

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Table 1. Methods Are Available for the Detection of *Candida*

method	example	advantages	disadvantages	ref
microscopic examination	nonspecific staining with calcofluor white (CFW)	serves as a rapid detection tool	• results must be validated against other culture-based methods owing to nonspecific binding • kills the organism; hence, drug resistance testing becomes impossible	36
	specific staining of Candida with fluorescent in situ hybridization (FISH)/antibodies	• reliable methods based on immunochemistry and genetic markers • rapid platform for specific identification of Candida spp. from the blood culture • high sensitivity and specificity	• cannot be employed directly with blood samples without culture, thus taking up to 2 weeks before giving positive results	37−39
conventional detection methods	carbohydrate assimilation and fermentation reactions	• tests can be performed easily on automated systems • faster than classical assimilation and fermentation methods • good identification results with an accuracy of 78.8−91.8% for API *Candida*	• requires up to 72 h incubation • some reports showed that this method misidentified various fungal species or provided inconclusive results	40,41
	culture-based Candida detection	most widely used methods for Candida detection in clinical applications	• time-consuming makes it unsuitable for rapid Candida testing • limited specificity and sensitivity, partly owing to differential gene expression of the targeted enzyme or substrate induced by changing environmental factors	42
	blood culture	• standard methods for fungal detection • combination with CHROMagar provides a better technique for the colony formation and detection of various Candida subtypes	• time-consuming • not suitable for rapid detection of *Candida* • not easily amenable to the use of miniaturized POC by patients or caregivers	42,43
	serological detection (immunoassay-based techniques)	• resourceful method • determine the presence of antibodies against fungal antigens • rapid	• low specificity and cross-reactivity with other antibodies/antigens • multistep including cell lysis, extraction, purification, and amplification of NA	44,45
	NA detection techniques	• robust, sensitive, and specific bioassays • present a viable platform for the detection of *Candida*	• time-consuming	
nonconventional techniques	lab-on-a-chip (LOC) device	• microfluidic LOC devices enhance early detection and identification of fungi in the bloodstream • used in resource-constrained settings without centralized laboratories • digital microfluidics is a robust platform • a sensitivity of 56% (compared to 69% of conventional PCR real-time analysis) has been achieved within 45 min	• a sensitivity of 56% (compared to 69% of conventional PCR real-time analysis) has been achieved within 45 min • high cost, low sensitivity, and specificity (e.g., commercial platform SeptiFast)	46−48

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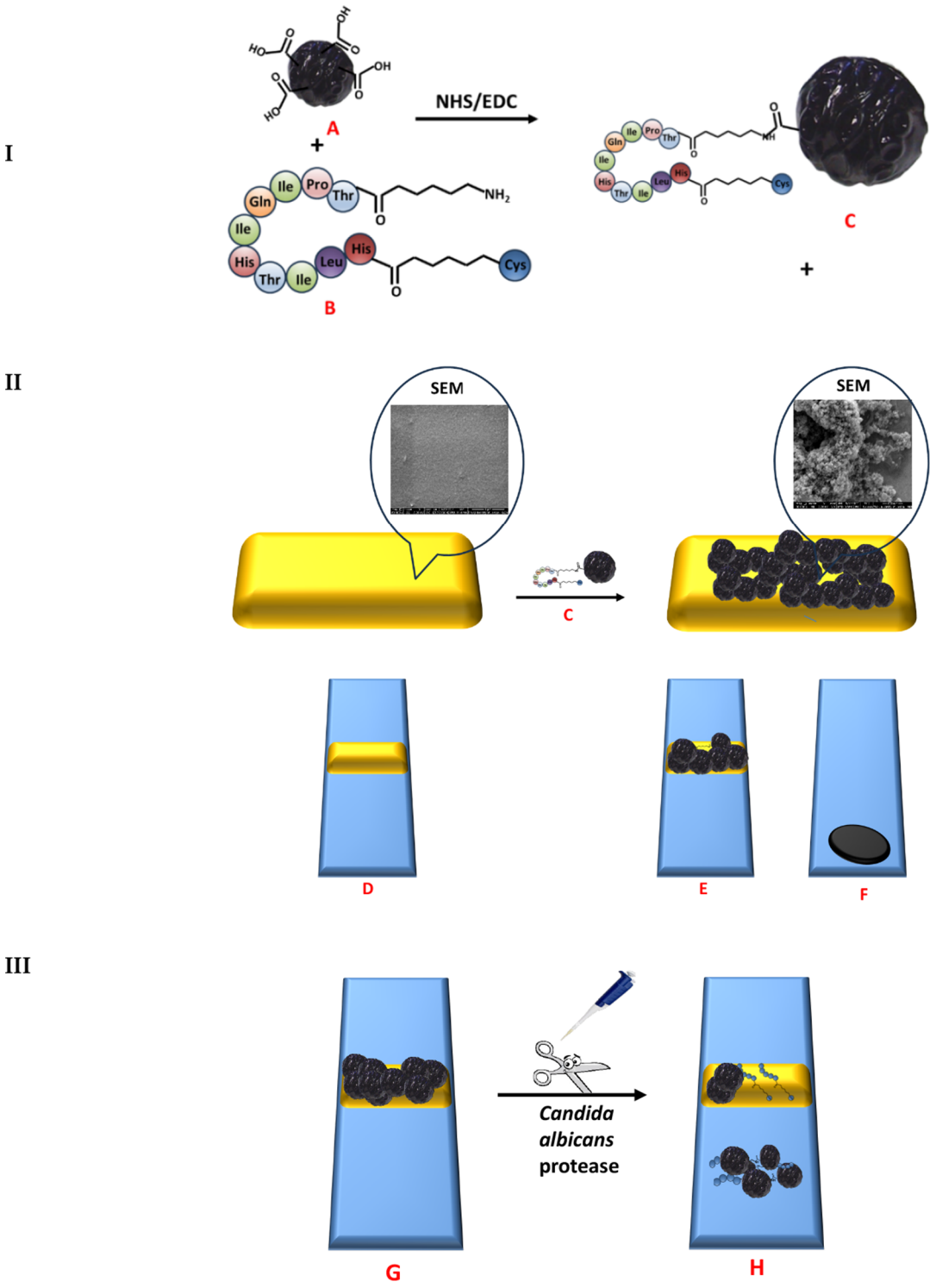

