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Recent advances towards point-of-care devices for fungal detection: Emphasizing the role of plasmonic nanomaterials in current and future technologies

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ABSTRACT

Fungal infections are a significant global health problem, particularly affecting individuals with weakened immune systems. Moreover, as uncontrolled antibiotic and immunosuppressant use increases continuously, fungal infections have seen a dramatic increase, with some strains developing antibiotic resistance. Traditional approaches to identifying fungal strains often rely on morphological characteristics, thus owning limitations, such as struggles in identifying several strains or distinguishing between fungal strains with similar morphologies. This review explores the multifaceted impact of fungi infections on individuals, healthcare providers, and society, highlighting the often-underestimated economic burden and healthcare implications of these infections. In light of the serious constraints of traditional fungal identification methods, this review discusses the potential of plasmonic nanoparticle-based biosensors for fungal infection identification. These biosensors can enable rapid and precise fungal pathogen detection by exploiting several readout approaches, including various spectroscopic techniques, colorimetric and electrochemical assays, as well as lateral-flow immunoassay methods. Moreover, we report the remarkable impact of plasmonic Lab on a Chip technology and microfluidic devices, as they recently emerged as a class of advanced biosensors. Finally, we provide an overview of smartphone-based Point-of-Care devices and the associated technologies developed for detecting and identifying fungal pathogens.

1. Introduction

Bacteria and fungi represent an important category of microorganisms. Although most of them are used by mankind in the food and pharmaceutical industry, some strains are pathogens, causing diseases and infections in humans (Santos et al., 2010). Due to mismanagement and excessive use of antibiotics and immunosuppressants in the last couple of years, the number of patients infected with antibiotic-resistant or susceptible fungi has increased dramatically (Xia et al., 2022).

But how common are fungal infections? The Fungal Kingdom is massive, with 3.8 million species of fungus worldwide, while only about 120000 have been classified up to now ("What are Fungi?"). All these fungal species can be divided into two categories. First, there are the helpful ones – the "healthy" fungus – that people consume: the Portabella mushrooms, Shiitake mushrooms, Pine mushrooms, and so on. In the same category, there are the fungi species responsible for the preparation process of wine, beer, and even bread (Chai et al., 2022). Still, most importantly, without fungi, there would be no antibiotics like penicillin and ciclosporin ("What are Fungi?"). Moreover, fungi live in symbiotic relationships with plants, ensuring the degradation of organic materials and providing the reuse of nutrients from dead organisms to live plants and animals. On the other hand, harmful fungi can't be ignored, as they affect our day-to-day lives. A great variety of fungi attack and affect plants worldwide. Although one may think this does not affect us humans, fungal infections of plants have a substantial economic impact. Billions of dollars are lost yearly due to the lack of fruit and vegetable production and harvest damage (Botina A et al., 2019). Moreover, many fungi strains can infect and produce toxins in

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fruits and groceries. These toxins are harmful and can be fatal, while also promoting the development of cancer when people come in contact with and ingest them (De Lucca, 2007). Climate change is also an important factor supporting the worldwide spread of fungi that needs to be considered. Furthermore, many animal and plant species will likely become extinct due to resistant and deadly fungal infections (Fisher et al., 2020; "What are Fungi?").

However, the risks of climate change influencing the development of new and resistant fungi pathogens are higher than ever, and the "silent" enemy should not be further ignored. Due to the worldwide rise in temperature and climate changes, the Fungi Kingdom is constantly evolving to survive in every condition, even at higher temperatures. At the end of March 2023, the first-ever case of a human infected with plant fungi was reported (Dutta and Ray, 2023). A 61-year-old man from India went to the hospital with a recurring cough, hoarseness of voice, difficulty swallowing, a sore throat, and fatigue. It was later investigated and the doctors found a paratracheal abscess in his neck, diagnosing the man with *Chondrostereum purpureum*, a plant fungus infection. The man was successfully treated and the pus was completely removed and sent to the World Health Organization (WHO) for further analysis. Even though it was thought that plant fungi could not spread and infect humans, this report makes the scientific community aware of the danger of fungi pathogens undergoing mutations. Despite this scenario best describes an episode of "The Last of Us" Sci-Fi TV series, it is a natural and severe threat waiting to expand and affect the world.

1.1. Prevalence

Invasive fungal diseases represent a serious and emerging global threat that is constantly expanding. Regardless of the recent progress in medicine and antifungal treatments, multi-drug-resistant fungi are emerging. Moreover, the lack of prevention, diagnostic analysis, and appropriate treatments, especially in low-income regions, jeopardizes human health, endangering, above all, people who have serious health problems, a weak immune system, or who undergo broad-spectrum antibiotics or immuno-suppressors treatments.

It is estimated that there are over 150 million fungal infections around the world, with a mortality rate of 1.7 million deaths every year (Kainz et al., 2020). In a global prevalence estimation, that studied the yearly occurrence of fungi diseases, 3000000 cases of chronic pulmonary aspergillosis were found, together with 7000000 cases of invasive candidiasis, ~250000 cases of invasive aspergillosis, ~500000 cases of *Pneumocystis jirovecii* pneumonia, ~10000000 cases of asthma caused by fungus and over 1000000 cases of fungal keratitis (Brown et al., 2012; Denning, 2015; Vallabhaneni et al., 2016). Despite emerging as a global health problem and threatening the health of millions of people, fungi infections receive minimal attention compared to other pathogens (Bongomin et al., 2017). Bacteria and especially viruses attracted a lot of attention in recent years, overshadowing fungi. Scientists, doctors, and medical representatives worked together, dedicating significant effort to the detection of viruses, especially since 2020 when the SARS-CoV-2 global pandemic emerged. D. Petrovszki et al. focused on the neuroinvasive capacity of the SARS-CoV-2 virus, specifically the S1 spike glycoprotein, which they believed contributed to neurological symptoms, as its levels are linked to the severity of the respiratory and neurological infection (Petrovszki et al., 2022). In their study, the authors investigate the path of the SARS-CoV-2 S1 protein across *in vitro* cell culture models of both the blood-brain and intestinal barriers using an optical biosensor, thus providing insights into its impact on biological barriers. Moreover, the same group of researchers described an innovative biosensing system, specifically designed to detect bacterial cells from real low-volume fluid samples (Petrovszki et al., 2021). Their detection approach integrates a dielectrophoretic cell-capturing technique with the precision of evanescent-field sensing for living *E. coli* bacteria cell detection from artificial urine samples. Still, fungi detection represents a top priority, as these silent pathogens are just as dangerous as viruses and bacteria infections.

WHO released in 2022 a "Priority List" regarding fungal pathogens (Fisher and Denning, 2023). All presented fungi strains, such as *Candida auris*, *Aspergillus fumigatus*, *Candida tropicalis*, *Mucorales,* and *Cryptococcus gatti*, were divided into 3 categories: i) the critical priority group, ii) the high priority group and iii) the medium priority group. This list represents a significant step in our quest to raise awareness of this silent threat that fungi infections constitute, while hopefully being used to improve early diagnosis methods and improved alternative treatments.

Common fungal diseases that affect people around the globe are fungal nail infections, ringworm, *Candida* infections of the mouth, throat, and esophagus, and the most common one, *vulvoganinal candidiasis*, also known as "vaginal yeast infection". There are also a few fungal infections that must be taken into account when traveling to certain areas, such as *Blastomycosis* – usually met in the United States and Canada, *Cryptococcus gatti* – a fungal infection that thrives in subtropical regions around the world; *Paracoccidioidomycosis* – affects mostly men that work outdoors in parts of Central and South America – and *Histoplasmosis* – a fungal species that is linked to birds and bat droppings. Moreover, patients with a compromised immune system due to HIV, cancer, or organ transplants are susceptible to fungal infections that become deadly as the immune system lacks the ability to combat these pathogens. *Aspergillosis*, *Candida auris*, *Pneumocystis pneumonia,* and *Mucormycosis* are just a few of these threatening infections ("Types of Fungal Diseases | Fungal Diseases | CDC," 2019).

F. Bongomin et al. collected data regarding the prevalence of different fungi from all over the world and analyzed them from a statistical point of view (Bongomin et al., 2017). Based on their data, we analyzed the burden of 7 different fungi infections around the globe and assigned them to each continent. We determined the total burden and the rate of cases per 100000 residents of each infection, thus establishing which continent carries the highest burden and also which fungal strain causes the most harm in each analyzed region (Fig. 1). However, an exact estimate of the global burden and the rate of each fungal infection is impossible to assess as no available database contains this information. Most data remains undiscovered, especially in less-developed countries. Moreover, to this day, more data regarding the prevalence of fungi infection needs to be reported for Australia and Antarctica.

Asia ranks first, with the highest fungi infection burden, 45.65 % of all the acquired data. Europe falls in second place, with 19.55 %, followed by South America (13.52 %), Africa (11.76 %) and North America (4.27 %). Surprisingly, when the incidence rate per 100000 people was analyzed, Europe came first, with a rate of 4902,57/100000. Asia came second, with a rate of 2260,09, followed by South America (rate of 2113,26), North America (rate of 1745,22), and finally Africa (rate of 1378,43). However, although 77 % of Russia is located in Asia and the rest of 23 % in Europe, the presented statistics exclude Russia, as it fits separately. Russia experiences a total burden of 474885, representing a 402,46/100000 residence rate.

Of all the 7 fungi infections – *Candidaemia*, Invasive *Aspergillosis*, *Pneumocystitis jirovecii* pneumonia, chronic pulmonary *aspergillosis*, Allergic bronchopulmonary *aspergillosis*, severe asthma with fungal sensitization and *Fungal Keratitis* – Asia is most affected by Allergic bronchopulmonary *aspergillosis*, with a burden of 1804853 (rate of 681,4/100000) (Agarwal et al., 2014; Batac and Denning, 2017; Beardsley et al., 2015; Ben and Denning, 2015; Chayakulkeeree and Denning, 2017; Gugnani et al., 2017; Huh et al., 2017; Jabeen et al., 2017; Khwakhali and Denning, 2015; Taj-Aldeen et al., 2015; Tilavberdiev et al., 2017), while Europe, South America, Africa, and North America are most affected by severe asthma with fungal sensitization.

The Fungal Infection Trust organization updated 2019 a list regarding the prevalence of fungal infections around the globe ("How Common are Fungal Diseases.pdf"). Oral infections, commonly known as oral thrush, own a burden of ~1.9 million people worldwide ("Global HIV & AIDS statistics — Fact sheet"). In comparison, Candida infection of the esophagus affects ~537000 people around the globe ("How

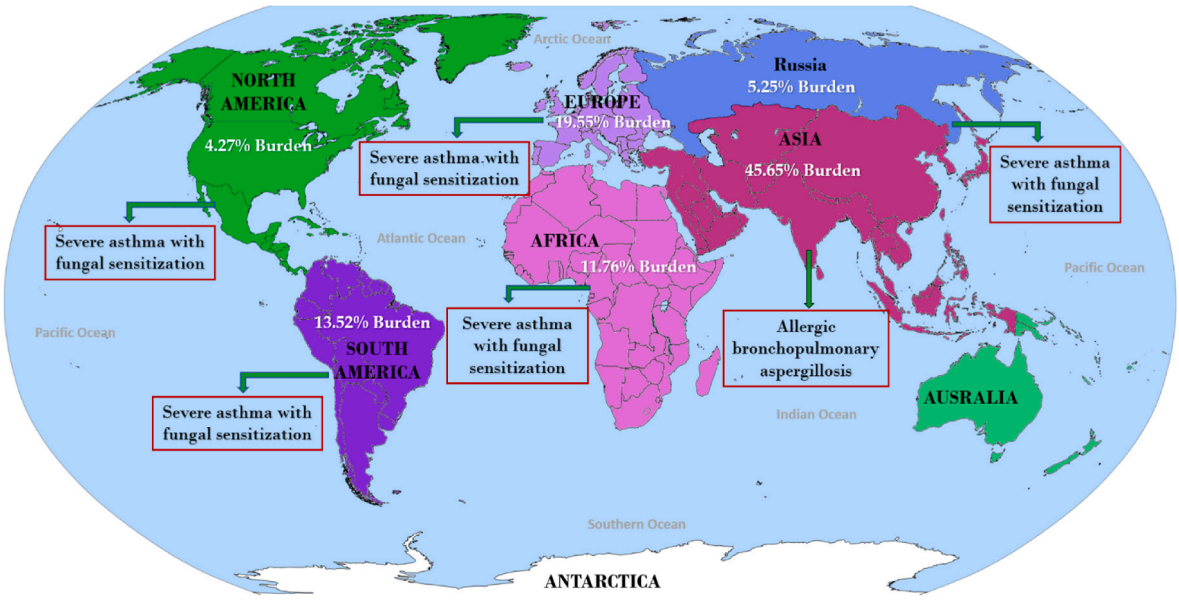

Fig. 1. World map representing the prevalence of fungi infections. Seven different fungi infections were analyzed, and the burden per continent was established, together with the infection rate per 100000 people (Bongomin et al., 2017). Australia and Antarctica were not considered, as insufficient data is reported in the literature.

Common are Fungal Diseases.pdf"). Vulvovaginal candidiasis impacts the lives of ~138000 million women annually (Denning et al., 2018; Foxman et al., 2013), whereas recurrent vulvovaginal candidiasis burdens ~372 million women over their lifetime (Denning et al., 2018). *Candidaemia*, a Candida infection of the blood, owns a rate of 2–26 infections per 100000 people (Arendrup, 2010; Cleveland et al., 2012; Puig-Asensio et al., 2014), with a mortality rate between 30 and 55% (Brown et al., 2012). Invasive *aspergillosis* affects especially patients who have leukemia, lymphoma, severe illness, or who have undergone organ transplants. It affects over 10 million people in Europe, the USA, and Japan. While the annual burden of invasive *aspergillosis* was reported to be 350000, the mortality of this fungal infection is over 50%, even if the infection is treated accordingly ("How Common are Fungal Diseases.pdf"). *Cryptococcal meningitis*, a fungal infection that spreads from the lungs to the brain, has a high incidence in AIDS patients, with a burden of 223100 cases and a high mortality rate, over 15% of all AIDS deaths around the world (Rajasingham et al., 2017).

Another fungal infection that primarily affects the lungs of individuals with AIDS is *Histoplasmosis*. It is estimated that 50 million people have been in contact with this fungal infection, with an additional burden of ~500000 new infections every year ("How Common are Fungal Diseases.pdf"). Allergic bronchopulmonary *aspergillosis* poses a burden of ~4.8 million people affected by active asthma (Denning et al., 2013). This fungal infection is widespread in India but affects the global population (Agarwal et al., 2014). Moreover, another fungal infection affecting people with asthma is fungal sensitization. The estimated burden of Severe Asthma with fungi sensitization is estimated at 6.5 million adults worldwide (Brown et al., 2012; Denning et al., 2014). Thus, this fungal infection is rarely documented in children. A report from India demonstrated that of 100 children with severe asthma, 60% of them were sensitized to fungi (Gupta et al., 2018). Another report from Russia investigated 120 children with poorly controlled asthma and highlighted that 30% of them were sensitized to fungi, in particular to *A. fumigatus* ("How Common are Fungal Diseases.pdf"). Additionally, chronic pulmonary *aspergillosis* has a burden of 1.2 million cases in people suffering from tuberculosis (Denning et al., 2011). Finally, cutaneous fungal infections, represented by skin, nail, and hair infections, burden 1 billion people worldwide ("How Common are Fungal Diseases.pdf").

1.2. Economic impact

In recent years, the burden caused by fungal infections around the globe has been significantly increased. Fungal infections affect people, animals, and plants, and are a global threat to the world's economy.

The economic burden of fungal infections can be analyzed *via* 3 perspectives (Table 1): i) the perspective of patients, who are the "payers", as they experience all medical costs, from treatment with antifungals to hospitalizations, medicine, and all the other costs associated with the post-discharge; ii) the perspective of the hospital facilities who support only the costs of medications and diagnostic tests; iii) the perspective of the society that suffers the impact of the lack of

Table 1
Evaluation of cost burden of fungal infections on public health institutions, patients, and society. All costs vary depending on the region and a mean estimation was made. All the data is likely underestimated, given the lack of complete economic impact reports.

