Review Article

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Catalytic defense against fungal pathogens using nanozymes

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Abstract: Fungal infections are still a major challenge for clinics, resulting from the resistance of drug-resistant fungi and the toxicity of antifungal drugs. Defense against fungal invasions *via* enzymatic catalysis has been found in nature. The use of nanozymes, as artificial enzyme mimics, may be a promising strategy to induce fungal death due to their advantages such as tunable catalytic activity, high stability, low cost, and easy preparation. Here, the importance of natural enzymes in the defense against fungi is outlined. The progress in antifungal performance and potential application of nanozymes and the related antifungal mechanisms are also summarized. Finally, the perspective and challenges in this field for future study, pointing out that nanozyme-based catalytic therapy represents a promising alternative strategy for antifungal treatment, are highlighted.

Keywords: nanozymes, antifungal, reactive oxygen species, enzymatic therapy, catalytic defense

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1 Introduction

Nearly 1.7 billion people suffer from fungal infections around the world, and systemic fungal infections lead to 1.5 million deaths each year because of their high mortality rate [1–3]. Although antifungal drugs are effective against fungal infections, they have some side effects like high liver and kidney toxicity. Importantly, single- or multi-drug-resistant fungi have emerged, associated with antibiotic abuse [4]. Therefore, it is urgent to develop new antifungal drugs.

Natural enzymes extracted from plants or microbes can degrade fungal cell walls and even destroy the integrity of fungal cells. Natural enzymes in humans with antibacterial, anti-inflammatory, and anti-oxidative abilities have been used in treating gastrointestinal diseases and leukemia [5,6]. However, natural enzymes have some disadvantages such as poor stability, high cost, and difficulty in mass production [7], which limit their application. To solve these problems, artificial enzymes have been developed as alternatives to natural enzymes. Fe_3O_4 nanoparticles (NPs) with intrinsic peroxidase (POD)-like activity were reported in 2007. Subsequently, Yan's team put forward a new concept, "nanozymes," which are artificial enzyme mimics with the unique properties of nanomaterials as well as catalytic functions [8]. Since then, incredible growth has been witnessed in research on nanozymes, suggesting their scientific significance and broad application prospects.

Nowadays, nanozymes are available that have the inherent characters of nanomaterials including photothermal effects [9], fluorescence, and infrared imaging [10] and show hundreds of enzyme-like activities, like those of oxidoreductase, hydrolase, isomerase, and lyases. In addition, nanomaterials have many applications in the biomedical field, such as tumor treatment [11], antioxidant therapy, detection and diagnosis [12], antibacterial [13], and antiviral therapy [14]. At present, studies on the antifungal effects of nanozymes are still in an exploratory stage. In this review, we summarize how natural enzymes existing in nature play an important role in avoiding

fungal invasion. Then, the antifungal activities and potential applications of nanomaterials with enzyme activity are highlighted (Figure 1), and different mechanisms for killing fungus are explained. In addition, the features and advantages of nanozymes in combating fungal infections, compared to natural enzymes and antifungal drugs, have also been reviewed. The authors here term the way against the microbial pathogens through catalysis as "catalytic defense," which might facilitate enzymatic therapy for infectious diseases. In this study, catalytic defense means that fungal pathogens can be combated through catalysis mediated by nanozymes or natural enzymes. Finally, the future challenges and perspectives in the use of nanozymes for antifungal applications are discussed in detail. The authors expect that this overview of nanozymes with antifungal activity will be helpful in the design of novel antifungal agents.

2 The importance of natural enzymes in antifungal activity

Fungal infections lead to serious effects on health or cause massive economic loss. However, nature has ways of mutually reinforcing and neutralizing such threats. Different organisms avoid fungal invasions by producing enzymes like chitinase, POD, and β-1,3-glucanase (found in microbial species, plants, and insects) to degrade the fungal cell wall, which is mainly composed of protein, polysaccharides, and lipids; among which chitin and β-1,3 glucan are the main targets. Most of the various reported species achieve destructive lysis of fungal cell walls through the catalytic activity of their extracellular enzymes (Table 1).