Figure 1. *Candida albicans* diagnostic platform. (I) Conjugation of the peptide substrates with magnetic nanobeads: (A) carboxyl magnetic nanobeads, (B) peptide substrate, and (C) nanobeads−peptide conjugates. (II) Sensing platform preparation and immobilization of nanobeads−peptide conjugates: (D) gold sensing platform, (E) front view of the *C. albicans* sensing platform, and (F) back view of showing the circular magnet. (III) Biosensing of *C. albicans* culture supernatant proteases: (G) ready to use sensing platform (black color), and (H) *C. albicans* detection (restoration of the golden color).

It is worth mentioning that *C. albicans* infection in hospitals is a challenge to deal with owing to the potential presence of this fungus on inanimate surfaces such as implants, prosthetic devices, and pacemakers. Patients are most likely to acquire *C. albicans* by transmission from healthcare professionals.[20] In cases of infection, patients frequently need to undergo supplementary surgical interventions to eliminate the source of infection to control *Candida* effectively.[20] Accordingly, infections with *Candida* remain a major threat in hospitals due to high morbidity and mortality rates, which underscores the need for rapid point-of-care (POC) diagnostic tools.

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Table 1 illustrates the methods available for detecting *Candida*. In brief, the classical methods involve observing clinical symptoms such as white vaginal discharge and itching, using microscopy to examine the appearance of colonies, and tracking changes in pigmentation within a chromogenic culture.[11] However, these procedures cause discomfort and suffering for the patient. Furthermore, the varied morphologies of vaginal microorganisms and subjectivity make detailed examination of these organisms imprecise and inaccurate.[11] Other methods include the "gold-standard" culturing method, yet this process is labor-intensive, requires a large sample volume, and typically takes at least 2−4 d. This delay is unacceptable in emergencies and in situations of a critical illness. Researchers have applied other approaches that rely on nucleic acid (NA) identification via DNA microarray techniques. Fortunately, these techniques are more sensitive and faster,[21] but they are tedious and require complex procedures including extraction, purification, and amplification. Other detection methods involved the integration of loop-mediated isothermal amplification (LAMP)-based approaches with microfluidic-based platforms, which led to the development of compact, self-contained, and rapid POC assays for the detection of *Candida*, as it does not require thermal cycling and thermal ramping.[22,23] Compared to traditional analytical methods, a nonconventional lab-on-a-chip diagnostic array allows patients to monitor their own health conditions. It is, however, crucial to consider whether low-cost diagnostic platforms, such as a POC tool that complies with market demands and the public health agencies guidelines, can be manufactured readily. For example, paper-based microchip technologies[24−31] integrated with optical/electrical sensing modalities may open a promising avenue in the development of POC diagnostics tools to expand the potential for preemptive (or early) therapy.

Colorimetric sensing platforms have garnered significant attention in contemporary scientific studies. Traditional colorimetric platforms for fungal detection include, but are not limited to, LAMP,[32] horseradish peroxidase tetramethylbenzidine (TMB)-mediated assays,[33] and surface plasmon resonance-based techniques.[34] While they are effective, these methods may require heating (LAMP), several manual manipulations, and access to a laboratory. As an alternative approach, nanomaterials are commonly used in biosensors. Nanomaterials improve the sensitivity and lower the limits of detection of a platform by expanding the available surface area for immobilizing a biorecognition element.[35] Some colorimetric platforms offer simple and direct results without the need for additional equipment or sample preparation, but they may lack specificity in distinguishing between fungal species. Incorporating gold nanoparticles into lateral flow platforms for the detection of fungal pathogens has shown promise, but integration with expensive equipment for PCR analysis can be a barrier to widespread adoption.