	Economic impact	Ref.
Public health institutions	4000–7000 € - antifungal drugs 8000–52000 € - hospitalization 265000–83000 € - all management costs	(Cornely et al., 2008; Dominguez-Gil et al., 2007; Mele et al., 2002; Sidhu et al., 2009; Slobbe et al., 2008; Stam et al., 2008)
Affected patients	10500–25500 € - fungal burden Over 50000 € - antifungal medication, hospitalization, additional charges $6.7 billion - hospitalization costs in the United States, just in 2018 $7.5 billion – medical costs in the US in 2019	Slobbe et al. (2008) (Jean-Pierre Gangneux et al.) (Rayens et al., 2022) (Benedict et al., 2022)
Society	$14 billion/year – lost productivity in women due to vulvovaginal candidiasis $870 million – lost productivity $2.1 million – lost workdays $3.2 billion – premature deaths	Denning et al. (2018) (Benedict et al., 2022)

productivity of the infected patient and also the future loss of productivity and incapacity to work caused by aggressive and invasive fungal infections (Drgona et al., 2014).

As hospitals and health institutions experience the costs of only antifungal drugs and hospitalization, the economic burden is between 4000 and 7000 € for antifungal medication, and an additional 8000–52000 € for the hospitalization/day for each patient (Cornely et al., 2008; Dominguez-Gil et al., 2007; Mele et al., 2002; Sidhu et al., 2009; Slobbe et al., 2008; Stam et al., 2008). E. Rayens et al. (2022) revealed an economic burden of $6.7 billion for hospitalization, in the US in 2018. They also investigated the rate of fungal infections by sex. They revealed that men are 1.4–3.4 times more likely to be diagnosed with a fungal infection than women.

The patients experience a much higher economic burden caused by fungal infections. A study conducted by Bruynesteyn et al. revealed that the average treatment costs for caspofungin and amphotericin B lay between 9700 and 11700 £, while the drug costs were between 4600 and 6400 £ in the UK (Bruynesteyn et al., 2007).

Invasive *aspergillosis*, a fungal infection with a high mortality rate, is affecting people with weak immune systems based on chemotherapy treatment for acute leukemia (Drgona et al., 2014). An observational study by L. Slobbe et al. Found that patients face high costs when exposed to invasive aspergillosis (Slobbe et al., 2008). While patients undergoing chemotherapy experience a 57750 € burden, this cost becomes greater – over 68000 € – when they become suspects of fungal infection, and the cost rises as high as 83300 € when the infection with *aspergillosis* is detected and treated. Moreover, one has to consider the loss of productivity and motivation to work while undergoing antifungal treatment, with the average duration of therapy being 104 days.

Gangneux et al. studied the economic burden of 50 patients experiencing invasive fungal infections while suffering from acute myeloblastic leukemia (Jean-Pierre Gangneux et al.). They concluded that, although patients received hospitalization for 45 days, the total duration of antifungal treatment was 198 days and the costs endured by patients were high. The mean total costs due to fungal infections are located around 51000 €; this includes the antifungal medicine – 35000 €, charges of hospitalization – over 13500 €, and over 1300 € for each additional hospitalization.

Vulvovaginal candidiasis, also known as “vaginal yeast infection”, is a mucosal infection that threatens women worldwide. It is estimated that 75% of women worldwide are affected by this fungal infection at least once in their lifetime (Denning et al., 2018). In contrast, a majority of these women, over 135 million (Fisher et al., 2020), are affected by recurrent vulvovaginal candidiasis, which can occur as frequently as 4 or more times per year. It has a significant mortality rate, affecting millions of women (Gonçalves et al., 2016; Sobel, 2016). Every episode of vulvovaginal candidiasis can be triggered by a variety of factors, such as the use of antibiotics, sexual activity, hormone replacement therapy, and many others, and unfortunately, the pathogenesis of recurrent vulvovaginal candidiasis still lacks a deep understanding (Bongomin et al., 2017; Sobel, 2005). The global annual prevalence is estimated to be over 3000 per 100000 women, while in developed and high-income countries, the economic burden caused by incapacity to work and loss of productivity reaches around 14 billion dollars per year (Denning et al., 2018).

A group of researchers from Atlanta, Georgia, analyzed the economic burden of various fungal diseases in the US for 2019 (Benedict et al., 2022). They estimated the total financial cost to be 11.5 billion dollars in 2019 alone, with the direct medical costs representing $7.5 billion, two-thirds of the total burden. They were also determined to investigate the total productivity costs, estimated to be a total of $870 million – approximately $2.1 million for lack of productivity and missed workdays – and premature deaths accounted for a loss of $3.2 billion. Of this total economic burden, they determined that $2.6 billion accounted for noninvasive candidiasis, $1.2 billion for dermatophytosis, $1.8 billion for *aspergillosis,* and other various mycoses had a $2.1 billion burden.

2. Plasmonic nanomaterials – unique properties and applications

Plasmonic nanoparticles (NPs) generally indicate noble metal NPs that, due to their reduced dimension (1–100 nm) exhibit the phenomenon of localized surface plasmon resonance (LSPR) consisting of the oscillation of the free electrons in metals. The small dimension of plasmonic NPs determines a high ratio of surface atoms to bulk atoms. Consequently, the surface atoms are responsible for the overall properties. A further consequence of the reduced dimensions of plasmonic NPs is the Electromagnetic (EM) confinement of the optical wave interacting with them. The EM confinement underlies the LSPR phenomenon.

In particular, in the presence of an oscillating EM field, namely EM radiation at a specific frequency, free electrons, in the conduction band of metals, oscillate collectively. The oscillation frequency is defined as plasmon resonance.

In detail, the external electric field exerts a force on the conductive electrons, displacing them from their equilibrium positions and creating a net charge difference. Electrons try to compensate through a restoring force giving rice to dipolar oscillations occurring at plasmon resonance.

Plasmon oscillations within plasmonic NPs can be conceptualized as a mass-spring harmonic oscillator influenced by the resonant energy of incident light waves. In this dynamic interplay, the electron cloud undergoes oscillations resembling a dipole, aligned parallel to the electric field of the EM radiation.

For a spherical metallic NP, the NP size (R), and the extinction cross section (C_{ext}) value as a function of the wavelength of the impinging light (λ) are related, according to the following equation:

$$C_{ext} = \frac{24\pi^2 R^3 \varepsilon_m^{3/2}}{\lambda} \frac{\varepsilon''}{(\varepsilon' + 2\varepsilon_m)^2 + \varepsilon''^2} \quad \text{Equation 1}$$

At the plasmon resonance wavelength (λ_{res}) C_{ext} reaches its maximum value.

This equation also evidences that the value of the λ_{res} depends on the dielectric constant of the medium (εm). Consequently, metallic NPs can be employed as excellent optical transducers, developing optical biosensors. Moreover, as the LSPR phenomenon localizes and concentrates the EM field, metallic NPs can be exploited for electrochemical biosensing and Surface-Enhanced Raman Scattering (Liz-Marzán, 2004).

The progress in the plasmonic field has driven remarkable advancements in sensitive and ultra-sensitive detection, even at a single particle or single molecule level. Significant breakthroughs in the last decade regarding the nano-synthesis of plasmonic materials have facilitated the development of NPs with different sizes and shapes, depending on the desired application. Additionally, noble metal NPs, such as gold (AuNPs) and silver (AgNPs), possess exceptional plasmonic properties and have been widely used for the detection of analytes without the addition of specific labels in biological environments (S and S, 2016). They are ideal candidates for designing and developing label-free nanosensors as they are highly sensitive to changes in the refractive index. The shift observed in the LSPR peak correlates with the change in the refractive index of the surrounding medium (Langer et al., 2015). Hence, the characteristics of the LSPR depend on several factors, such as the NP’s size, shape, surface structure, and aggregation level. All these factors influence the interaction of the NPs with the analyte. This distinctive property of plasmonic resonance originates from the oscillation of plasmons or the collective movement of conduction band electrons concerning fixed positive ions, which are commonly found in noble metals (S and S, 2016).

Fungal pathogens represent a worldwide threat not only to plants and animals but also to human health. Clinical fungal infections have become more common in the last decade, owing to the misuse of antibiotics, glucocorticoids, and various medical interventions (Xia et al., 2022). Moreover, an alarming number of people suffer from fungal

allergens that are known to be the root of lung-associated pathogens (K. Hussain et al., 2020). Plasmonic NPs, due to their unique properties can significantly contribute to the development of novel biosensing technologies able to detect and discriminate between fungal pathogens rapidly is crucial to preventing and accurately treating patients.

Biosensors represent devices that exploit chemical or biological reactions to detect specific target analytes and they typically involve a receptor and a transducer. Generally, the receptor binds to the target analyte molecule and the transducer translates the chemical/biological reaction into a detectable signal (Brahmkhatri et al., 2021). The efficiency of the as-designed sensor is assessed based on its selectivity, response time, limit of detection (LOD), and the linear detection range (Mao et al., 2017). Utilizing plasmonic NPs has been well-documented lately in the quest to design biosensors that meet the as-presented characteristics. Over time, NP applications have diversified, thus producing nanomaterials that are efficient, low-cost, sensitive, miniaturized, and environmentally friendly. Various types of NPs, especially AuNPs (Ferrari, 2023; Hegde et al., 2022) and AgNPs (Ivanišević, 2023; Tan et al., 2020), have been explored for their unique ability to undergo multifunctional surface functionalization and exhibit target-specific binding properties, leading to the development and designing of a wide array of plasmonic NPs-based biosensors.

The surface of metal particles is characterized by amphiphilic organic molecules that occupy surface sites through coordination bonds. These molecules, identified as capping agents, control the size and shape of NPs and ensure their colloidal stability in a specific medium. This characteristic allows for the chemical manipulation of metal NPs. Indeed, through appropriate chemical procedures, it is possible to partially replace or modify the capping agent. These modifications have several purposes, such as: making the metal particles soluble in a medium of different polarity, enabling their integration into polymer matrices, and introducing specific probe molecules. The introduction of probe molecules makes plasmonic transducers more specific and sensitive.

Furthermore, surface functionalization paves the way for the development of miniaturized devices based on metal NPs, which can also integrate microfluidic channels (Chen et al., 2016).

The versatility of metal NPs makes them suitable for use in wearable devices as well. All these devices can work as sensing platforms, supporting specific processes (such as a chemical reaction or aggregation process) that, for example, result in a color change or generate an electrochemical signal that can be read by a portable device such as a smartphone. These are known as Point-of-Care (PoC) devices.

PoC devices can be extremely helpful for fungi and mycotoxins identification as they can be used in environments where monitoring the presence of fungi and mycotoxins is crucial for precisely identifying the infections but also for preserving foodstuff, ensuring safety in the whole agri-food chain with a positive economic impact.

3. Current fungi detection and identification technologies

The most common approach in healthcare facilities to identifying fungal strains is based on morphological characterization of those strains that possess reproductive structures.

Morphological characterization is carried out by microscopy and consists of the observation of fungi appearance in a specific tissue section and the identification of a specific morphological pattern. This approach allows the direct observation of an infected tissue or fluid and enables to discriminate among septate non-septate molds or yeast and it is often associated with histopathological analysis. These methods allow the direct observation of a sample derived from a patient and allow to obtain information regarding the host response.

Unfortunately, this method has significant limitations, being ineffective in identifying fungal strains that do not possess reproductive structures, and even in different fungal strains that have similar morphologies.

Also, fungal cultures are very effective for pathogen identification and useful for gathering information on fungal resistance, but the analysis requires days and can be also subjected to contamination.

Matrix-Assisted Laser Desorption Ionization-Time of Flight - Mass spectrometry (MALDI-TOF) and Fluorescence *in situ* hybridization are two analytical methods both used in healthcare facilities that are extremely accurate and fast, although they present some limits regarding quantification.

Other approaches are based on real-time polymerase chain reaction (PCR) for molecular diagnosis, enzyme-linked immunosorbent assay (ELISA), electrochemiluminescence for immunoassays, and, lately, fluorescence detection is being largely used in the detection of viruses and bacteria (Carter et al., 2020; Kim et al., 2021; Lee et al., 2020).

In particular, ELISA along with chromatographic methods are used for the analysis of food commodities. They are sensitive, reproducible and fast. Moreover, ELISA tests are suitable for screening, while chromatographic methods can be automatized (Pandey et al., 2023). However, these methods require expensive and dedicated instrumentation and well-trained personnel.

Unfortunately, all these methods possess some drawbacks, such as the high amount of time they need to detect and identify the fungal strain, the low sensitivity they possess, and also the need to be operated by qualified and certified personnel. This has sparked significant interest in diagnostic platforms designed as Point-of-Care (PoC) devices that can enable early disease detection worldwide. While current PoC solutions, such as paper-based devices, offer rapid, affordable, and user-friendly detection platforms, they suffer from insufficient sensitivity and selectivity (Petronella et al., 2023). As a crucial tool for disease management, especially in developing regions, PoC diagnostics devices are continuously evolving to overcome the limitations of existing detection methods. Recently, plasmonic-based platforms have emerged as promising new candidates for next-generation diagnostics, aiming to alleviate the burden of infectious diseases (Li et al., 2019).

With this in mind, spectroscopic, colorimetric, lateral-flow immunoassay, and electrochemical detection techniques (Fig. 2) constitute excellent tools for high accuracy and rapid pathogens detection that can overcome all these drawbacks (Erukhimovitch et al., 2005).

3.1. Detection of fungi and mycotoxins: current challenges and possible solutions

As discussed in the previous sections, fungal infections severely impact human health and the economy. Reliable, user-friendly, and cheap detection methods are a powerful weapon to contrast the threats associated with fungal infections. Moreover, it is essential to consistently monitor the presence of toxigenic fungi and mycotoxins to ensure biosafety across the entire agri-food chain, including on farms. In this framework, the main challenges are mostly related to the time required to obtain a response and the specificity and selectivity of the analytical method. Indeed, detection methods conventionally used in healthcare facilities, such as culture and microscopy examination, are not quantitative, are laborious, time-consuming, and have limited sensitivity and specificity. Moreover, some analytical methods, such as the MALDI-TOF, although specific, require a high initial cost for supporting the purchase of the instrument and highly specialized personnel (Mendonça et al., 2022).

In most cases, these analytical protocols cannot be implemented in a portable device. The portability is a fundamental requirement for the rapid and on-site detection of fungi and mycotoxins in operative contexts, spanning from medical offices to food supply chains.

Nanomaterials, particularly plasmonic nanomaterials, have inherent chemical-physical properties that make them the ideal building blocks for the realization of multifunctional, robust, and compact devices.

Their high surface-to-volume ratio is a crucial prerequisite for miniaturization and, therefore, for producing plasmonic devices. Noble metal nanostructures absorb visible light and respond to the alteration of

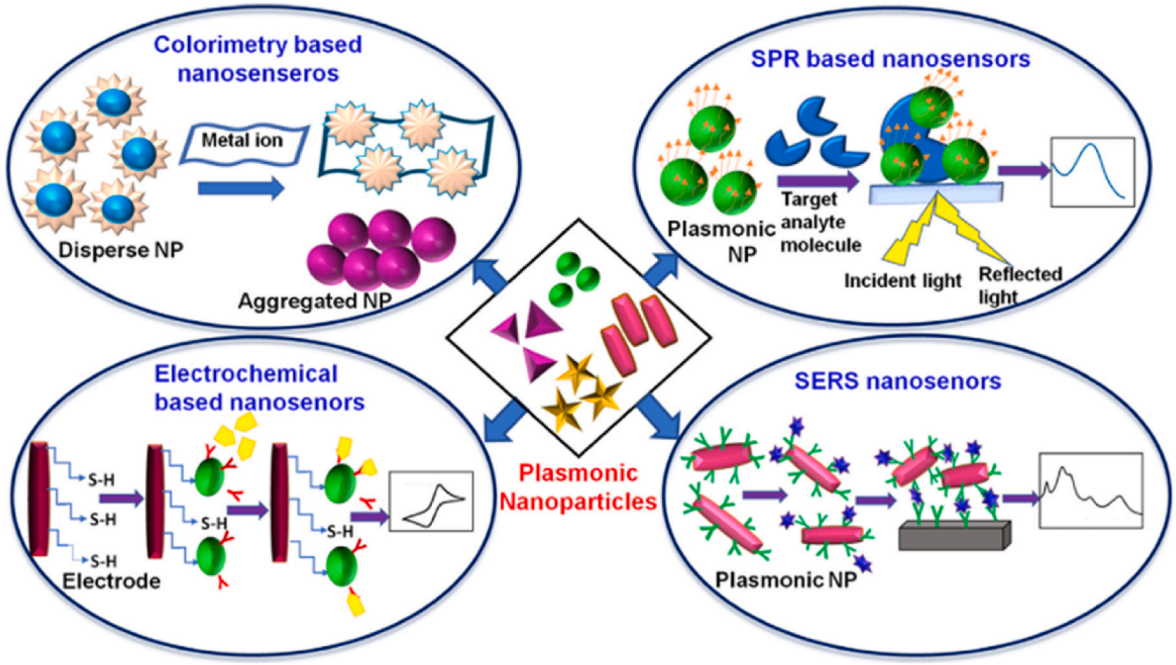

Fig. 2. Schematic illustration displaying plasmonic-based colorimetric, electrochemical, spectroscopic, and SERS nanosensors employed as accurate tools for pathogens detection (Brahmkhatri et al., 2021).

their surrounding environment by altering their spectroscopic properties, such as their color. For this reason, they respond to the challenge of user-friendliness. They allow the merging of highly analytical performance with simplicity of operation in compact devices intended for non-highly specialized personnel. The high surface-to-volume ratio results in an elevated number of surface-active sites that can be functionalized with probe molecules that recognize a defined target, thus overcoming the issue of specificity and selectivity. The high electric field generated in the proximity of plasmonic NPs can be exploited to boost the sensitivity of the detection method.