Accordingly, further research is necessary to improve diagnostic, preventive, and therapeutic strategies to reduce the considerable *candida* infection-related morbidity and mortality. The current study aimed to develop a sensitive, specific, and low-cost colorimetric biosensor capable of detecting the presence or absence of *C. albicans*. This diagnostic technique is based on the incorporation of recent nanotechnology advances in the field of biosensors. The detailed protocol steps in the sensor preparation are shown in Figure 1. The sensing mechanism is based on the proteolytic activity of *C. albicans* proteases on a specific peptide substrate, sandwiched between magnetic nanobeads and a gold surface on the top of a solid paper support. An external magnet fixed at the back of the sensor accelerates the cleavage of the nanobeads−peptide conjugates. This dissociation process reveals the golden color of the sensor surface visible to the naked eyes as shown in Figure 1.

2. EXPERIMENTAL SECTION

2.1. Materials and Reagents. Magnetic nanoparticles (MNPs), surface coated with carboxylic acid groups Turbobeads, Zurich, Switzerland (mean diameter 50 nm, Sigma-Aldrich); *N*-hydroxysuccinimide (NHS); 1-(3-(dimethylamino)propyl)-ethyl-carbodiimide (EDC); and pH test strips 0−14 pH (P4786) were purchased from Sigma-Aldrich (Dorset, UK). A self-adhesive magnetic A4 sheet (Product Code: 15000SHEETA4) was purchased from the Polarity Magnets Company (Wickford, UK). Peptide substrates (NH_2-Ahx-TPIQIHTILH-Ahx-Cys) were synthesized by the Pepmic Co., Ltd. (Suzhou, China) with >98% purity. A Greiner multiwell plate sealing film (A 5596) was purchased from Sigma-Aldrich and coated with a thin layer (30 nm) of gold using a sputtering machine at the School of Engineering at King Abdullah University of Science and Technology (KAUST, Thuwal\Saudi Arabia). Sabouraud dextrose agar (SDA); Sabouraud dextrose broth (SDB); brain heart infusion (BHI); and De Man, Rogosa, and Sharpe (M.R.S.) broth were purchased from Oxoid (Amman, Jordan). Sterile filters (0.22 μm) were purchased from Millipore (Amman, Jordan). The wash/storage buffer (10 mM Tris base, 0.15 M sodium chloride, 0.1% (w/v) bovine serum albumin, 1 mM ethylenediaminetetraacetic acid, 0.1% sodium azide, pH 7.5) and the coupling buffer (10 mM potassium phosphate, 0.15 M sodium chloride, pH 5.5) were prepared from chemicals of analytical grade.

2.2. *Candida* Species Stock Culture Preparation. *Candida albicans* ATCC 10231 (*C. albicans*), *Candida glabrata* ATCC 90030 (*C. glabrata*), *Candida parapsilosis* ATCC 90018 (*C. parapsilosis*), and *Candida dubliniensis* ATCC 44508 (*C. dubliniensis*) were procured from the American Type Culture Collection (ATCC), (Manassas, Virginia, USA). Each fungus was rehydrated in accordance with the guidelines provided by the ATCC culture guide. In brief, for *Candida* species, the contents of a freeze-dried lyophilized ampule were rehydrated using sterile distilled water to create a suspension, which was then allowed to remain undisturbed at RT for a minimum of 2 h. Then, the suspension was thoroughly agitated and an aliquot (100 μL) was inoculated onto SDB (Oxoid, U.K.). The inoculum was incubated and regularly observed for growth, which usually manifested within 2−3 d but may differ depending on the strain. Different *candida* species were subcultured in SDA (Oxoid, U.K.). After 2 d, separated colonies (2−5 colonies) were transferred into SDB supplemented with 15% glycerol and stored at −20 °C to be used later.

2.3. *Candida* Species Pure Broth Culture Supernatant Proteases. An aliquot (0.5 mL) of the stock culture was added to SDB (4.5 mL) and incubated for 3 d at 30 °C for *C. albicans* ATCC 10231 and for 5 d at 25 °C for all other *Candida* strains (*C. glabrata*, *C. parapsilosis*, and *C. dubliniensis*). Then, 5 and 10 mL of freshly prepared SDB were added to *C. albicans* and cultures of other strains, respectively, to reach a turbidity of approximately 1 Mcfarland (except for *C.*

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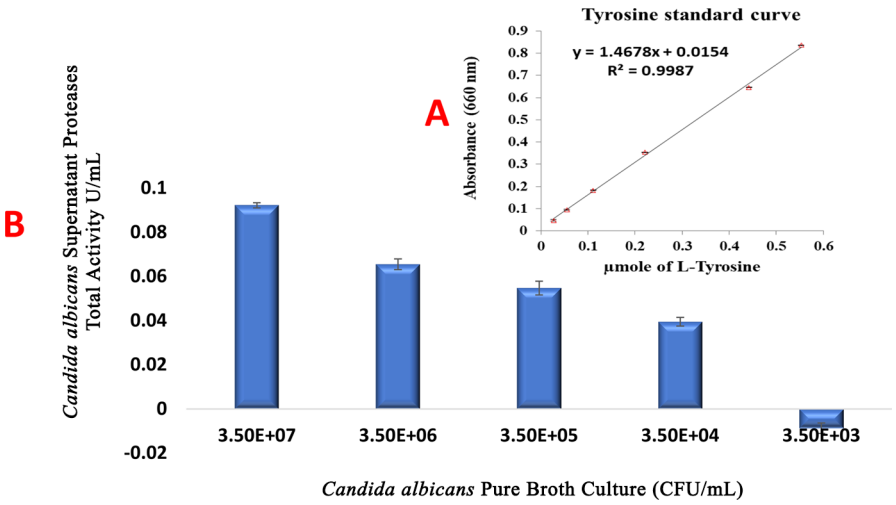