The utilization of plasmonic nanostructured materials in the analytical domain, particularly for the identification of fungi and mycotoxins, holds substantial potential with an unpredictable outcome. In particular, developing plasmonic-based systems for multiplex analysis can unlock the full potential of plasmonic nanomaterials. Achieving this goal involves exploring easily scalable and secure methods for generating nanostructured surfaces with diverse probe molecules, utilizing microfluidic technology. Another avenue for multiplex analysis is surface functionalization with probe molecules that adhere to specific crystallographic planes. Addressing this challenge is possible, with robust computing systems and artificial intelligence support, to identify the most effective pair of crystallographic plane and probe molecules. Moreover, incorporating active materials like liquid crystals can significantly enhance the detection limit of spectroscopic methods, such as absorption and fluorescence. Plasmonic nanomaterials can also excel in developing wearable Point-of-Care devices for data collection in the agri-food supply chain. Monitoring is essential for prevention, and these devices can serve as anti-waste tools, promoting a conscientious and sustainable use of food resources.

3.2. Surface Enhance Raman Spectroscopy detection

Surface Enhance Raman Spectroscopy (SERS) is an extremely powerful detection method that allows the identification of specific molecular fingerprints, based on the Raman Scattering of molecules deposited on plasmonic nanostructures. The use of arrays of plasmonic nanostructures to support the analyte enables the amplification of the analytical signal, due to the extremely intense electric field associated with the plasmonic nanostructure. SERS has been extensively used for the identification of fungal strains. While traditional techniques used for pathogen detection present high sensitivity, they are often time-consuming and expensive. SERS instead, represents an excellent detection method for biological application, as it is highly sensitive and cost-efficient (Ambartsumyan et al., 2020). However, it is worth noting that the SERS-based detection technique has several downsides. The first problem would be the production of an inexpensive, chemically stable, and reliable SERS substrate that possesses also uniformly high enhancement and reproducibility through the whole substrate, which is hard to achieve. Secondly, a significant enhancement of sensitivity and reproducibility may occur only when statistical analysis methods are applied together with SERS-based biosensors (Ambartsumyan et al., 2020; Xia et al., 2022).

In the field of fungi biosensing, J. C. Ramirez-Perez et al. investigated the impact of three different sizes of AgNPs – 10, 30, and 60 nm – on the SERS signal of four fungal strains: *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticilliodes* (Ramirez-Perez et al., 2022). This study demonstrated that the SERS detection and identification of microorganisms is mainly dependent on the size of the metallic NPs, on which the pathogens adsorb. The wet chemical method was employed to synthesize AgNPs with three different sizes and a small-angle X-ray was employed to determine their size. Furthermore, the SERS spectra demonstrate an NP size-dependent intensity increase in the Raman fingerprint. In particular, the 60 nm AgNPs had almost twice the intensity on the SERS spectra of each fungus, compared to the 30 nm AgNPs. This SERS-active system revealed high sensitivity, an enhancement factor of 4.4×10^7, reproducibility, stability over 2 months, and the ability to differentiate with high accuracy between four types of filamentous fungi.

The same group of authors developed a combined SERS-based detection and statistical analysis method (Perez et al., 2021). They used 60 nm AgNPs as a SERS substrate, to amplify the Raman signal from the molecules in close vicinity to the metal NPs. The analytical readout was combined with statistical analysis, and principal component analysis (PCA), to analyze the data thus determining, identifying, and discriminating three fungal entomopathogens, IBCB 66 *Beauveria bassiana*, IBCB 130 *Isaria fumosorosea* and IBCB 425 *Metarhizium anisopliae*.

The study focuses on obtaining specific SERS spectral fingerprints of each fungi strain, followed by a statistical comparison and differentiation of all spectral data. The specific 550–1700 cm^{-1} fingerprint region was analyzed *via* PCA technique and a discrimination accuracy of over 99% between the three entomopathogens was obtained.

Because of the more than excessive use of antibiotics and immunosuppressants, fungal infections are becoming more common in clinical settings and the mortality rate of patients, infected with drug-resistant pathogens, is rising at an alarming rate. *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis* are fungal strains isolated from Candida infections, that possess the highest mortality rate in patients. Fortunately, a SERS-based detection method combined with a statistical analysis method that processes a huge number of data, able to detect, identify and distinguish fungal strains, has been reported recently (Gu et al., 2022). The three fungal Candida strains, *C. tropicalis*, *C. glabrata,* and *C. parapsilosis* – all resistant and sensitive to common drug treatment, were trapped with magnetic Fe_3O_4@PEI NPs and mixed with triangular AgNPs for an enhanced Raman signal. The SERS detection was rapid and non-invasive, the cell wall was preserved through this method, and the fingerprint of each fungal strain was then statistically analyzed. This SERS-based detection method in collaboration with statistical analysis has proven to be very effective, having an accuracy of over 99% and a fast detection time, of only 90 min. Moreover, the same identification method was used by S. Hu et al. in detecting *C. albicans*, *C. tropicalis,* and *C. krusei* fungi (Hu et al., 2021). In this work, three DNA samples were extracted from the fungi without damaging the cell wall, after which they were mixed with magnetic Fe_3O_4@PEI NPs and with positively charged AgNPs for SERS identification. The same statistical analysis was applied to the SERS fingerprints and the method was reported to be even more rapid and accurate, with the detection being completed in 40 min and the accuracy reaching 99.8%.

Furthermore, fungi metabolism determines the production of mycotoxins. Mycotoxins are extremely important due to their high toxicity at very low concentrations. *Ochratoxin A* (OTA) represents a group of mycotoxins produced by *Aspergillius carbonarius*, *Aspergillius ochraceus,* and *Penicillium verrosum*. When these fungi grow in optimal temperature and humidity conditions, OTA is produced. If food (e.g., wheat, corn, barley, coffee, fruit, rice, meat) is stored or processed in inappropriate conditions, the OTA contaminates it by provoking, upon ingestion, nephropathies, immunotoxicity or cancer, as investigated in animal studies (Bui-Klimke and Wu, 2015). Indeed, the International Agency for Research on Cancer (IARC) classified the OTA as a possible human carcinogen molecule (Group 2B) (Chen et al., 2018; Penalva-Olcina et al., 2022). In 2006, The European Food Safety Authorization, together with Tolerable Weekly Intake, regulated the exposure to OTA of 120 ng per kg body weight. The resulting regulation declared that the OTA concentration must not exceed 5 ppb in cereals, 2 ppb in wine and juices, and 0.5 ppb in baby food. FDA regulated OTA at 1 ppb, on account of its exceptional adverse effects (Rodriguez et al., 2020).

Therefore, in the last years, the scientific community focused on designing and implementing sensitive and selective detection devices to monitor mycotoxins. R. Gillbert et al. reported a selective SERS-based sensor able to detect OTA using gold (Au) film as SERS substrate (Gillibert et al., 2018). The proposed detection method uses SERS spectroscopy to acquire spectra fingerprints of different OTA concentrations, that were analyzed and discriminated using statistical techniques. Hence, this study achieved a picomolar limit of detection using a simple and low-cost SERS-based detection method. Moreover, by analyzing the other two non-specific molecules, the SERS combined with the statistical analysis method proved to be highly selective for OTA.

In the same year, Y. Hernández et al. also developed a label-free nanoprisms aptasensor-based SERS detection method for OTA (Hernández et al., 2020). They designed an OTA detection sensor based on a specific aptamer and gold nanoprisms as Raman signal enhancement (Fig. 3. Left). The research group was able not only to detect OTA in the laboratory from solution samples but also to analyze real samples, from coffee and wheat. After all the SERS spectra were obtained, a multivariate analysis method was employed to process all spectra and to discriminate the samples with OTA in the 10–250 ppb range. This SERS-based detection method proved to have a high sensitivity and also excellent selectivity, as two other common mycotoxins – *aflatoxin B1* and an oligonucleotide – were analyzed but not detected.

R. S. Rodriguez and coauthors investigated two different mycotoxins, *deoxynivalenol* (DON) and OTA, *via* multiplex SERS detection (Rodriguez et al., 2020). They focus their research on these two mycotoxins, as they are relevant targets due to their dangerous effects on food, livestock, and humans. FDA indeed limited the amount of DON to 1 ppm for humans. The presented biosensing technique is based on a multiplex detection scheme of a linear polymer affinity agent, capable to attract and immobilize DON and OTA, both separately and simultaneously, on film over nanosphere SERS substrate (Fig. 3. Right). The individual detection of both toxins was followed by the simultaneous detection of DON and OTA without further chemometric analysis. Ultimately, both mycotoxins were able to interact and were captured by the linear polymer affinity agent at the limit concentration regulated by the FDA.

Aflatoxin B1 (AFB1) belongs to the same group of harmful mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* which are highly toxic and cause contamination of agricultural products. In 2020, a group of scientists from China developed an ultra-sensitive SERS aptasensor for AFB1 detection (H. He et al., 2020). They combined

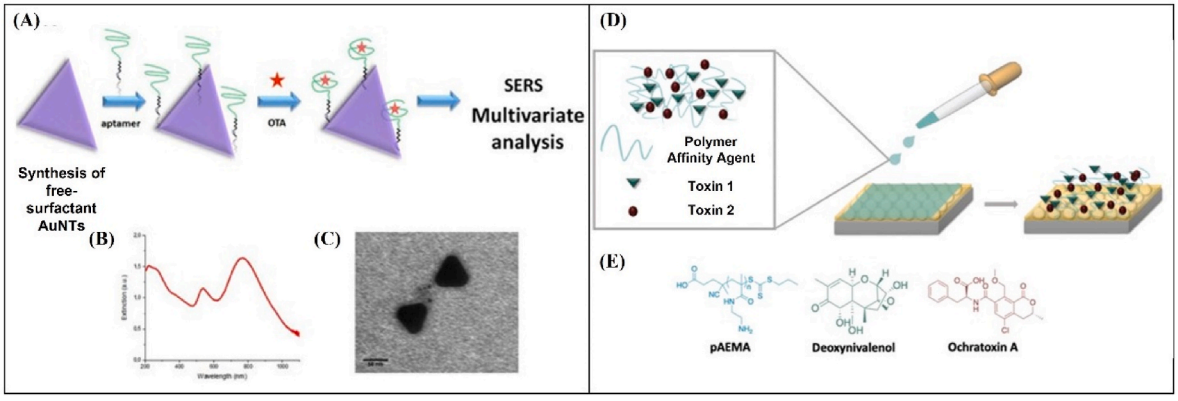

Fig. 3. Left: (A) Scheme of the proposed nanoaptasensor for the detection of OTA *via* SERS in combination with multivariate analysis. Characterization of the AuNTs synthesized by **(B)** UV–Vis–NIR spectroscopy and **(C)** transmission electron microscopy (Hernández et al., 2020). **Right:** Illustrative image of multiplex detection on film over nanosphere (FON) SERS substrates. **(D)** Image of in-solution interaction occurring with a polymer affinity agent and two small molecule toxins. The then complexed molecules attach to the gold FON surface. **(E)** Molecular structures for the methacrylamide polymer (pAEMA) and two small molecule toxins used in this study (*deoxynivalenol* and *ochratoxin A*) (Rodriguez et al., 2020).

magnetic Fe_3O_4@Au nanoflowers (NF) with Au@Ag nanospheres (NS) as sensitive SERS aptasensors that do not require additional chemometrics analysis for the detection and discrimination of fungi strains. The aptasensor with an increased Raman signal was developed by aptamer recognition of SH-cDNA-modified Fe_3O_4@Au NF and SH-Apt-modified Au@Ag NS. The SH-cDNA modified magnetic NF behaved as a capture probe for AFB1, while SH-Apt modified Au@Ag NS were employed as reporter probes. The preferred conjugation of AFB1 triggered the release of Au@Ag from the magnetic NF and an AFB1 concentration-dependent decrease in the SERS signal was obtained. On that account, an ultralow detection limit was achieved, 0.4 pg x mL^{-1} in a wide linear range of 0.0001–100 ng x mL^{-1}. Moreover, real peanut oil samples were analyzed, and proved that the presented SERS-based aptasensor is a promising detection technique with great sensitivity and selectivity.

In the subsequent year, N. M. Santhosh et al. proposed an innovative SERS-based detection strategy to identify four toxic mycotoxins produced by fungi species (M. Santhosh et al., 2021). The approach they followed was different, employing carbon-based nanostructures as templates for SERS substrates. Making use of plasma-enhanced chemical vapor deposition, the authors designed four different carbon-nanostructures, that differ in growth-time. All four carbon nanostructures act as templates for a 100 nm Au film deposition, as the morphology of the as-designed carbon nanostructures possess multiple hot spots that increase the vibrational Raman signal they are decorated with gold. The surface with the best optical response was the one prepared at the shortest time of plasma treatment and was used as a SERS substrate to collect Raman fingerprints spectra of four mycotoxins: *Aflatoxin B1* (AFB1), *Fumonisins*, *Zearalenone* (ZEN) and *Alternariol*. This particular substrate achieved a SERS intensity enhancement of 5 x 10^7, had a thickness of 300 nm, and was made of vertical carbon nanotubes decorated with Au films. Being a highly sensitive detection method, 500 ppb of each mycotoxin was enough to be analyzed *via* SERS. Later on, the PCA statistical analysis technique was employed and all SERS spectra were successfully discriminated. This plasmonic SERS-based detection method combined with statistical analysis data processing proved to be time-efficient, reliable with a great discrimination ability, and a limit of detection between 4 x 10^{-9} and 5 x 10^{-9} M.

A very efficient strategy for detecting and discriminating three fungal species was proposed by N. E. Dina et al. (2018). They proposed a sensitive SERS-based detection method combined with chemometrics analysis to discriminate between three clinically relevant filamentous fungi species: *Aspergillus fumigatus* sensu stricto, cryptic *Aspergillus fumigatus* complex species, and *Rhizomucor pusillus*. The mentioned three fungi species belong to the most relevant fungi pathogens because they are accountable for most cases of invasive fungal infections in humans. Thereby, the team implemented a very rapid and reliable detection method based on label-free SERS identification by using colloidal AgNPs as Raman signal enhancers. The 25 nm AgNPs were synthesized directly onto each fungal exoskeleton and all SERS spectra were acquired using two excitation laser lines – 532 and 633 nm. It is worth mentioning that all fungal isolates were derived from patient samples. Each SERS spectra fingerprint brought information that was used for strain identification. The whole spectra data set was later evaluated by a combination of three chemometric techniques and by the end, the presented detection method proved to be extremely fast, less than 5 min, accurate, and reliable upon fungal species discrimination.

Even though standard colloidal AgNPs are commonly used as SERS substrate for an enhanced Raman signal, E. Hahm et al. proposed a combined silica NPs core and AgNPs assembly as SERS active substrate for fungi detection (Hahm et al., 2020). They successfully synthesized silica-metal-NPs nanostructures – SiO_2@AgNPs –, with excellent size stability and reproducibility, that exhibit several hot spots, marking this nanocomplex with higher sensitivity and greater SERS signal as standard AgNPs. With this in mind, they used this nanostructure in *Alternariol* detection by SERS analysis. Being a mycotoxin of fungi pathogens, *Alternariol* is highly threatening because it contaminates a wide variety of processed food products and possesses very high thermal stability. Based on this study, the as-prepared SiO_2@AgNPs exhibit a highly reproducible SERS detection of *Alternariol*, with a limit of detection of 4.83 nM, and could be considered an exemptional tool for further rapid fungi strain detection.

H. Jin et al. developed a synthesis of "flower" shaped silver colloidal NPs to detect and compare different types of pollens, bacteria, and fungi commonly found in bioaerosols (Jin et al., 2020). The Raman-based detection method, combined with statistical analysis of all the spectra, was able to rapidly detect and identify 14 types of pollen, two types of bacteria, *E. coli* and *Staphylococcus aureus* (*S. aureus*), and one fungi strain, *C. albicans* with an accuracy of 97.3%. All pollen strains were investigated *via* Raman spectroscopy, but due to their weak signal and fluorescent background, the bacterial and fungi strains were mixed with colloidal AgNPs for SERS enhancement of the peak intensity. All Raman spectra were further statistically analyzed through PCA to reduce the total data size and to improve data processing time.

SERS detection followed by statistical analysis data processing proved to be a very efficient strategy to detect and compare similar pathogens that exhibit few biological differences that escape the common detection methods. S. Hu and coauthors reported a 100% accuracy detection and differentiation method for the two most common fungi species that cause cryptococcosis among humans, *Cryptococcus neoformans* and *Cryptococcus gattii* (Hu et al., 2020). The authors observed that the two fungi strains have a negative surface charge, hence they employed positively charged spherical AgNPs, that could self-assemble on the fungal cell wall surface through electrostatic attraction, as SERS substrate. Based on the obtained SERS spectra, some minor differences could be observed between *C. neoformans* and *C. gattii*, therefore they applied a classification method to distinguish the SERS spectra of the two fungi species.

While some studies focus on comparing different nanostructures as SERS enhancements (Witkowska et al., 2019), other studies focus on SERS detection and identification of human pathogens (Witkowska et al., 2016). Four human fungal pathogens species, such as *Aspergillus flavus*, *Trichophyton rubrum*, *Candida krusei,* and *Scopulariopsis brumptii* were investigated by combining AgNPs/FTO-based SERS technique with PCA statistical analysis not only to distinguish the four fungi strains, but also to detect them in human skin samples. They used the PCA technique to extract biochemical information from each fungal species and to perform statistical classifications of the investigated microorganisms together with identifying them in clinical samples. The submitted research provided a successful SERS-based and PCA method to detect, identify, and discriminate four fungi strains with 98% accuracy.

Another very efficient strategy to detect and discriminate between fungal strains was reported by Z. Guo et al. (2021). The authors reported a label-free SERS detection technique combined with four pattern recognition statistical analysis methods to identify and accurately discriminate between five fungi strains, responsible for apple corruption and spoilage (Fig. 4). *Penicillium paecilomyces*, *Penicillium chrysanthemum*, expanded *Penicillium expansum*, *Aspergillus niger,* and *Alternaria* were mixed with gold nanorods (AuNR) and left interacting for 3h. After that, SERS spectra were collected and all spectra data was statistically analyzed *via* all four pattern recognition techniques. The proposed design was able to successfully identify all five apple spoilage fungi strains and the fungi fingerprints were discriminated with an excellent accuracy of 98.31%.

Going one step forward, T. Szymborski et al. proposed a new and innovative SERS detection platform modified with a femtosecond laser (Szymborski et al., 2021). The proposed design overcame the drawback of colloidal SERS substrates that do not present stability in their homogeneity over time, by treating a silicon substrate with a femtosecond laser. Therefore, by modifying the surface of the silicon substrate with the femtosecond laser, the ablation process occurs, making the surface vastly uniform and homogenous. Further, the Raman signal was

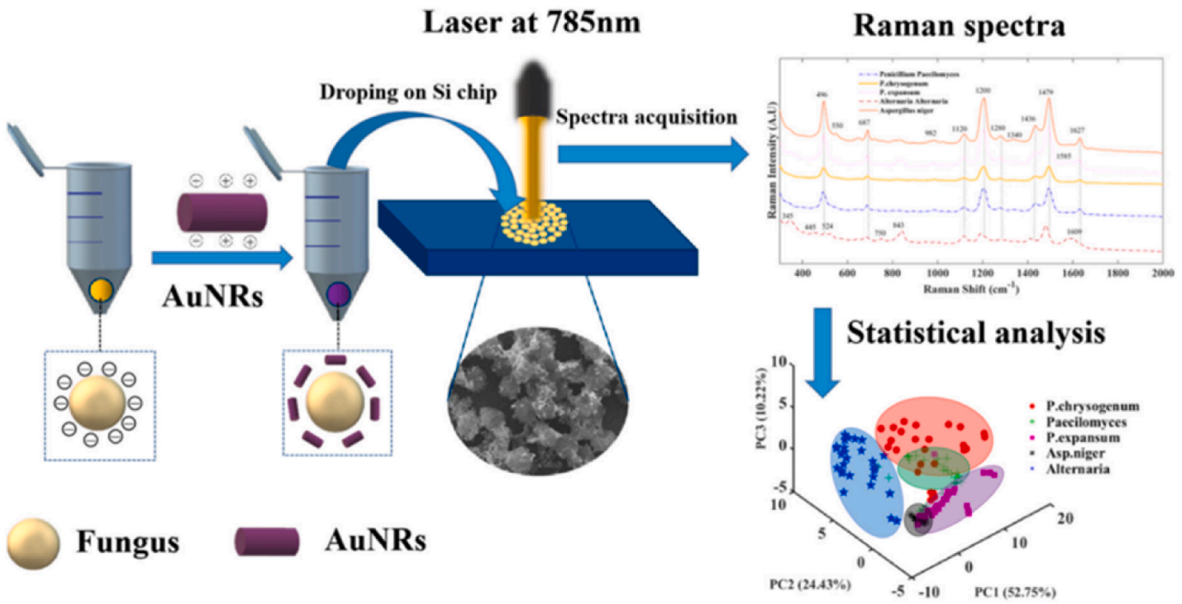

Fig. 4. Schematic illustration of SERS measurements on fungus using gold nanorods. The positively charged AuNRs are firstly adsorbed on the fungus cell wall by electrostatic attraction. Then the obtained SERS spectra were analyzed by PCA and other algorithms for the discrimination of five dominant apple spoilage fungi (Guo et al., 2021).

enhanced with a 10^8 factor by conjugating a 100 nm layer of silver on the modified silicon surface. Proceeding with this technique, the authors investigated three strains of human opportunistic fungal pathogen *Candida* spp. – *Candida glabrata*, *Candida albicans* SN148 and *Candida albicans* BWP17. Making use of the SERS spectra of each Candida spp., the authors employed chemometric analysis – PCA statistical analysis – to identify and distinguish the three fungi strains. The statistical analysis successfully discriminated the *C. glabrata*, *C. albicans* SN148, and *C. albicans* BWP17 with a 97% accuracy. Moreover, the SERS-based detection method combined with PCA analysis proved to accurately discriminate the three fungal strains from Gram-positive bacterial samples with *Staphylococcus aureus*, with a successful indication rate of 83% between fungi and bacterial samples.

3.3. Colorimetric detection

Colorimetric assays detect the analyte by observing a color change perceptible by the naked eye. Colorimetric detection methods show the advantages of being cost-effective, providing a fast response, while not requiring specialized personnel. Therefore, they are highly desired and reliable detection methods in operational fields. Plasmonic NPs have attracted a lot of attention for the development of colorimetric biosensors for several reasons: i) they absorb light in the visible wavelength range with a high extinction coefficient. It means that a very low concentration of plasmonic NPs is sufficient to appreciate a color change; ii) due to the LSPR phenomenon, plasmonic NPs are extremely sensitive to the alteration of their chemical environment and the variation of their interparticle distance or morphology. Indeed, the variation of interparticle distance determines plasmon coupling effects between neighboring NPs, which turns into a drastic color change. Third, the etching or growing plasmonic NPs can also trigger a color change. The etching/growing process can be promoted or hindered by the presence of a specific molecule in the assay design. The fourth reason behind the success of plasmonic NPs in developing colorimetric biosensors is their high surface-to-volume ratio and their possibility to modify their surface chemistry by chemical exchange procedures or by covalent bonding. Such a feature allows the functionalization of the surface of plasmonic NPs with other molecules of interest (e.g., biorecognition elements), suitable for detecting the desired analyte. Due to the strategic surface functionalization, the analyte can induce electrostatic, hydrophobic, or covalent interactions. This leads to a color change promoted by plasmon coupling phenomena caused by NP aggregation or assembly processes (Petronella et al., 2022; Tang and Li, 2017). Although numerous colorimetric biosensors, with remarkable sensitivity, recovery, and speed, have been developed to become established diagnostic tools (Fig. 5), only a small percentage is available in the market (Maddali et al., 2021). This could be attributed to challenges and drawbacks such as lower sensitivity compared to ELISA and PCR techniques, and the complexity involved in accurately quantifying concentrations of the

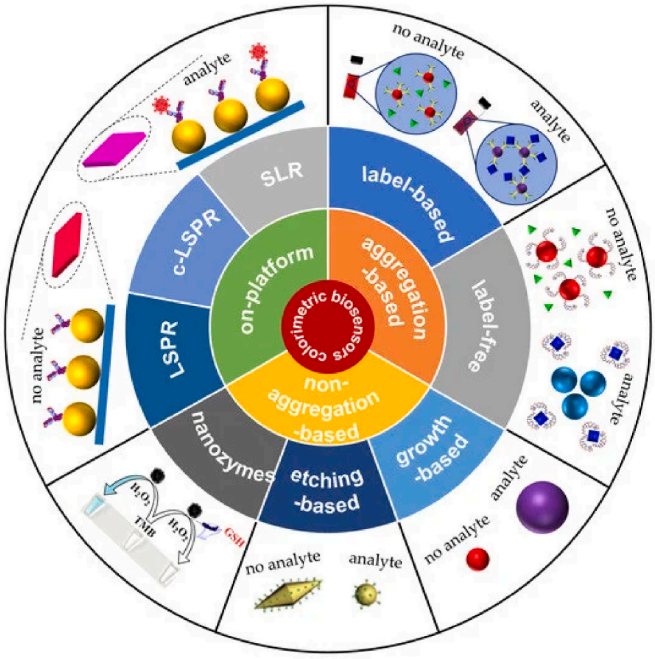

Fig. 5. Schematic illustration classifying the plasmonic-based colorimetric biosensors into on-platform-based and colloid-based types, including both aggregation-based and non-aggregation-based approaches (Acunzo et al., 2022).

target analytes. Hence, continuous efforts are needed to enhance the practicality and reliability of these devices (Acunzo et al., 2022).

Y. He et al. realized an ingenious procedure for the colorimetric detection of OTA, achieved by generating Mn^{2+} that induces the aggregation of AuNPs (Y. He et al., 2020). The authors loaded the surface of magnetic beads (MBs) with an OTA (OTA_{apt}), a biotinylated complementary DNA (or cDNA, a DNA that hybridizes the OTA aptamer), and streptavidin-modified alkaline phosphatase (SA-ALP). ALP is an enzyme that converts the ascorbic acid 2-phosphate into ascorbic acid. The presence of OTA determines the generation of an OTA-OTA_{apt} complex and the consequent release of the ALP-cDNA. At this stage, the ALP-cDNA is magnetically separated from the MBs, and the ALP enzyme is free to reduce the MnO_2 in Mn^{2+}. As sketched in Fig. 6. **Left,** Mn^{2+} ions determine the aggregation of AuNPs and, therefore, the appearance of blue color in the solution. The proposed procedure is suitable for qualitative identification of OTA and quantitative OTA detection, evaluated considering the decrease of AuNPs plasmon intensity as a function of the OTA amount. Interestingly, the proposed biosensing system showed a LOD of 5 nM.

Fusarium species produce, through their metabolism, another mycotoxin, the *Zearalenone* (ZEN), that an AuNPs-based colorimetric aptasensor can identify. The procedure relies on a catalytic reaction involving the 4-nitrophenol and AuNPs functionalized with a thiol-modified oligonucleotide sequence (CS) (Taghdisi et al., 2018). The reaction involves the utilization of a walking aptamer (selected for the ZEN identification) having a sequence complementary to the ZEN aptamers. In the absence of ZEN, the walking aptamer hybridizes with the CS on the AuNPs surface, forming a double-stranded DNA (dsDNA). When the Exonuclease III (EXOIII) is introduced, it digests the 3′-terminus of dsDNAs so that the walking aptamer can bind onto other strands of the CS, generating a second dsDNA that, in turn, is degraded by the EXOIII to remove the CS from the AuNPs completely. The surface atoms of the AuNPs are now free to reduce the 4-nitrophenol to the 4-aminophenol, thus determining the disappearance of the yellow color associated with the 4-nitrophenol, turning the batch colorless. Conversely, when the analyte solution contains the ZEN, the latter takes up the walking aptamer due to the high affinity between the aptamer and the target molecule. In this condition, the surface of AuNPs is still covered by the CS that cannot be chemically modified by the EXOIII, as it reacts only with dsDNA. Therefore, the 4-nitrophenol cannot be reduced by the Au and the solution's color keeps its yellow hue (Fig. 6. Right).

A further colorimetric method for ZEN identification was developed by S. Sun et al., who exploited the peroxidase mimic activity of AuNPs towards the 3,3′,5,5′-tetramethylbenzidine (TMB) that turns from colorless to blue in the oxidized state (Sun et al., 2018). In this assay, the AuNPs surface is functionalized with the ZEN aptamer that occupies their surface-active sites, preventing the TMB oxidation by the surface Au atoms. The aptamer is removed from the AuNPs surface when the ZEN-ZEN aptamer complex is generated. Accordingly, the surface of AuNPs is unshielded and free to oxidize the TMB, causing a color change. Such a strategy provides a 10 ng/mL LOD and was used to detect ZEN in corn and corn oil samples.

Moreover, yet another interesting colorimetric method was designed to detect the spores of *Penicillium Italicum*, a mold that damages citrus production during storage and transportation (Yazgan et al., 2018). The development of this colorimetric test relies on the use of AuNPs suitably synthesized to load sugar derivatives on their surface (s-AuNPs). Such sugar derivatives exhibit intense activity toward specific fungal lectins, thus providing specificity. The colorimetric test involves immobilizing fungal spores on a glass surface and introducing s-AuNPs stabilized with different sugar derivatives. First, the glass substrate is activated with a silane to bind a 4-(N-Lactosyl) aniline, a ligand that captures the *P. Italicum* spores, promoting immobilization on the substrate. The immobilized spores were stained with s-AuNPs and rinsed toughly at this stage. The rinsing step removes the s-AuNPs having a surface functionalization unsuitable for binding *P. Italicum* lectins so that AuNPs that carry the sugar derivative can bind the *P. Italicum* spores and remain immobilized on the glass slide, providing a peculiar pinkish color.

3.4. Lateral flow immunoassay

Lateral flow assays (LFA) are a class of biosensing systems whose readout is essentially the appearance of color indicating the presence of the target analyte. LFA are the ideal PoC biosensors for their rapid response times, reduced cost, and extremely simple use. Indeed, LFA is used as a diagnostic tool in many applications such as medical diagnosis, agriculture, food control, forensic science, and military (Jiang et al., 2019). Even though LFA represents one of the simplest, quickest, and most cost-effective PoC diagnostic platforms, its potential has yet to be fully realized. Despite the development of various LFA detection devices, none has achieved sensitivity and quantification levels comparable to traditional methods such as ELISA and PCR. Generally, conventional colorimetric LFA is approximately 1000-fold less sensitive than the traditional standard laboratory tests, needing additional confirmatory

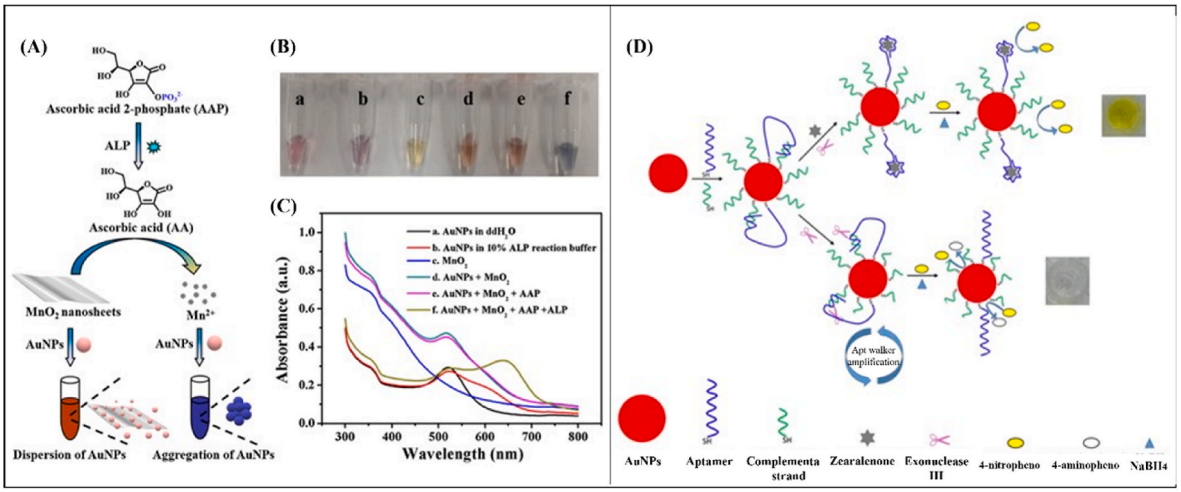

Fig. 6. Left: (A) Schematic of colorimetric detection of OTA achieved by the salt aggregation of AuNPs in the presence of the OTA aptamer and its complementary DNA strain. **(B)** Photograph of the six samples analyzed and their corresponding color. **(C)** Corresponding absorption spectra of the above six samples (Y. He et al., 2020). **Right: (D)** Colorimetric assay for detecting the ZEN realized by using a walking aptamer as a biorecognition probe. The reduction of 4-nitrophenol by the surface Au atoms promotes the color change (Taghdisi et al., 2018).

testing in a laboratory setting to accurately confirm negative results (Gupta et al., 2022).