Figure 2. Proteolytic activity of different *C. albicans* culture supernatants measured by the Sigma's nonspecific protease activity assay. (A) Tyrosine standard curve. (B); Activity of *C. albicans* proteases determined in terms of units (μmol of tyrosine equivalents released from casein min^{-1}).

albicans, where it was 2 Mcfarland to make a 10-fold serial dilution). The count of *Candida* broth suspension was checked by the viable cell count method.[49] *C. albicans* cultures were then incubated for 4 d at 30 °C, and all other *candida* strains were incubated at 25 °C for 2 d. The fungal count was estimated to be 3.5×10^{10} CFU/mL for *C. albicans*, 2.0×10^{8} CFU/mL for *C. glabrata*, 3.0×10^{7} CFU/mL for *C. dubliniensis*, and 5.0×10^{7}CFU/mL for *C. parapsilosis*.

The total extracellular protease was obtained from each fungus by centrifuging the PBC solution at 3500 × g (Eppendorf centrifuge 5424 R, Germany) for 10 min. Then, the supernatant was filtered using a 0.22 μm sterile syringe filter (Millipore, Middlesex, U.K.) to get the crude protease solution, which is directly proportional to each fungus concentration. The proteolytic activity of the proteases was evaluated using the universal, nonspecific casein assay and was determined as micromoles of tyrosine equivalents released from casein per minute.[50] In this assay, casein acts as a universal protease substrate. When digested by proteases, tyrosine is liberated along with other amino acids and peptide fragments. Then, the free tyrosine will react with the Folin−Ciocâlteu reagent to produce a quantifiable, blue-colored chromophore at 660 nm. Proteases with higher activity will digest casein and release more tyrosine, generating more intense chromophores and higher absorbance values. These values will then be compared to a tyrosine standard curve, which is generated by reacting known quantities of tyrosine with the Folin−Ciocâlteu reagent to correlate changes in the absorbance with the amount of tyrosine. From the standard curve, the activity of protease in the *C. albicans* culture supernatant can be determined in terms of units.

An increase in each *C. albicans* proteolytic activity strength was correlated to C. candida culture concentration (CFU/mL), as shown in Figure 2. To determine the lower limit of detection, serial dilutions were tested to identify the minimum protease concentration that was unable to cleave peptide magnetic nanobead composites attached to the sensor's golden surface, i.e., the sensor's golden surface is invisible to the naked eye due to the intact SAM layer.

2.4. *Candida* Clinical Isolates. Eight strains of *Candida* isolated from high vaginal swab clinical samples were obtained from the Islamic Hospital (Amman, Jordan) microbiology laboratory and from Precision Medical Laboratories (PML; (Amman, Jordan)). The authenticity of samples was verified by VITEK 2 Compact 15 at the Specialized Hospital (Amman, Jordan). Clinical isolates were provided as colonies on SDA. Then, 3−5 colonies were suspended into SDB (5 mL) and incubated for 2 d at 37 °C.

2.5. Preperation of *Lactobacillus acidophilus* (L. acidophilus) and *Gardnerella vaginalis* (*G. vaginalis*) Primary Broth Culture (PBC). *Lactobacillus vaginalis* ATCC 49540 (*L. vaginalis*), *Gardnerella vaginalis* ATCC 14019 (*G. vaginalis*), and *Lactobacillus acidophilus* ATCC 36031 (*L. acidophilus*) were procured from the American Type Culture Collection (ATCC), (Manassas, Virginia, USA). The freeze-dried pellet of each bacterium was rehydrated according to the guidelines provided by the ATCC culture guide. In brief, the *L. acidophilus* pellet was rehydrated in lactobacilli MRS broth (0.5 mL), while the *G. vaginalis* pellet was rehydrated in NYC III broth (0.5 mL). Then, each bacterium suspension was added to a test tube containing the corresponding broth (5 mL) to produce *L. acidophilus* and *G. vaginalis* PBCs, which was incubated at 37 °C for 24−48 h in an atmosphere of 5% CO_2.

2.6. Conjugation of the Peptide Substrates to Magnetic Nanobeads. As shown in Figure 1-I, the suspension of magnetic nanobeads (1.0 mL, 20 mg/mL) was mixed with the peptide substrate solution (1.0 mg/mL, NH2-Ahx-TPIQIHTILH-Ahx-Cys), coupling agent EDC (0.57 mg/mL), and NHS (12 μg/mL) (Figure 1-I). The mixture was rotated gently at room temperature for 48 h. The uncoupled peptides were removed by washing the beads 3 times using a wash buffer. Finally, the nanobeads were stored at 4 °C.[46,51,52]

2.7. Sensing Platform Preparation and Immobilization of Nanobeads−Peptide conjugates. Figure 1 shows the *C. albicans* protease detection and quantification sensing platform, which is made from a rectangular piece of gold-plated sealing film (~1.5−2 mm × 3 mm) (Figure 1-IID). This platform was mounted with nanobeads−peptide conjugates suspension (30 μL) and left to dry at room temperature (Figure 1-IIE) for 30 min, after which an external magnet (12.5 × 12.5 × 5 cm), strength (3360 and 573 T), was passed over the immobilized sensing platform to attract unbounded nanobeads−peptide conjugates. At this point, the biosensor will be visibly black (Figure 1-IIE) due to the formation of a

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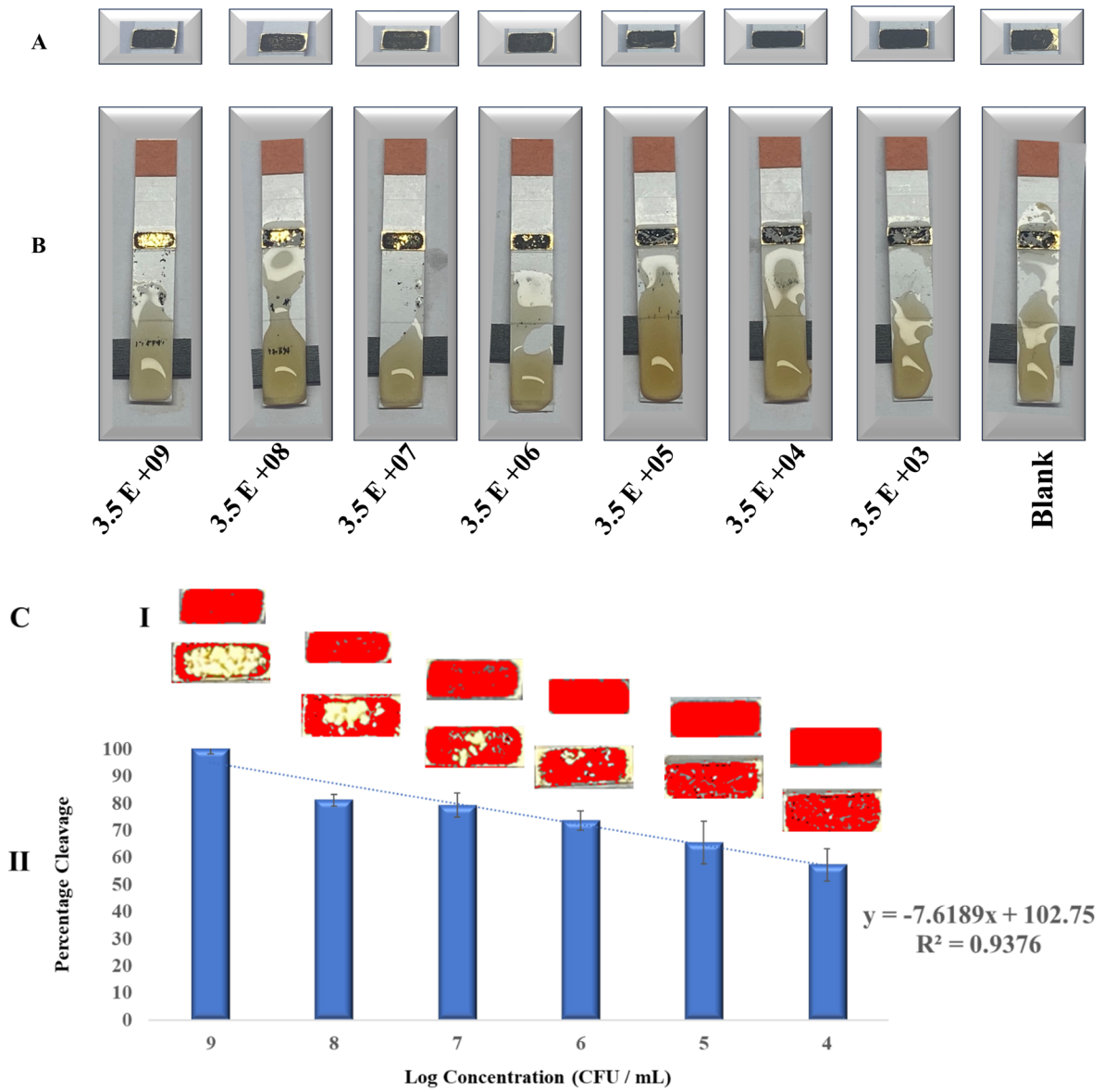

Figure 3. Colorimetric *C. albicans* sensor probe. (*C. albicans*-specific substrate peptide covalently bound to magnetic nanobeads). (A) Biosensor chip functionalized with the magnetic nanobeads−specific *C. albicans* proteases peptide substrate. (B) Biosensing process under the effect of different *C. albicans* culture supernatant protease solutions protease concentrations. (C) Correlation of different *C. albicans* culture supernatant proteases obtained from *C. albicans* cultures of different concentrations (CFU/mL) and the percentage cleavage of magnetic nanobeads−peptide moieties. (I) Color intensity of the nanobeads−peptide area on the sensor surface as processed by image J software before and after application of *C. albicans* protease. (II) Mean percentage cleavage ± coefficient of variation.

self-assembled monolayer on the gold sensing platform through adsorption by means of a covalent S−S linkage. The color changes from gold to black after immobilization with the nanobead−peptide conjugates, providing a colorimetric sensing platform. To accelerate the separation of the cleaved nanobeads−peptide conjugates, the magnetic paper was stacked at the back face of the sensing support at 2−3 mm beneath the sensing platform (Figure 1IIF). This magnet will collect the cleaved nanobead−-peptide conjugate fragments, restoring the sensor's original golden color.