In LFA, the analyte flows through a membrane by capillary action. In the general scheme, such a membrane embeds three pads: the sample, the conjugation, and the adsorption pad. The analyte is collected in the sample pad where, in some cases, it undergoes a pretreatment. Then, the sample migrates, by capillary, toward the conjugation pad where the probe system (that can contain the specific probe biomolecules and NPs) is immobilized to form the complex prompt for the visual detection of the analyte. After that, the mixture flows towards the test zone, namely a membrane where the affinity assay takes place because it embeds primary biorecognition molecules. Finally, the adsorption pad collects the excess fluid (Mak et al., 2016). The sample pad works to ensure that the analyte is in the optimal chemical-physical condition to be tested according to the LFA design, while in the conjugation pad, the first analyte-bioreceptor interaction takes place and induces the release of the labeled detection bio-receptors that reach the test pad where they bind the capture bioreceptor, generating the line that can be visualized by the naked eye or by an optical reader (Parolo et al., 2020).

A. O. Abdelrazig et al. developed an LFA to detect *Colletotrichum truncatum* - a fungus affecting chili seeds (Abdelrazig et al., 2021). The LFA is designed to identify the actin DNA of the *Colletotrichum truncatum* by using AuNPs, conjugated with the complementary sequence of the actin DNA, as a detection probe (AuNPs-DNA). The capture probe consists of mesoporous silica NPs that are modified with a polyelectrolyte multilayer to load an avidin-tagged DNA sequence (the MSN-capture probe). The AuNPs-DNA were immobilized on the hybridization area of the Fusion 5 Paper, and used as LFA support, while the MSN capture probe was deposited on the test zone of the Fusion 5 paper. When the solution containing the DNA target diffuses across the Fusion 5 paper by capillary, it hybridizes with the AuNPs-DNA, giving rise to an AuNPs-dsDNA complex that, when interacting with the MSN-capture probe, develops an intense red color, due to the generation of a sandwich DNA hybridization complexes (MSP-dsDNA-AuNPs). The excess of the conjugate continues to migrate until the MSP captures it in the control zone. The genetic material of *Colletotrichum truncatum* is therefore transduced in the appearance of an intense color. The photographic images of the Fusion 5 paper at different actin DNA concentrations were converted into gray-scale images and analyzed using the ImageJ software, thus allowing a quantitative correspondence between the *Colletotrichum truncatum* DNA amount and the color intensity.

Another worthwhile AuNPs-based LFA biosensor, valuable in the field of agriculture, was designed to detect the *Phytophthora infestans* a fungal pathogen that late blight in potatoes and tomatoes (Zhan et al., 2018). In particular, this biosensing system integrates universal primer-mediated asymmetric PCR, to obtain an optimal amount of *single-stranded DNA (ssDNA)* and the LFA platform (Fig. 7). In the LFA platform, AuNPs, functionalized with a thiolate DNA probe, were deposited in the conjugation pad. At the same time, the test line and the control line were prepared by depositing on a nitrocellulose membrane two streptavidin-biotinylate capture probes (T and C, respectively) at a distance of 6 mm. The ssDNA from the PCR moves, by capillary, to the conjugate pad where it hybridizes with the probe DNA loaded on the AuNRs surface. The resulting complex reaches the nitrocellulose membrane where, in the test area, it interacts with the streptavidin-biotinylated capture probe T and C. Here, if the DNA-modified AuNPs have been coupled with the DNA of the *Phytophthora infestans*, the hybridization between the complex and the streptavidin-biotinylated capture probe T occurs with the consequent development of an intense red band determined by the accumulation of AuNPs. The excess of the complex migrates to the control line, generating a further red line due to the hybridization with the streptavidin-biotinylated capture probe C.

LFA was also investigated as a diagnostic tool for human fungal infections. One of the most concerning fungal infections is the candidiasis provoked by the *Candida albicans*. An AuNPs-based LFA was developed

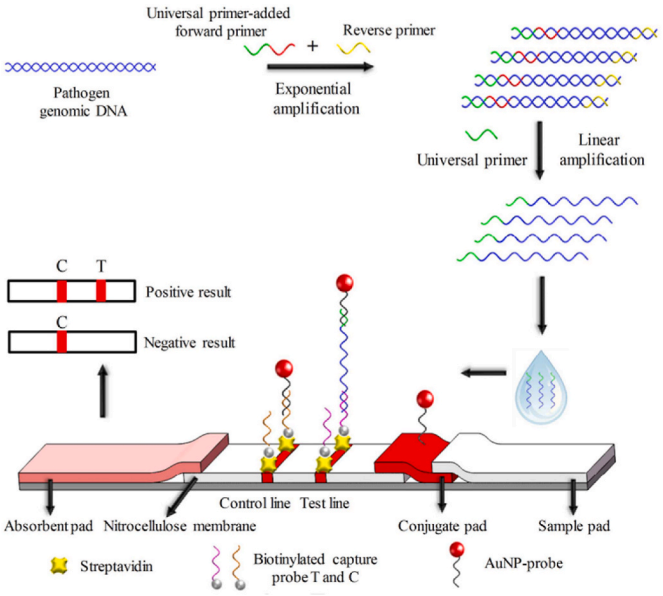

Fig. 7. Schematic illustration of the operating principle of LFA developed for detecting the genomic DNA of *Phytophthora infestans* (Zhan et al., 2018).

by Z. X. He et al. (2016). The biosensor identifies an antibody developed upon *Candida albicans* enolase infection. In this case, AuNPs were functionalized with human IgG, resulting in a colored probe system. Recombinant enolase and goat anti-IgG were immobilized in test and control lines of the Nitrocellulose membrane. The serum sample containing the Candida antibody was deposited on the sample pad and recognized as an anti-human IgG antibody on the surface of AuNPs (30 nm in size), generating a complex. Then, the complex migrated on the test pad, where the recombinant enolase captures it, thus giving rise to a red band. The excess of AuNPs-IgG-anti-IgG absorbed in the control line provided a second red line (positive test). Only one red line in the LFA area proved a negative test.

Test strip biosensors show effectiveness and advantages analogous to LFA assays. Y. Wu et al. exploit the size and shape-dependent optical properties of AuNPs to give rise to an immunochromatographic test strip biosensor for the multiplex analysis of four mycotoxins - *FumonisinB1* (FB1), ZEN, OTA, and *aflatoxinB1* (AFB1) - contaminating corn and corn related product (Wu et al., 2020). To this end, authors synthesized four kinds of AuNPs with different morphology: gold nanospheres (AuNSs, red); gold nanocacti (AuNCs, purple), gold nanoflowers (AuNFs, blue), and hyperbranched AuNPs (AuPBs, black). Each NP was bioactivated with an antibody, specific to identify the respective mycotoxin. In this colorimetric strip test, the identification of the target analyte is not achieved by the development of an intense color. However, the biosensor is conceived to provide a coloration as pale as the concentration of the target analyte increases. Indeed, the four conjugation pads contain AuNPs functionalized with monoclonal antibodies (MAbs), thus producing the bioconjugates AuNPs@mabs. The bioconjugates are embedded on four distinct T lines, because of the competitive antigen pre-immobilized on each T line respectively. From a visual standpoint, the lines with a different color and a different contrast appeared on the four T lines. In this configuration, the target mycotoxin generates the complex AuNPs@mab-mycotoxin. When the antigen complex is sprayed on the T line, the generation of AuNPs@mab-antigen complexes is hindered, because the mabs Fab sites are occupied by the mycotoxin. Consequently, as the mycotoxin concentration increases, the occurrence of the colored AuNPs@mab probe decreases, thus decreasing the color intensity, as represented in Fig. 8.

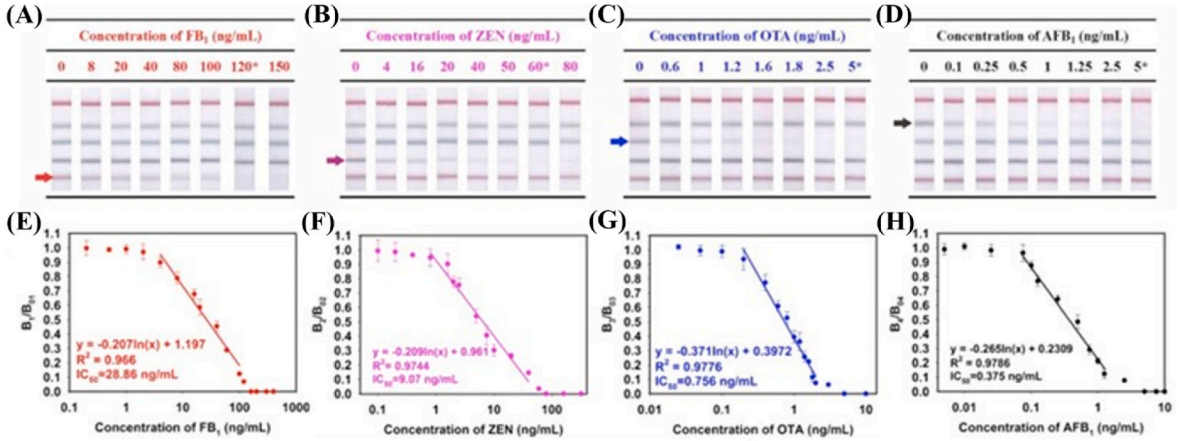

Fig. 8. Strip tests of the multiplex colorimetric detection of four mycotoxins realized using antibody-functionalized AuNPs with different morphologies – therefore the different colors. The color intensity decreases as the mycotoxin concentration increases. Stereograms (A–D) and calibration curves (E–H) were obtained by measuring the optical density (Wu et al., 2020).

3.5. Spectroscopic detection methods (absorbance and fluorescence detection)

Absorption and fluorescence spectroscopies are among the most diffused read-out techniques for the design of plasmonic NP-based biosensors. In the field of sensing and biosensing, as for the SERS, the widespread use of absorption spectroscopy relies on the high refractive index sensitivity of plasmonic NPs to alteration of their surrounding medium, as described by the Ganz theory (Mayer and Hafner, 2011) and confirmed by the considerable number of literature papers reporting LSPR sensors and biosensors, exploiting the shift of the plasmon band as a function of the refractive index variations (Chang et al., 2023; Petronella et al., 2022; Solra et al., 2020; Zaccagnini et al., 2023; Ziai et al., 2022a, 2022b). The attention collected by absorption spectroscopy in LSPR biosensor development is driven not only by the affordability of the instrumentation and by the user-friendliness, but also by the needlessness of fluorescent labels or enzymes to catalyze specific chemical reactions as in the case of colorimetric biosensors. Indeed, the plasmonic probes for absorption spectroscopy consist of the plasmonic optical transducers (Ag, Au, Cu NPs) suitably bio-conjugated with a bio-recognition element (nucleotide sequence, protein, peptide, antibody) providing specificity and selectivity. However, the LSPR-based biosensors also present some drawbacks, particularly in their ability to discern between specific and non-specific interactions with the sensor surface. Even after thorough washing, some non-specifically bound components may remain, requiring the use of reference material or control samples for correction (Ahmed et al., 2010). Moreover, fluorescent-based assays frequently encounter challenges due to their weak signal at low analyte concentrations, necessitating the use of sophisticated, expensive, and bulky instruments to uphold high detection sensitivity (Semeniak et al., 2023).

Absorption spectroscopy was also used for fungal pathogen biosensing through the optical determination of mycotoxins. Most biosensing platforms, based on plasmonic NPs for fungi/mycotoxin determination, find applications in food processing and agriculture.

The OTA mycotoxin was optically detected by using AuNSs as optical transducers. As the refractive index sensitivity of AuNSs is limited concerning one of the anisotropic metallic NPs, the LSPR wavelength shift occurs only upon AuNS aggregation. For this reason, the paper of B. Liu et al. (2018) reported the spectroscopic OTA detection and quantification, mediated by AuNSs, by playing on the salt aggregation (induced by NaCl) of AuNSs functionalized with an OTA aptamer (OTA_{apt}), in the presence of OTA molecules. In particular, AuNSs stabilized by the OTA_{apt} (AuNSs-OTA_{apt}), in the presence of NaCl preserve their colloidal stability because of the shielding action ascribable to the oligonucleotide on the AuNSs surface. Consequently, their LSPR peak keeps its wavelength at 520 nm and its absorption intensity (A_{520}). When OTA and NaCl are introduced in the reaction batch, the OTA-O-TA_{apt} complex forms, thus partially removing OTA_{apt} from the AuNSs surface. Therefore, the AuNS surface starts partially losing its colloidal stability, due to the effect of NaCl. The NaCl, indeed, triggers the salt aggregation by competing with citrate functional groups that instead ensure colloidal stability to AuNSs. The gradual removal of OTA_{apt} from the AuNSs surface and the consequent partial aggregation of AuNSs, proven by TEM and DLS analysis, give rise to a further optical signal at 630 nm, having a defined absorption intensity (A_{630}). Hence, the authors detected a linear relationship between the logarithm of OTA concentration and the absorption intensity ratio (A_{630}/A_{520}). In particular, as the OTA concentration increases, the A_{630}/A_{520} increases, due to AuNSs aggregation. Interestingly, for OTA concentrations higher than 10^{-8} g/mL, the A_{630}/A_{520} ratio decreases with the increase in OTA concentration. Such a phenomenon is explained considering that for low OTA concentration, a fraction of OTA_{apt} remains on the AuNSs surface protecting NPs from complete aggregation, while for higher OTA concentration all the OTA_{apt} are involved in the generation of the OTA-OTA_{apt} complex inducing the complete AuNSs aggregation.

An alternative absorption spectroscopy method for OTA detection, exploiting AuNPs as optical transducers, was proposed by B. Lee et al. (2018). In their work, the authors developed OTA aptasensors using gold nanorods (AuNRs), instead of AuNSs, because of the high sensitivity of anisotropic plasmonic NPs to refractive index variation. To develop a biosensor with a lower detection limit, and enable its utilization in the operational field, the AuNRs were immobilized on an optical fiber, and then the AuNR optical fiber was bioactivated with a thiolate OTA_{apt}. The biosensing performance was evaluated by dipping the bioactive optical fiber in a solution containing OTA and measuring the absorption spectrum after a defined incubation time, as schematized in Fig. 9. Top. The shift of the longitudinal LSPR (more sensitive to alteration of the chemical environment concerning the transverse LSPR) increases linearly with the OTA concentration. Such spectroscopic detection method showed specificity and was also applied to a real sample of grape juice obtaining spectroscopic shifts comparable to the ones measured for standard OTA solutions, considering also the matrix effect.

A similar procedure for OTA detection was previously reported by J. H. Park et al. (2014). In this case, the biosensing support was a flat glass slide instead of an optical fiber. The resulting system reported a LOD of less than 1 nM, while also being reusable up to seven cycles.