2.8. Biosensing of *C. albicans* Culture Supernatant Proteases. The *C. albicans* culture supernatant protease solution (100 μL) was dropped over the functionalized sensing platform (Figure 1-III). During the enzymatic cleavage reaction, the cleaved nanobead−peptide conjugate fragments would be attracted by the permanent magnet, allowing for a qualitative evaluation of the tested sample (Figure 1-IIIH). Furthermore, a quantitative evaluation could also be performed by testing the proteolytic activity of *C. albicans* protease obtained from *C. albicans* pure broth cultures of different concentrations ranging from 3.5 × 10^{10} to 350 CFU/mL, as illustrated in Figure 3 A,B.

The ImageJ program developed at the National Institute of Health[53] was used to construct the calibration curve (Figure 3C). Following the operation of the biosensor, a photograph of the biosensor surface was captured using a smartphone and saved in a JPEG format. Using the RGB stack command of ImageJ, each photograph was split into red, green, and blue channels. Red channel images were selected for further processing. The color threshold was adjusted manually to highlight the black beads in red (Figure 3CI). The Set

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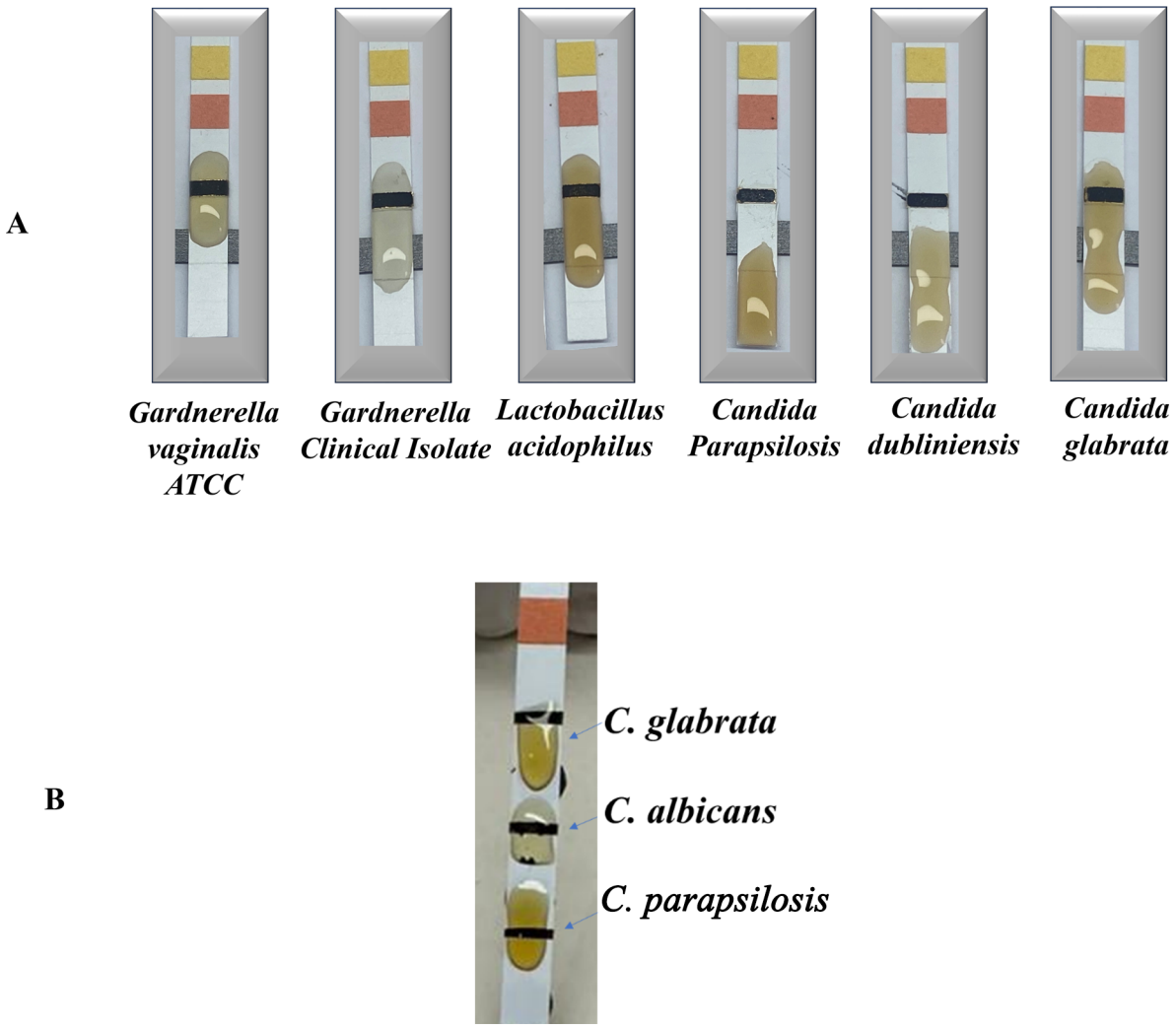

Figure 4. Colorimetric *C. albicans* nanobead paper-based biosensor (A) under the effect of different microorganism culture supernatant proteases and (B) under the effect of different *C. albicans* culture supernatant protease solutions protease concentrations.