Among the mycotoxins produced by the metabolism of *Fusarium*, the *Deoxynivalenol* (DON), is a worldwide food contaminant that can cause several undesired effects on human health. An absorption spectroscopy

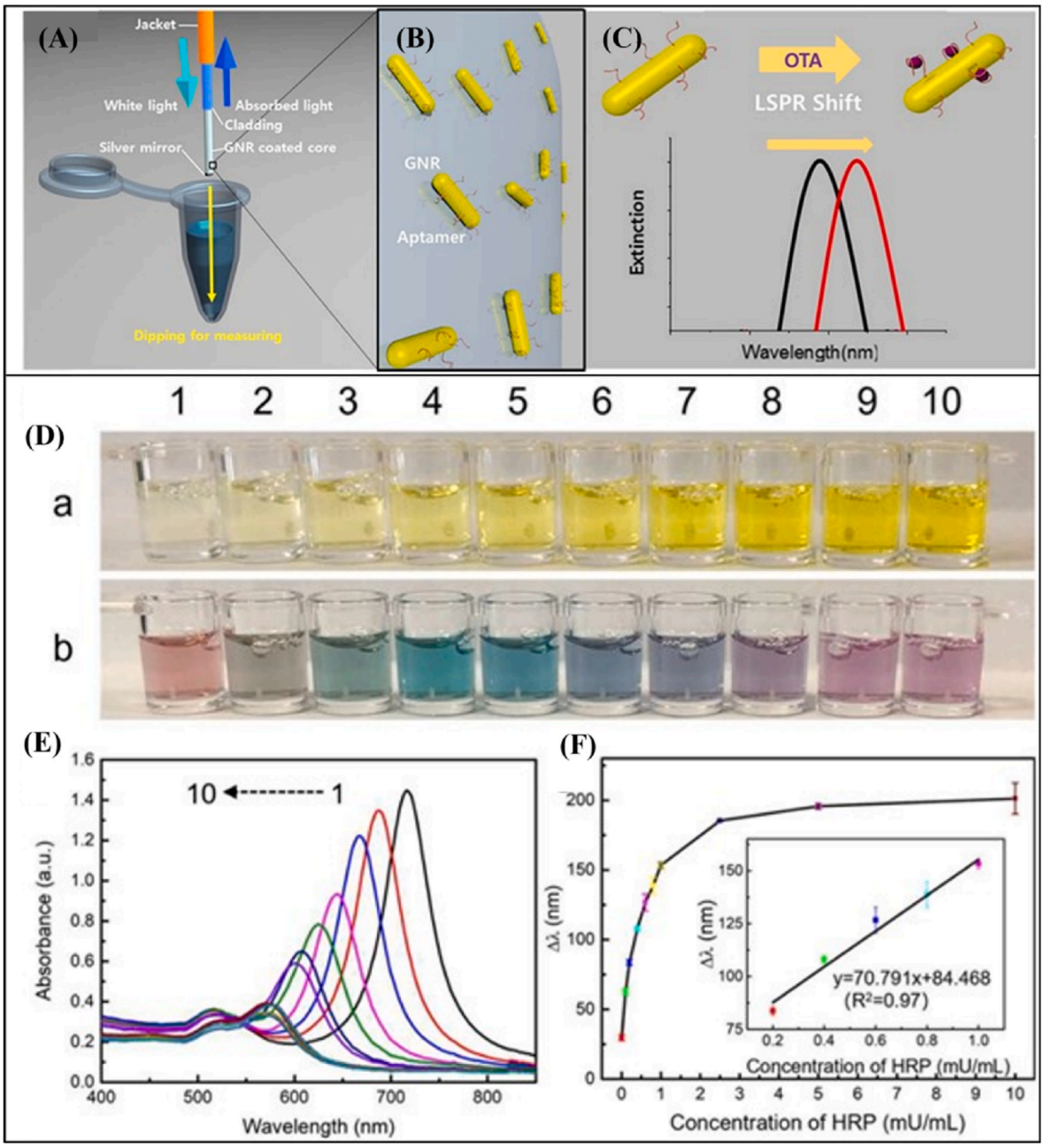

Fig. 9. Top: Schematic of the LSPR-based method for the detection of OTA, based on the functionalization of an optical fiber with AuNRs. The assay uses a thiolate OTA_{apt} as a biorecognition element (Lee et al., 2018). **Bottom:** Refractometric analysis of DON based on the change of the absorption spectroscopy features of AuNBPs as a function of their morphology. The assay is designed so that the presence of the DON promotes the generation of the TMB^{2+} that etches the AuBPs determining a well-perceptible color change that depends on the DON concentration (Guo et al., 2023).

assay for the detection of DON was recently developed using gold nanobypiramids (AuBPs) as optical transducers. The assay enables the concomitant visual and spectroscopic detection of the DON thanks to the unique optoelectronic properties of AuBPs. Such nanostructures are characterized by two sharp and elongated tips that concentrate the EM field in an extremely localized area (De Sio et al., 2021). Moreover, the LSPR of AuBPs exhibits very narrow absorption bands. These features make AuNBP excellent optical transducers for absorption spectroscopy-based assays. This study used magnetic beads modified with a monoclonal antibody that shows affinity towards the DON as a DON carrier. Horseradish peroxidase DON antigen is then linked to the magnetic beads forming a complex that is captured by the TMB enzyme. As the horseradish peroxidase concentration increases, the oxidation rate of the TMB to TMB^{2+} increases, intensifying the yellow color of the solutions. When AuBPs are introduced in the solution, the TMB^{2+} etches the AuBPs, thus determining a well-perceptible color change (Fig. 9. Bottom), due to the variation of the AuBPs aspect ratio. The optical shift of the longitudinal LSPR of AuBPs was then correlated to the DON concentration by a linear regression that allowed authors to calculate a detection limit of 57.93 ng/mL (Guo et al., 2023).

As for the absorption spectroscopy-based biosensor, fluorescence-based biosensors provide a color change caused by an environmental or chemical alteration. While absorption spectroscopy measures the intensity of absorbed EM radiation at a specific wavelength, fluorescence spectroscopy quantifies the EM radiation, at a specific wavelength (emission wavelength λ_{em}) emitted by a molecule after the absorption of light at the excitation wavelength (λ_{exc}). Fluorescence spectroscopy is an analytical tool that is more sensitive than absorption spectroscopy and is

also affordable and user-friendly. Plasmonic NPs do not exhibit fluorescence emission, but as they possess a high extinction coefficient, they are often used as quenchers to design fluorescence-based biosensors (Kim et al., 2016; Lee and Kang, 2023).

However, metallic NPs with dimensions of 2–5 nm, known as metallic nanoclusters (NCs), possess fluorescence properties and, therefore, can be used as fluorescent labels (Kaushal et al., 2020; Li et al., 2014).

For OTA determination, X. Lv et al. used AuNPs as quenchers in a fluorescence recovery-based assay (Lv et al., 2017). Authors capped AuNPs with a fluorescent probe consisting of a suitable OTA_{apt} modified with the carboxyfluorescein (FAM), resulting in the OTA-FAM probe. The probe shows a λ_{em} at 520 nm that overlaps the LSPR of the AuNPs. The resulting OTA-FAM-capped AuNPs do not show fluorescence signals, as AuNPs absorb the photons emitted by the OTA-FAM at 520 nm. Such a phenomenon is known as fluorescence resonance energy transfer (FRET). The presence of OTA, due to the high affinity OTA-OTA_{apt} alters the conformation of the OTA-FAM. The consequent desorption of the OTA-FAM probe from the AuNPs surface, scratches the conditions determining the FRET, thus recovering the FAM fluorescence. The introduction of NaCl increases the fluorescence intensity because of the salt aggregation. Indeed, AuNP aggregates have fewer surface-active sites available for the OTA-FAM absorption. The decreased number of OTA-FAM probes on the AuNP surface weakens the FRET phenomenon. Such an assay enables OTA quantification by correlating the fluorescence recovery at 520 nm (the difference between the fluorescence intensity after and before the OTA introduction) to the OTA concentration. The linear correlation between these two quantities allowed authors to calculate a detection limit of 22.7 nM.

In another plasmonic NPs-based fluorescence assay, OTA was quantified through the in-situ synthesis of copper NPs that exhibit a λ_{em} at 625 nm (He et al., 2019). The scheme of such an assay utilizes a biotinylated OTA_{apt} hybridized with a DNA strand (bio-OTA_{apt}-DNA) as a biological probe. The probe is absorbed on streptavidin-modified magnetic particles through streptavidin-biotin recognition. The introduction of OTA, producing the OTA-OTA_{apt} complex, releases the DNA strand that is subsequently exploited as a primer for making a poly (thymine) strand. The production of the poly(thymine) strand is induced by the specific enzyme named deoxynucleotidyl transferase. The poly (thymine) strand is isolated from the reaction batch by applying a magnetic field to exploit as a stabilizing agent for producing luminescent CuNCs. CuNCs can be synthesized by the ascorbic acid's mild reduction of Cu^{2+} ions. As the OTA concentration increases, the amount of the released DNA boosts, and consequently, a poly(thymine) strand with a higher length is produced. The poly(thymine) length enhances the CuNCs' fluorescence intensity. Such a dependence allowed authors to correlate the CuNCs fluorescence intensity to the OTA concentration in a linear dynamic range from 2.5 nM to 250 nM with a detection limit of 2 nM.

Ratiometric assays are additional approaches for designing fluorescence-based biosensing systems, improved by plasmonic NPs. Such a biosensing strategy relies on the read-out of two fluorescence signals and shows the benefit of limiting the risk of a false positive response. The detection of OTA was achieved by a ratiometric fluorescence aptasensor recently published by H. Guo et al. (2022). The two fluorophores involved in this assay are a green emitting CuNCs (λ_{em} at 510 nm) and an orange-emitting molecule (the *N*-methyl mesoporphyrin IX or NMM, λ_{em} at 610 nm). In this assay, polydopamine NSs (PDANSs) were used as quenchers. The assay is conceived so that the increase of the OTA concentration decreases the intensity of green emission (I_{510}) and increases the intensity of orange emission (I_{610}). In this way, the ratio I_{610}/I_{510} value is linearly dependent on the OTA concentration. To achieve this goal, CuNCs were synthesized and functionalized with a DNA strand (cDNA) complementary to the one of the OTA_{apt}, so that the OTA_{apt} and the cDNA hybridize in a duplex structure providing a defined I_{510} value along with a weak I_{610} associated with the NMM. The PDANS do not affect the I_{610}/I_{510} ratio. The addition of OTA results in the formation of a G-quadruplex structure involving the OTA, and the OTA_{apt} (q-OTA_{apt}-OTA), upon the release of the OTA_{apt} from the duplex assembly with the cDNA. The NMM is an intercalating agent effective for the G-quadruplex structures. Accordingly, its intercalation in the OTA-OTA_{apt} complex determines an enhancement of the I_{610}. Such a condition promotes the absorption of the cDNA-CuNS on the PDANSs and the consequence quenching of the I_{510} value. This CuNC-based ratiometric aptasensor provides a linear dynamic range between 0.05 ng/mL and 100 ng/mL, and a detection limit of 0.045 ng/mL. Moreover, it was successfully used for determining OTA in several food samples, including wine, grape juice, and wheat.

In a different study, the AFB1 was detected by a FRET aptasensor (Wang et al., 2021). In this case, the selected plasmonic NPs are Ag nanocubes (AgNCB) that possess the property to quench the fluorescence of an aptamer-modified ZnS nanocrystal, which exhibits a blue emission around 430 nm. A thiol-modifying aptamer, able to hybridize AFB, was immobilized on the AgNCB surface *via* a thiol-Ag coordination bond. The AgNCB's unoccupied surface sites were saturated with the *6-mercapto-1-hexanol.* The cDNA is instead bound to the ZnS surface. In this configuration, a hybrid architecture AgNCB-APT-cDNA-ZnS is formed in a fashion that quenches the emission of the ZnS by FRET. The AFB, recognizing its aptamer, smashes the assembly of the AgNCB-APT-cDNA-ZnS, recovering the blue emission of the ZnS NPs.

A further plasmonic NP FRET configuration for AFB detection involves quantum dots as fluorophores and AuNPs as fluorescence quenchers (Xiong et al., 2023). The binding of the AFB to its target aptamer determines AuNP aggregation. The introduction of the quantum dots, due to electrostatic attraction between negatively charged AuNPs and positively charged quantum dots, quenches the emission intensity of quantum dots. The fluorescence quenching is as low as the concentration of the AFB increases.

3.6. Electrochemical detection

Electrochemical detection methods are widely used in biosensor design due to several advantages including sensitivity, selectivity, low cost, and fast response. Electrochemical detection methods are suitable for developing portable instruments, often integrated with smartphone apps, and therefore are highly useful for biosensor utilization operational environment. This is a relevant advantage for on-site detection of fungi or mycotoxin in contexts where they can constitute a health threat, such as hospital facilities, workplaces, and food production/supply chains.

One of the most investigated electrochemical analytical methods for fungi detection are voltammetry and amperometry which measure the current as a function of the applied potential (Lin et al., 2022). The biosensing performance is significantly affected by the features of the electrode materials. Plasmonic NPs are often used to increase the investigated methods' sensitivity. Indeed, plasmonic NPs increase the electrode surface area, achieving a high biomolecule loading on the electrode surface, improving the conductivity, and being biocompatible, preserve the chemical-physical properties of the probe biomolecule (and therefore its biological activity); finally, plasmonic NPs show excellent electrocatalytic properties (Sohrabi et al., 2022; Theyagarajan and Kim, 2023). However, there are a few drawbacks that make the realization of electrochemical sensors challenging, such as i) achieving a low LOD; ii) mitigating the non-specific adsorption of interfering substances; and iii) ensuring the reproducibility and stability of the biosensor within complex real matrices (Ferrag and Kerman, 2020).

Plasmonic NPs were extensively used for modifying electrodes and developing mycotoxin electrochemical biosensors with improved performance. To this end, W. Chen et al. demonstrated the ability of AuNPs to amplify the electrochemical signal of an aptasensor designed for detecting the OTA (Chen et al., 2018). The biosensing platform exploits two bioconjugates based on 20 nm AuNPs. The first (AuNPs-1) behaves

as a probe-bioconjugate, and the second (AuNPs-2) is an electrochemical signal reporter. AuNPs-1 consists of AuNPs linked to two oligonucleotides: a bridge probe that immobilizes the AuNPs-1 on a gold electrode and a capture probe (CP2) that is hybridized with the OTA aptamer. The AuNPs-2 is conjugated to a ferrocene (Fc) tagged SH-signal probe (SSP). In the presence of OTA, the biorecognition between the OTA and its aptamer induces the hybridization between the CP2 on the AuNPs-1 and the SSP on the AuNPs-2. Consequently, the AuNPs-2, carrying the Fc, is immobilized on the electrode surface. The AuNPs-2 immobilization dramatically amplifies the electrochemical signal associated with the OTA presence. The proposed AuNPs-based electrochemical platform displayed a LOD of 0.001 ppb and a linear range between 0.001 ppb and 500 ppb.

Fumonisin B1 is another mycotoxin produced by *Fusarium proliferatum* and *Fusarium verticillioides*. It is dangerous for food products and is considered a carcinogenic molecule by the IARC. The sensitive and rapid detection of Fumonisin B1 was achieved by L. Lu et al., who modified a screen-printed carbon electrode (SPE) with gold NPs and polypyrrole NPs deposited on a reduced graphene oxide film (Lu et al., 2016). Such a hybrid architecture (Fig. 10), provides the SPE improved biocompatibility, and a higher number of surface sites able to host the antibody, selected as biomolecular probes for developing such an electrochemical immunosensing platform. The authors used AuNPs to increase the electric conductivity of the SPE. The resulting electrochemical immunosensing platform allowed to detect the Fumonisin B1 by voltammetry with a detection limit of 0.2 mg/L. Interestingly the AuNPs modified SPE was successfully tested for the quantification of Fumonisin B1 in samples of spiked corn.

A further mycotoxin produced by the *Fusarium* is the ZEN. An effective method for detecting the ZEN was proposed recently by B. Zhou et al., who developed a molecularly imprinted electrochemical sensor on a glassy carbon electrode (Zhou et al., 2023). The electrode consists of oxidized graphene nanoribbons and AuNPs that were electro-deposited on a glassy carbon electrode. The authors claimed an interplay effect between AuNPs and graphene nanoribbons that amplifies the electrochemical signal. Moreover, differential pulse voltammetry measurements demonstrated a higher sensitivity for the detection of the ZEN, for the electrochemical sensor prepared with the molecularly imprinted polymer, concerning a similar sensor prepared with a non-molecularly imprinted polymer. The resulting sensor showed a LOD of 0.34 ng/mL.

The unique conductive properties of plasmonic nanostructures were also exploited to develop an electrochemical biosensor to determine human fungal pathogens classified by the OMS as high priority as *Candida albicans*. In particular, a gold layer was grown on iron oxide particles, resulting in plasmonic-magnetic core@shell architecture (da Silva-Junio et al., 2022). The resulting Fe_3O_4@Au heterostructure generated a self-assembled monolayer on a gold electrode to detect *Candida Albicans* and *Candida tropicalis* by cyclic voltammetry and impedance spectra. In this work, the Temporin-PTA peptide was used as a biorecognition molecule. The discrimination among the investigated microorganisms relies on the different absorption to the Fe_3O_4@Au electron, which resulted in a different electrochemical signal.

Beyond producing carcinogenic mycotoxins, the *Aspergillus* also causes invasive aspergillosis. AuNPs were used for developing an electrochemical biosensor as a diagnostic tool for aspergillosis. The biosensor can detect the occurrence of genetic materials associated with the *Aspergillus*. The readout of the oligonucleotide hybridization is realized by determining the electrochemical signal of the toluidine blue as a suitable intercalating agent and indicator molecule. In this work published by I. Bhatnagar et al. (2018), the electrochemical performance of an Au electrode was improved by assembling a monolayer of AuNPs stabilized by the chitosan molecules. The electrode characterization demonstrated that AuNPs improve the electron transfer ability of the electrochemical probe.