Measurements dialogue was checked for "Color threshold." The percentage of cleavage was calculated as follows:

$$\% \text{ Cleavage} = \left(\frac{\text{Biosensor golden surface following protease application}}{\text{Biosensor golden surface at the highest protease concentration}}\right) \times 100$$

ImageJ analysis was performed by two researchers, who followed the proposed protocol. The two researchers measured the golden surface area. To gain insights into the reliability of the assay, the coefficient of variation (CV) was calculated and found to be ≤1, as shown in Figure 3C, illustrating low variance in the distribution of data.

3. RESULTS AND DISCUSSION

Sensitive and rapid detection of *C. albicans* can play a major role in the effective management of fungal infection and the monitoring of treatment. Despite considerable advances in the detection of *C. albicans*, there are still some potential shortcomings in developing a cheap, robust, speedy, simple, compact, and portable POC detection platform. Today, biosensing technologies offer numerous advantages, such as high accuracy, unique sensitivity, robustness, cost-effectiveness, speed, and convenient operation with potential miniaturization.[54,55] Colorimetric biosensors are popular because of their simplicity and affordability. These strategies allow for the identification of specific substances or biomolecules through visible color changes, eliminating the need for complex data processing.[56] This ease of use makes them particularly useful in overcoming location-based limitations in testing, which is crucial for advancing healthcare in remote areas and improving disease prevention and diagnosis. Our research contributes to this field by introducing a cost-effective, peptide-based biosensor,[57] specifically designed for the colorimetric detection of *Candida albicans*. This biosensor stands out for its affordability and suitability for field use and resource-limited environments. The integration of magnetic nanobeads enhances both the sensitivity and the specificity of the detection, resulting in more accurate and reliable outcomes. Additionally, the paper-based design of our biosensor simplifies its application and reduces the need for complex equipment. Compared to more expensive and intricate biosensors from previous studies, our approach offers practical and cost-effective advantages while maintaining strong performance, making it an ideal choice for on-site testing in various settings.

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3.1. Sensor Fabrication and Sensor Testing. The colorimetric biosensor (Figure 1) was created using a specific peptide substrate conjugated with carboxy-terminated magnetic nanobeads and covalently bound to a gold surface that when cleaved by *C. candida* proteases, a change in color will be observed visually. The peptide substrate used, TPIQIHTILH, was chosen to be cleaved specifically by *C. candida* proteases only. The peptide substrate attached to magnetic nanobeads[54] will enhance sensitivity owing to the large surface area of the nanobeads, which allows more peptide molecules to bind.[58] Moreover, conjugation of the peptide with magnetic nanobeads will prevent bead aggregation and oxidation, while retaining their magnetic properties.[59]

The *C. candida* peptide substrate (Ahx-TPIQIHTILH-Ahx-Cys) was modified with a hydrophobic flexible 6-aminohexanoic acid (Ahx) moiety on both termini (Figure 1-IB). The N-terminus was conjugated to magnetic nanobeads (Figure 1-IC). The cysteine residue at the C-terminus allows the gold−sulfur interaction for the establishment of a self-assembled monolayer (SAM) of peptide and magnetic nanobeads on the surface of the gold sensor (Figure 1-II). For optimal monolayer performance, the amount of the peptide substrate needed to be labeled with magnetic nanobeads had been studied previously.[46,60] In brief, the amount of the peptide substrate required for optimum conjugation with magnetic nanobeads depends upon the substrate's molecular weight (MW) and its relative affinity for the magnetic nanobeads as well as the surface area of the beads.[61] This amount could be calculated theoretically using the following equation.[61]

$$S = (6/\rho Sd)(C)$$

where S = the amount of the representative protein substrate required to achieve surface saturation (mg protein/g of microspheres), ρS = density of the solid sphere (g/cm^3), d = mean diameter (μm), and C = capacity of the microsphere surface for a given protein (mg protein/m^2 of sphere surface). However, in this study, we have found that the theoretical amount was substantially high. Accordingly, a lower concentration was used to avoid any steric effects or nonspecific binding.

The suspension of peptide−nanobead conjugates was mounted over the golden chip (Figure 1-IIE) and allowed to air-dry for 30 min to ensure proper immobilization of the peptide−nanobeads and coating of the gold surface. At this stage, the biosensor platform is predominantly black in color. Later, an external magnetic field was passed over the sensor surface to remove any nonimmobilized peptide−nanobeads. The sensor was then ready to detect *C. albicans* proteases (Figure 1-IIIG).