3.6.1. *Summary tables*

Fungal infections are an important health affection requiring accurate, early, and efficient detection. Late diagnosis, as well as misdiagnosis, compromise the patient's health. In this section, we report significant progress toward using spectroscopic methods, electrochemical, and various biosensing, and biodetecting systems as promising tools for rapid and accurate detection of various fungal pathogens. The recent advances in the development of noble metal NPs show promising results as SERS enhancement, as they reported good stability and excellent reproducibility. Hence, a large diversity of SERS detection platforms was exploited. They overcame the challenge of detecting and discriminating fungi strains in a time-dependent, cost-effective, selective, and sensitive manner. Moreover, low-cost, sensitive, user-friendly, and affordable are just a few advantages that characterize the colorimetric and spectroscopic detection methods, placing them at the top of the list for fast and reliable fungi strain detection techniques. Table 2 characterizes the advantages, as well as the limitations, of each detection method described above. Furthermore, for a detection method to be effective, WHO implemented a set of characteristics and guidelines that these methods need to hold – the ASSURED criteria (Otoo and Schlappi, 2022): Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free and Deliverable to end-users. Table 3 classifies the fungi strains mentioned above, each with what device was detected, the limit of detection (LOD) or sensitivity, and finally, for each detection method we mentioned which ASSURED criteria are met and which are not.

4. Integrated microfluidic point-of-care devices for fungi recognition

Rapid detection of fungal infections with high accuracy is essential, while time-effective and sensitive detection methods urgently need to be developed. To precisely choose the correct treatment, and avoid risks to the patient's life, it is essential to rapidly identify pathogens for an early

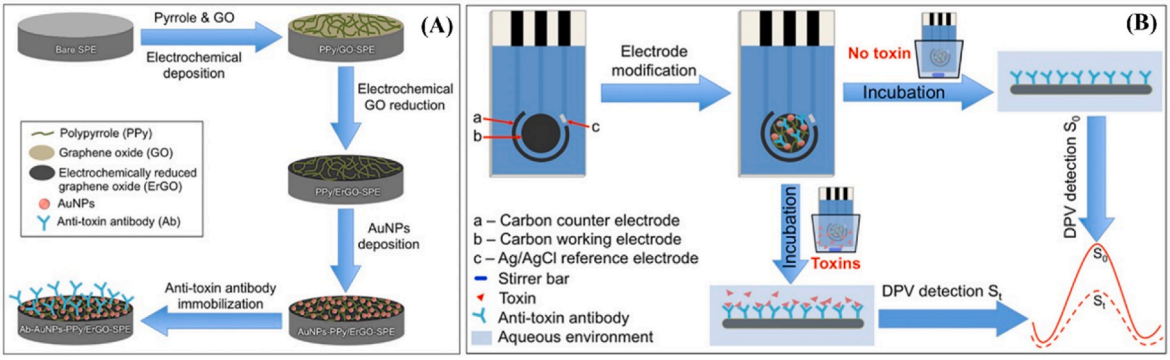

Fig. 10. (A) Sketch of the modification of the screen-printed carbon electrode with Au NPs and the subsequent bioactivation with the antibody specific against the target mycotoxin *Fumosin* B1. **(B)** Schematic of the procedure followed for the electrochemical immunosensing of the *Fumosin* B1 (Lu et al., 2016).

Table 2
The five detection methods described above, each with several known advantages and limitations in fungal species detection and discrimination.

Detection Method	Advantages	Limitations	Ref.
SERS	Fast response Non-invasive High sensitivity High specificity Reliable Multiplexing potential	For good LOD/ sensitivity and good discrimination → the need to combine SERS with statistical analysis methods Costly Sample complexity Low quantification of the concentration The need for SERS substrate to enhance the Raman signal	(Ambartsumyan et al., 2020; Xia et al., 2022)
Colorimetric detection	Simplicity Cost-effective Fast response Not requiring specialized personnel Visual response	Low specificity Limited sensitivity Sample Interference Lack of detailed information No discrimination between fungi strains Need of fluorescent labels/enzymes to catalyze specific reactions	(Acunzo et al., 2022; Petronella et al., 2022; Tang and Li, 2017)
Lateral-Flow Immunoassay	Ideal Point-of-Care biosensor Fast response Cost-effective Extremely simple use Portable Low sample volume	Limited sensitivity Low specificity Lack of detailed information Low quantitative capability	(Jiang et al., 2019; Gupta et al., 2022; Jeon et al., 2024)
Spectroscopic detection methods (absorption and fluorescence spectroscopy)	Affordable instrumentation User-friendly High selectivity Great specificity Molecular information Non-invasive Real-time monitoring	Sample preparation Spectra complexity Photobleaching Environmental effects Limited structural information Quantification challenges	(Ahmed et al., 2010; Chang et al., 2023; Petronella et al., 2022; Semeniak et al., 2023; Solra et al., 2020; Zaccagnini et al., 2023; Ziai et al., 2022a, 2022b)
Electrochemical detection	High sensitivity High selectivity Cost-effective Fast response Portable	Sample complexity Electrode functionalization Specialized instrumentation	(Ferrag and Kerman, 2020; Lin et al., 2022; Sohrabi et al., 2022; Theyagarajan and Kim, 2023)

diagnosis and treatment plan. Moreover, it has been determined that, for a successful treatment with high chances of recovery, antifungal medication should be administered in the first 6 h after symptoms occur in patients (Fuchs et al., 2019), thus rapid detection and identification of fungi strains is essential. Current clinical applications involve techniques related to the conventional cultivation of cell culture, the gold standard for identifying fungal infections. However, this conventional method has many disadvantages, such as a high delay in diagnosis and also a significant incidence of false-negative outcomes (Reinhart et al., 2012). Plasmonic Lab on a Chip (LoC) devices are a class of advanced, cost-effective, and efficient biosensing devices able to rapidly detect and identify various pathogens. Thanks to their unique features, they represent the new perspective of novel PoC diagnostic tools. Hence, LoC technology implies the miniaturization and implementation of common laboratory analysis into a single, portable, and easy-to-use sensor device, able to perform multiple analyses at once without the need for qualified personnel and a fully equipped laboratory (Atkins et al., 2023; Morris, 2013). The LoC technology is currently being employed in various domains, from biosensing to biotechnology and microfluidics, having a broad range of possible applications, such as premature molecular diagnosis, cell culture analysis, and even DNA/RNA detection and amplification (Jiang et al., 2017). Moreover, plasmonic paper-based devices own impressive properties: i) high specific surface area; ii) great flexibility; iii) robustness, and iv) versatility, making them ideal tools for detecting low concentrations of target samples (Kim et al., 2018; Lee et al., 2011).

Driven by further improvement of the LoC properties, integrated microfluidic devices have been developed in the last few years. Microfluidic systems are automated sensing devices, designed with microchannels that allow low volume of liquid samples – femtoliters – to flow in specific patterns, for drug interactions with various cells or specific molecules and drug efficiency studies, as well as for studying various biological interactions (Nasseri et al., 2018). These microfluidic automated systems possess miniaturized components, from micro-pumps to micro-scale inlet valves and outlet drains (Sin et al., 2013). The rapid analysis time, extremely small sample volume, and the real-time detection of pathogens are only a few advantages that make microfluidic devices attractive biosensing tools (Nikoleli et al., 2018). Not only are they ideal sensing devices for pathogens, but they also gained interest in various domains such as chemistry, microbiology, the food industry, and the military (Campion et al., 1980; Jeong et al., 2018; Sackmann et al., 2014; Zhang et al., 2018). Moreover, there has been a significant interest in the development of integrated plasmonic-based microfluidic devices (López-Muñoz et al., 2017). The plasmonic microfluidic sensing devices can simultaneously detect and identify multiple samples of interest, overcoming the single analyte detection, thus allowing detection at the ultrasensitive level (Wu et al., 2015). One of the main advantages of metal NPs, specifically AuNPs, is based on the simultaneous implementation of multiple analysis techniques in one single detection method. Therefore, the unique LSPR response based on even the smallest change of the analyte environment, together with the metal NP property to absorb and scatter light, design the accurate and effective label-free detection of pathogens and other molecules of interest (Chateau et al., 2015). Hence, making use of microfluidics, sensing devices that present short response time, and high sensitivity, are portable and automatic, are financially accessible, and can integrate a whole laboratory with multiple analyses inside one single chip have been developed (Geddes and Lakowicz, 2002). However, it is worth mentioning that different plasmonic-based microfluidic systems have several disadvantages, such as they are susceptible to environmental disturbances, channel cross-talk with low resolution, and complex system setups (Liu and Zhang, 2021).

Both, LoC technology and microfluidic devices have a tremendous impact in medical diagnosis applications. Therefore, by combining the technology of microfluidic devices with the LoC technology, a new class of devices is being developed, the PoC devices. PoC devices are miniaturized and portable devices used to rapidly analyze and detect various molecules of interest outside a conventional, fully-equipped laboratory (Giannitsis and Min, 2010; Volpatti and Yetisen, 2014). The WHO outlines PoC devices as affordable, sensitive, easy to use, rapid and robust, equipment-free, and able to deliver to end-users (Jagannath et al., 2022). The main purpose of the LoC PoC devices is to integrate the sample preparation, detection, identification, and analysis in a single integrated and easy-to-use chip.

W. Asghar et al. overcame the disadvantages and limitations of conventional detection methods based on blood culture and polymer chain reactions (PCA), by developing a novel immuno-based microfluidic device to rapidly detect *Candida albicans* (Asghar et al., 2019). The conventional detection method of fungal infection of the bloodstream requires ~10 mL of blood from the patient to be analyzed *via*

Table 3
Exploring fungal detection: fungi strain, detection device used and described above, LOD and sensitivity, and ASSURED criteria (**A**ffordable, **S**ensitive, **S**pecific, **U**ser-friendly, **R**apid and **R**obust, **E**quipment-free, and **D**eliverable to end-users) for each detection system.

Fungal species/ Mycotoxins	Device	LOD/Sensitivity	A	S	S	U	R	E	D	Ref
Aflatoxin B1	SERS	LOD: 0.4 pg x mL^{-1}	No	Yes	Yes	No	Yes	No	Yes	Rodriguez et al. (2020)
	SERS + PCA	LOD: 4–5 x 10^{-9} M	No	Yes	Yes	No	Yes	No	Yes	(Gillibert et al., 2018)
Alternaria alternata	SERS	High sensitivity	No	Yes	Yes	No	Yes	No	Yes	Ramirez-Perez et al. (2022)
Alternariol	SERS + PCA	LOD: 4–5 x 10^{-9} M	No	Yes	Yes	No	Yes	No	Yes	Gillibert et al. (2018)
	SERS	LOD: 4.83 nM	No	Yes	Yes	No	Yes	No	Yes	(H. He et al., 2020)
	SERS +4 pattern recognition methods	Identification and discrimination accuracy of 98.31%	No	Yes	Yes	No	Yes **3h**	No	Yes	(Hu et al., 2020)
Aspergillus flavus	SERS	High sensitivity	No	Yes	Yes	No	Yes	No	Yes	Ramirez-Perez et al. (2022)
	SERS + PCA	Identification and discrimination accuracy of 98%	No	Yes	Yes	No	Yes	No	Yes	(Jin et al., 2020)
Aspergillus fumigatus	SERS + chemometric analysis	–	No	Yes	Yes	No	Yes **<5min**	No	Yes	Hernández et al. (2020)
Aspergillus niger	SERS + 4 pattern recognition methods	Identification and discrimination accuracy of 98.31%	No	Yes	Yes	No	Yes **3h**	No	Yes	Hu et al. (2020)
Aspergillus parasiticus	SERS	High sensitivity	No	Yes	Yes	No	Yes	No	Yes	Ramirez-Perez et al. (2022)
Candida albicans	SERS + statistical analysis methods	Accuracy of >99.8%	No	Yes	Yes	No	Yes **40min**	No	Yes	Hu et al. (2021)
	SERS + PCA	Detection accuracy of 97.3%	No	Yes	Yes	No	Yes	No	Yes	(M. Santhosh et al., 2021)
	SERS + PCA	Identification and discrimination accuracy of 97% Discrimination accuracy of 83& between fungi and bacterial samples	No	Yes	Yes	No	Yes	No	Yes	(Witkowska et al., 2019)
Candida glabrata	SERS + statistical analysis methods	Accuracy of >99%	No	Yes	Yes	No	Yes **90min**	No	Yes	Gu et al. (2022)
	SERS + PCA	Identification and discrimination accuracy of 97% Discrimination accuracy of 83& between fungi and bacterial samples	No	Yes	Yes	No	Yes	No	Yes	(Witkowska et al., 2019)
Candida krusei	SERS + statistical analysis methods	Accuracy of >99.8%	No	Yes	Yes	No	Yes **40min**	No	Yes	Hu et al. (2021)
	SERS + PCA	Identification and discrimination accuracy of 98%	No	Yes	Yes	No	Yes	No	Yes	(Jin et al., 2020)
Candida parapsilosis	SERS + statistical analysis methods	Accuracy of >99%	No	Yes	Yes	No	Yes **90min**	No	Yes	Gu et al. (2022)
Candida tropicalis	SERS + statistical analysis methods	Accuracy of >99%	No	Yes	Yes	No	Yes **90min**	No	Yes	Gu et al. (2022)
	SERS + statistical analysis methods	Accuracy of >99.8%	No	Yes	Yes	No	Yes **40min**	No	Yes	(Hu et al., 2021)
DON	LSPR-based spectroscopic method	LOD: 57.93 ng/mL	Yes	Yes	No	Yes	Yes	No	Yes	Park et al. (2014)
Fumonisin	SERS + PCA	LOD: 4–5 x 10^{-9} M	No	Yes	Yes	No	Yes	No	Yes	Gillibert et al. (2018)
	Electrochemical detection	LOD: 0.2 mg/L	Yes	Yes	Yes	Yes	Yes	No	Yes	(Theyagarajan and Kim, 2023)
OTA	Colorimetric	LOD: 5 nM	Yes	Yes	No	Yes	Yes	Yes	Yes	(Y. He et al., 2020)
	LSPR-based spectroscopic method	LOD: 1 nM	Yes	Yes	No	Yes	Yes	No	Yes	(Liu et al., 2018)
	Fluorescence spectroscopy method	LOD: 22.7 nM	Yes	Yes	No	Yes	Yes	No	Yes	(Kaushal et al., 2020)
	Fluorescence spectroscopy method	LOD: 2 nM	Yes	Yes	No	Yes	Yes	No	Yes	(Li et al., 2014)
	Fluorescence spectroscopy method	LOD: 0.045 ng/mL	Yes	Yes	No	Yes	Yes	No	Yes	(Lv et al., 2017)
	Electrochemical detection	LOD: 0.001 ppb	Yes	Yes	Yes	Yes	Yes	No	Yes	(Tang and Li, 2017)
Penicillium chrysanthemum	SERS + 4 pattern recognition methods	Identification and discrimination accuracy of 98.31%	No	Yes	Yes	No	Yes **3h**	No	Yes	Hu et al. (2020)
Penicillium expansum	SERS + 4 pattern recognition methods	Identification and discrimination accuracy of 98.31%	No	Yes	Yes	No	Yes **3h**	No	Yes	Hu et al. (2020)
Penicillium paecilomyces	SERS + 4 pattern recognition methods	Identification and discrimination accuracy of 98.31%	No	Yes	Yes	No	Yes **3h**	No	Yes	Hu et al. (2020)
Rhizomucor pusillus	SERS + chemometric analysis	–	No	Yes	Yes	No	Yes **<5min**	No	Yes	Hernández et al. (2020)
Scopulariopsis brumpii	SERS + PCA	Identification and discrimination accuracy of 98%	No	Yes	Yes	No	Yes	No	Yes	Jin et al. (2020)
Trichophyton rubrum	SERS + PCA	Identification and discrimination accuracy of 98%	No	Yes	Yes	No	Yes	No	Yes	Jin et al. (2020)
ZEN	SERS + PCA	LOD: 4–5 x 10^{-9} M	No	Yes	Yes	No	Yes	No	Yes	Gillibert et al. (2018)
	Colorimetric	LOD: 10 ng/mL	Yes	Yes	No	Yes	Yes	Yes	Yes	(Sun et al., 2018)
	Electrochemical detection	LOD: 0.34 ng/mL	Yes	Yes	Yes	Yes	Yes	No	Yes	(Sohrabi et al., 2022)

blood culture incubators (Hall and Lyman, 2006). After that, additional analysis must be performed to improve the fungal infection differentiation (Adam et al., 2010). All these detection methods are time-consuming, requiring between 2 and 10 days to detect and identify the fungal strain, delaying the treatment of the patient. The authors from the United States developed a cheap and rapid microfluidic device, functionalized with specific polyclonal antibodies to detect *C. albicans* in PBS and blood (Fig. 11. Left). The capturing and identification of the fungi strain is time-effective and requires low blood sample volume, due to the size of the microfluidic channels. After the blood is collected from the patient, it is stained with a fluorescent protein and inserted in the microchannels that were previously functionalized with the antibodies. The unbound fungal metabolites are washed with PBS before analyzing the blood sample under a fluorescent microscope. The as-designed microfluidic device possesses excellent advantages, achieving a 60–80% capture efficiency in less than 2h, requiring a total of 10 CFU/mL sample.

In their quest to isolate and concentrate specific fungal pathogens from the blood, B. B. Fuchs et al. reduced the cell cultivation step – a very time-consuming part – by designing an integrated microfluidic device based on inertia focusing (Fuchs et al., 2019). Based on the microchannel diameter and the parabolic flow profile, two opposite forces are applied to the cells that flow inside the channels, thus resulting in the equilibrium position of the cells. Moreover, the morphology of cells influences the flow inside the microchannels, meaning the equilibrium position. Hence, fungal cells are commonly smaller than white blood cells (WBC). Based on this property of the microfluidic devices, the authors were able to separate and investigate the *C. albicans* flow and the WBC flow, by previously staining *C. albicans* with FITC (green fluorescence) and WBC cells with Calcein AM (orange fluorescence). The authors developed the Inertial Fungal Focuser (IFF), a PDMS-based microfluidic device able to rapidly separate specific fungal strains from the blood, allowing further detection and identification analysis for an improved diagnosis. While the fungal cells entered the IFF device in a random distribution, they then focused on the equilibrium position, separating themselves from the equilibrium position of the WBC. By determining the flow rate at 400 μL/min, the IFF device can isolate fungal strain from blood samples in under 125 min. However, the IFF device presents limitations. Although it can be used for isolating and concentrating fungal strains from the blood without sophisticated preparation steps, it is not ideal for blood cleansing and filtration.

Moreover, integrated microfluidic devices are exceptional tools not only for the identification of various pathogens, but also for testing and developing specific drugs to act against fungal infections based on their versatility, speed, and reduced sample consumption. Up to now, there are limited effective therapeutics to fight fungal infections, especially *C. albicans*. Of all four common drugs – fluconazole, amphotericin B, caspofungin, and terbinafine – there is not one to not exhibit toxicity and side effects (Ehrenkaufer et al., 2018). In response to this crisis, Le Qiang et al. designed a high throughput microfluidic screening device, combined with fluorescence imaging, to study the antifungal effect of the combined amphotericin B drug with 50.520 different molecules and to determine which small molecule can improve the amphotericin B's antifungal efficacy (Qiang et al., 2019). This microfluidic screening device consists of thousands of parallel microchannels between 3 and 5 μm, the standard width for *C. albicans* cells to flow through and to align one by one, to make it possible to record fluorescence microscopy images and to determine their vitality. In only one week they were able to test all 50.520 small molecules, overcoming by far the conventional time-consuming cell culture method. At the end of the study, they reported 10 small molecules that possess the ability to enhance amphotericin B's antifungal effect against *C. albicant* by over 30%.

There are a variety of fungal strains that do not cause whole-body infections, but that target a specific delicate organ, such as the lungs. There is a pressing need for novel and accurate *ex vivo* models to study the biological interaction between the fungi strain and the attacked organ. Based on this clinical need, integrated microfluidic chips with advanced and improved properties have been developed for an emerging new technology, Organ-on-a-Chip. Making use of this technology, Lung-on-a-Chip microplatforms have been employed for investigating and assessing lung-related respiratory infections, diseases, and various injuries in the last decade (Bennet et al., 2021; Shrestha et al., 2020). The microfluidic design allows the development of a miniaturized 3D lung model on the cellular level, for simulation of flow and breathing conditions and fungal infections-lung interactions (Huh et al., 2010; Huh, 2015). In this context, a group of researchers from the University of Wisconsin-Madison, USA, designed a biologically complex Lung-on-a-Chip model of the human bronchiole, together with a complete vascular system, airways, and extracellular matrix compartments to investigate pulmonary infections (Fig. 11. Right) (Barkal' et al., 2017;

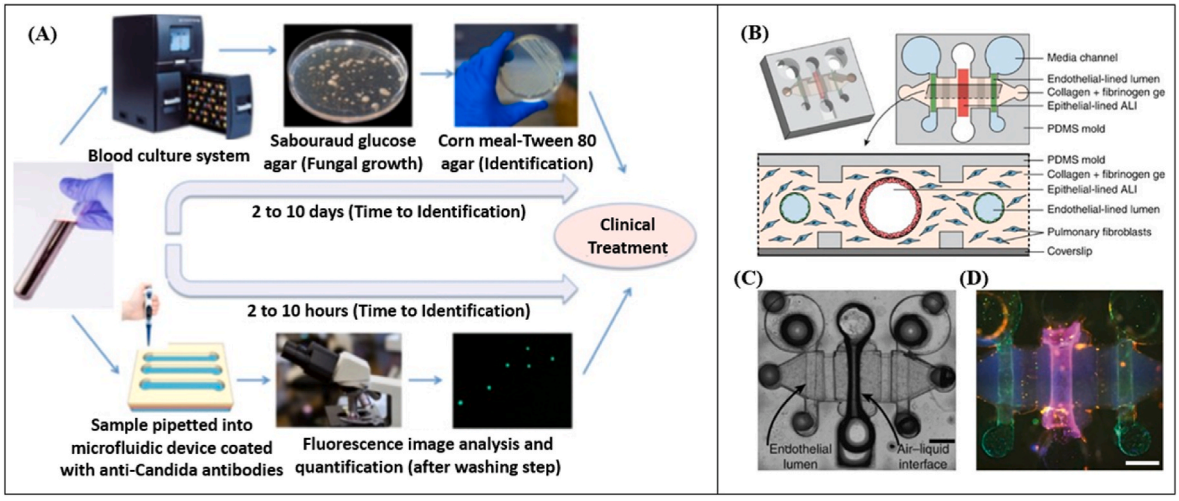

Fig. 11. Left: (A) Conventional fungal culture methods compared with the microfluidic chip detection method for *C. albicans*. Conventional clinical settings are time-consuming and require a high volume of samples, while the microfluidic device can detect the fungi strain from a low volume and the time for identification is between 1 and 2 h (Asghar et al., 2019). **Right:** Lung-on-a-Chip microfluidic device used for studying fungal respiratory infections. **(B)** Schematic representation of the Lung-on-a-Chip device that simulates the human bronchiole. **(C)** Representative microscopy image of the microfluidic device. **(D)** Microchannels coated with cells stained with Hoechst (blue, nucleus stain), anti-CD31 antibody (green, endothelial tight junction marker), and anti-EpCAM antibody (red, epithelial cell-cell adhesion marker). Scale bar is 500 μm (Barkal et al., 2017).

Monteduro et al., 2023). Although they integrated up to three host cell types and two microbial types – *A. fumigatus*, a fungi strain, and *Pseudomonas aeruginosa*, a gram-negative bacillus – the system is easy to use and accurate. The design allowed the volatile communication between the microbial population/fungi strain and the host model organ *via* a clickable extension. They studied the inflammatory response caused first by *A. fumigatus* fungal infection and then by a "double-infection" with *A. fumigatus* and *P. aeruginosa*. As the fungi and bacteria strains were separated, the communication between them and the host could occur only *via* volatile diffusion in a collagen matrix, simulating exactly the real bronchiole. Moreover, they also investigated and monitored the distribution of leukocytes as a result of the infection. The authors successfully demonstrated that the volatile interactions between the fungi strain and the bronchiole model are changed once in contact also with *P. aeruginosa*. Hence, they reported the novel advanced Lung-on-a-Chip device to facilitate insights about the multi-kingdom – bacterial-fungal-human – the entanglement of infections and diseases within the human organs.

5. Smartphone-based and commercially available portable point-of-care devices

The widespread use of smartphones simplified the access to information and data resources for individuals worldwide. As we navigate a time characterized by increasing healthcare demands, a need for time-effective disease detection, and the problem of remote healthcare access in some regions, the need to develop reliable, portable, sensitive, and rapid detection and diagnostic devices emerged. With this in mind, the development of smartphone-based PoC devices unfolds as a modern healthcare solution. Smartphones are nowadays globally accessible, rapid, convenient, and user-friendly, making them invaluable tools that revolutionize healthcare delivery, and improve patient outcomes while also advancing medical research in an interconnected world (Kanchi et al., 2018). To this day, smartphones proved their potential in visualizing, detecting, gathering, and sending data when combined with complementary devices (Li et al., 2018; Liao et al., 2016; Murphy and King, 2016; Rong et al., 2019). Early and accurate detection is crucial in the context of fungal infections, as fungal infections can be life-threatening. Smartphone-based devices own several advantages, such as easy accessibility to diagnostic tools, time-effective diagnosis, cost-efficiency, reduced laboratory workload, and the possibility of remote consultation (Pawar et al., 2023). In this context, we reviewed the smartphone-based POC devices and technologies developed to detect and identify fungal pathogens, along with offering insights into potential future developments, while addressing current limitations associated with these pathogen detection systems (Fig. 12).

In 2017, news broke out regarding a novel application (app) that helps young Australians monitor and control their Asthma ("Australian first"). A team of researchers, together with clinicians, were funded by Asthma Australia to develop the so-called "Kiss My Asthma" app, in the hope of helping young teenagers and adults manage their health affection. Based on the article, this app was developed as a response to a 2014 survey that monitored over 500 12-25-year-olds with asthma. The survey findings painted a dark picture: 63% struggled with poor asthma management, 52% were susceptible to mental health problems, whereas 56% mentioned how asthma limits their ability to enjoy life to the fullest.

PathoNostics is a company founded in The Netherlands, that provides a selection of real-time PCR kits, designed for the identification of various fungal infections ("PathoNostics - Company," n.d.). Their main goal is to rapidly detect fungal pathogens, while enabling healthcare professionals and researchers to initiate appropriate treatments, in an attempt to improve patient outcomes. They developed the AsperGenius® device ("AsperGenius® | PathoNostics," n.d.) for *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus flavus,* and other *Aspergillus* species and mutations detection and identification. Among the benefits

Fig. 12. Schematic representation of smartphone-based POC diagnostic devices (Pawar et al., 2023).

and advantages of this device, is the fast sample-to-result feature, together with the specific differentiation between the as-mentioned *Aspergillus* strain.

Hain Lifescience BmbH is a German company that provides a variety of products for medical diagnostics. Fungiplex® is a molecular diagnostic device developed to provide rapid and targeted results regarding fungal infections. There are currently three Fungiplex® devices commercially available: Fungiplex® Aspergillus, Fungiplex® Aspergillus Azole-R, and Fungiplex® Candida ("Fungiplex® - Product Overview"), each designed to detect specific pathogens or their most common mutations. Even though it is not directly smartphone-based, it can be easily integrated with smartphones for data analysis.

A group of microbiology researchers from New Delhi, India confirmed the successful use of smartphones and pocket magnifiers as PoC, rapid, cost-efficient, and reliable microscopic screening tools for *fungal keratitis* detection (Agarwal et al., 2015). While these accessories are not specific for fungi detection, they can be successfully used for various microscopic applications, including examining fungal samples. They investigated a tissue sample obtained by corneal scraping, from a case of mycotic keratitis and they proved that the smartphone-based PoC device obtained good microscopic images of the gram-positive organism with hyphae.

J. Guinea et al. evaluated the MycAssay™ Aspergillus assay for diagnosis of patients suffering from invasive aspergillosis, but that do not present hematologic cancer (Guinea et al., 2013). MycAssay™ Aspergillus is a commercially available device, based on the real-time PCR technique, that presents significant advantages compared to common fungal detection methods, such as portability, rapid diagnosis, and high specificity. They evaluated in a microbiology laboratory 322 low respiratory tract samples from 175 patients, by extracting Aspergillus DNA and amplifying it by MycXtra® and MycAssay™ Aspergillus. At the end of their study, they concluded that MycAssay™ Aspergillus owns a high sensitivity toward invasive aspergillosis diagnosis in patients without hematologic cancer. Moreover, the sensitivity increased when multiple samples were used.

Nonetheless, to this day, commercially available smartphone-based biosensors are often crafted annually and lack standardized specifications compared to traditional devices. Ensuring high sensitivity,

selectivity and reproducibility necessitates a user-friendly setup. Software applications should facilitate easy analysis and efficient transmission of diagnostic results. Moreover, optical sensors reliant on smartphones demand superior image resolution and precise sample classification. Additionally, incorporating biosensors into smartphones poses a notable challenge, particularly since certain smartphones may have restricted processing capabilities and battery longevity (Pawar et al., 2023). These limitations can significantly hinder the device's capacity to detect fungi effectively. Nevertheless, despite these obstacles, smartphone-enabled sensing devices hold immense potential for future PoC healthcare. Such diagnostic tools could potentially overcome the drawbacks encountered by traditional methods like PCR and ELISA, therefore enabling instant and cost-effective medical diagnostic tests.

6. Conclusions and future perspectives: shaping Tomorrow's diagnosis

This review discusses the recent progress on plasmonic NPs-based biosensors and the up-to-date PoC devices for fungal infection identification. In particular, the review points out the exceptional progress in PoC field, underlining both current trends and promising future technologies. The constantly increasing prevalence of fungal infections, along with the need for rapid and accessible diagnostic solutions, evidences the urgency of innovative research and development efforts. Current trends revealed many smartphone-based and portable commercially available diagnostic devices. They are both affordable and user-friendly, empowering healthcare providers to quickly diagnose fungal infections, thus enhancing patient care and reducing the burden on healthcare systems.

In resource-limited settings, there is a pressing need for rapid, user-friendly, easy-to-use, and cost-effective diagnosis methods to efficiently tackle infectious diseases through PoC testing. While conventional diagnostics methods, such as ELISA and PCR, offer dependable diagnosis and treatment monitoring, their reliance on well-equipped facilities and trained personnel makes them impractical for PoC testing, especially in resource-limited areas. Consequently, there is a growing interest in the development of diagnostic platforms suitable for PoC applications, aiming to enable and facilitate early fungal infection detection worldwide.

Moreover, molecular and immunological techniques, coupled with microfluidics and biosensors, have paved the way for sensitive, specific, and non-invasive fungal detection as PoC devices. However, they are hindered by insufficient sensitivity, selectivity, and overall reliability, underscoring the challenges encountered in PoC diagnostics. The rapidly increasing global prevalence of fungal infections, together with the urgent need for rapid and accessible diagnostic tools underscore the essential urge for innovative research in the field of fungal detection. Given their crucial role in disease management strategies, particularly in underdeveloped regions, PoC diagnostics are undergoing continuous enhancement to overcome the limitations of current methods and emerge as dependable tools suitable for implementation in all required settings. In addition, this review highlights several promising technologies capable of revolutionizing fungal detection. Advances in nanotechnology, microfabrication, and miniaturization are expected to yield even more compact, easy-to-use, and efficient diagnostic devices.

The ASSURED criteria proposed by the WHO for PoC devices serve as a valuable guideline for researchers when designing their devices. However, achieving these objectives can be particularly challenging, even for plasmonic-based devices. A significant concern revolves around the potential decrease in the specificity of plasmonic substrates when dealing with complex clinical samples. Thus, as advancements in nanofabrication continue, more automated and reproducible methods for generating complex nanostructured substrates are expected to arise. These substrates are highlighted across the review and are a crucial component across a variety of biosensing devices, making them an important part of future commercialization.

Further incorporation of specific technologies, such as paper-based, microfluidics, smartphone, and wearable devices can serve as a pivotal area of focus for advancing these biosensors. As these fields progress, they offer a clear trajectory toward achieving an ideal design that is simple, user-friendly, cost-efficient, portable, and automated. Furthermore, integrating AI and machine learning algorithms can enhance fungal identification and discrimination accuracy and speed, thus revolutionizing pathogen diagnostic capabilities. Future research should explore the clinical importance of the novel developed biosensors for fungal detection and discrimination, treatment monitorization, and equal healthcare access for countries all over the world. The advancements presented in the present work will have a crucial impact in improving diagnostic protocols and, consequently, patient life quality, reducing healthcare costs and family/society burden while advancing our general understanding of pathogens, ultimately contributing to a healthier and more resilient healthcare landscape.

CRediT authorship contribution statement

Daria Stoia: Writing – original draft, Conceptualization. **Luciano De Sio:** Writing – review & editing, Funding acquisition. **Francesca Petronella:** Writing – review & editing, Writing – original draft, Funding acquisition. **Monica Focsan:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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