Upon dropping the *C. albicans* (3.5×10^{10} CFU/mL) culture supernatant solution (50 μL) onto the biosensing platform, a colorimetric visual readout could be observed by the naked eye. As shown in (Figure 1-IIIH), the color changes from black to gold as a result of the cleavage of the peptide, indicating the presence of *C. albicans*. Notably, the magnet fixed to the reverse side of the sensor platform attracts the cleaved peptide−magnetic bead moieties.

Different concentrations of the *C. albicans* culture supernatant protease solutions were applied to the functionalized sensor to determine the amount of protease present (ranging from 3.5×10^{10} to 35 CFU/mL), as shown in Figure 4 A,B. As the concentration of protease increased, there was a corresponding increase in the bare gold area visible on the sensor. This was a result of the protease's proteolytic activity, causing dissociation of the peptide−magnetic beads complex (Figure 4B).

3.2. Specificity and Stability Testing. The specificity of *C. albicans* nanobeads-based strip biosensor was evaluated using supernatant protease solutions from other *Candida* species and other microorganisms. In this study, *G. vaginalis* ATCC 14019, *L. acidophilus* ATCC 36031, *C. parapsilosis* ATCC90018, *glabrata* ATCC 90030, *C. dubliniensis* ATCC 44508, and *Gardnerella* clinical isolate culture supernatant proteases were used to check possible cross-reactivity using a single and multiplex platform (Figure 3A, B). Interestingly, no visual change in the black color of the biosensor was observed when testing these organisms, indicating the specificity of the sensor. Accordingly, the *C. albicans* nanobead-based strip biosensor is a promising detection tool for *C. albicans* because of its low cost, specificity, and rapidity.

A stability study confirmed that the sensor maintains its stability for a minimum of 4 months at 4 °C. This temperature is universally applicable, offering a standardized approach that contrasts with the variability of room temperature across different regions. By maintaining the sensor at 4 °C, consistent and reliable performance can be ensured across diverse environments.

Every month, three ready-to-use sensor probes were used to detect *C. albicans* culture supernatant proteases. In every experiment, a blank sample (broth medium) was tested. The designed biosensor produced readings comparable to those of the initial results. This suggests that *C. albicans* sensor platform has a long-term stability of performance under the storage conditions.

The *C. albicans* biosensor's reliable performance across diverse environmental conditions was validated through a year-long experimental study conducted in our Jordanian laboratory. This research assessed the biosensor's performance under varying room temperatures that fluctuated with seasonal changes, ensuring its effectiveness across different environmental scenarios.

3.3. Biosensor Detection of Clinically Isolated and Standard *Candida* Proteases. PBCs of standard *C. albicans* ATCC 9643 and other *Candida* isolated from high vaginal swab samples were prepared and labeled with a code (S1−S6) to hide their identity for blind testing. Samples were assessed for the presence of *C. albicans*. As shown in Figure 5, a positive result for samples S1, S2, S3 and S4 was obtained. However, samples S5 and S6 gave negative results. Looking at the authenticity results as verified by VITEK 2 Compact 15 at the Specialized Hospital (Amman, Jordan), all clinical samples except S6 were identified as *C. albicans*. However, the S6 sample was verified as *C. glabrata*. The false negative results for the S5 sample could be related to the probability of misidentification by VITEK 2 Compact 15. A previous study by Kaur et al. compared Vitek-2 system performance with conventional and molecular methods and indicated that out of 172 isolates, 155 candida isolates were correctly identified, 13 were misidentified, and four were with low discrimination, whereas only one clinical isolate was misidentified with the conventional method. The average measurement of agreement between the Vitek-2 system and conventional methods was >94%.[62] Thus, further studies are required to compare the biosensing platform performance with those of other conventional and molecular methods.

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Figure 5. Colorimetric biosensor performance under the effect of standard *C. albicans* ATCC 9643 and other *candida* isolates from high vaginal swab samples.

4. CONCLUSIONS

This *C. albicans* colorimetric biosensor represents a significant advance in the field of diagnosis of fungal infection. This biosensing platform capitalizes on recent nanotechnology advances and offers a rapid POC solution to address the urgent need for the timely detection and management of *Candida* infections. This diagnostic biosensor operates by tracing the proteolytic activity of *C. albicans*, providing a visual color change upon cleavage of a specific peptide substrate labeled with magnetic nanobeads and immobilized over a gold sensing platform with a LOD of 3.5×10^3 CFU/mL. This approach offers several advantages, including simplicity, affordability (less than 2 $), rapidity (5 min), and portability, making it suitable for deployment in various healthcare settings, particularly in resource-constrained environments. Notably, this *C. albicans* biosensor distinguished *C. albicans* from other *Candida* species and microorganisms. Furthermore, the biosensor exhibited stability over an extended period, suggesting its potential utilization for long-term use. Looking ahead, our study shows promising results for the biosensor's rapid detection capabilities, but it will be essential to further evaluate the sensor performance across a broad range of microorganisms and clinical samples. The potential of biosensors to detect *candida* in various matrices, such as human serum and urine, represents a highly promising area for future research and development. This versatile technology could revolutionize healthcare by providing swift and accurate diagnosis of *candida* infections, including unconventional settings like mattresses. To fully capitalize on these benefits, ongoing research is vital to improve the effectiveness and efficiency of biosensors for *candida* detection in both clinical and nonclinical contexts.

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Notes

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