2.1 Natural enzymes originating from microbes

Chitinase and β-1,3-glucanase have been considered important hydrolytic enzymes to degrade fungal cell walls (Figure 2). It is reported that chitinase is distributed in Gram-negative bacteria [15], Gram-positive bacteria [16], and fungi [17,18]. Prapagdee *et al.* isolated *Streptomyces hygroscopicus* which can secrete chitinase and β-1,3-glucanase to suppress the growth of *Colletotrichum gloeosporioides* and *Sclerotium rolfsii* [19]. Similarly, cellulase and chitinase isolated from *Pseudomona* have great antifungal activity toward *Pythium aphanidermatum* and *Rhizoctonia solani* [20]. The alkaline serine protease gene ALP5 has been cloned from *Aureobasidium pullulans* and subsequently transformed into *Pichia pastoris* KM71P for expression. The strain possesses relatively high chitinolytic activity toward *Botrytis cinerea via* the activity of enzymes such as glucanases, chitinases, proteases, and hydrolases [18].

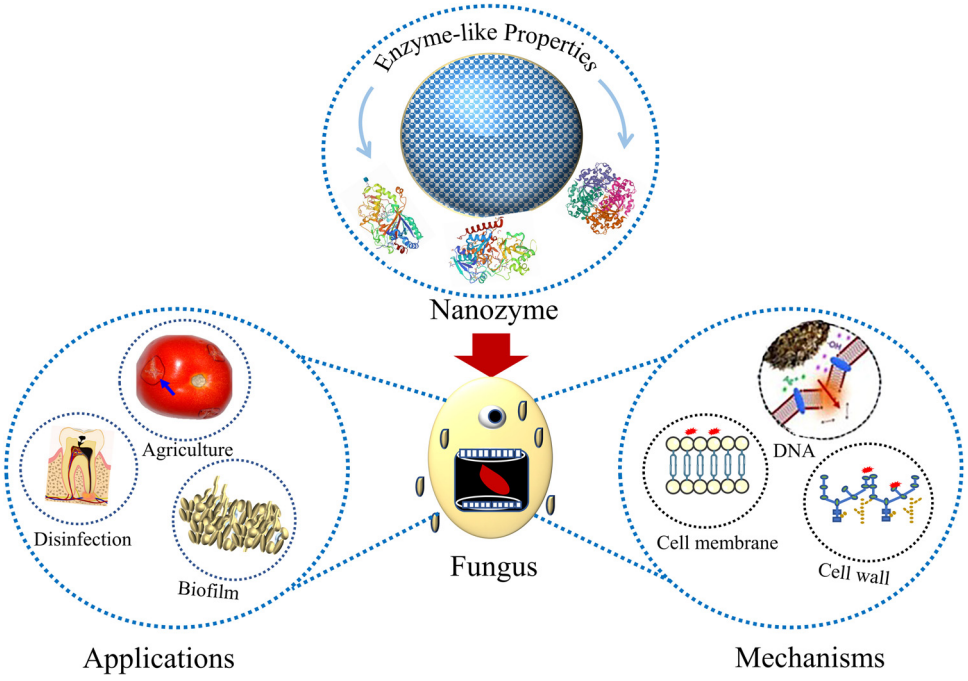

Figure 1: Schematic of nanozymes in antifungal applications and their mechanisms [45,49,58]. All images reproduced with permission.

Table 1: Summary of natural enzymes from various organisms with antifungal activity

Organisms	Natural enzymes	Target	Mechanism	References
Triticum aestivum	POD, OXD	*Fusarium culmorum*, *Trichoderma viride*, *Botrytis cinerea*	Inhibit hyphal growth	[23]
Marsdenia megalantha latex	Class III POD	*Fusarium oxysporum*, *Fusarium solani*	ROS, damage cell membranes	[24]
Calotropis procera latex	Cysteine peptidases	*Fusarium oxysporum*	ROS, damage cell membranes and cell walls	[26]
Avena sativa	Chitinase	*Penicillium roqueforti*	Degrade cell walls	[21]
Indigenous *Actinomycetes*	β-1,3-glucanase, chitinase	*Colletotrichum gloeosporioides*, *Sclerotium rolfsii*	Degrade cell walls	[19]
Pseudomonas strains	Chitinases, cellulases	*Pythium aphanidermatum*, *Rhizoctonia solani*		[20]
Trichoderma harzianum	Chitinases	*R. solani*, *B. cinerea*, *Phytophthora citrophthora*	Degrade cell walls	[17]
Bacillus pumilus	Chitinases	*Fusarium graminearum*, *Bipolaris sorokiniana*	Inhibit hyphal growth	[85]
Serratia marcescens	Chitinases	*Didymella applanata*		[86]
Aureobasidium pullulans	Alkaline serine, protease	*Penicillium expansum*, *B. cinerea*, *Monilinia fructicola*, *Alternaria alternata*	Damage cell walls	[18]
Termites	β-1,3-glucanase	*Wood-rot fungi*		[27]
FE	Cellulase, chitinase, β-1,3-glucanase, lichenase, xylanase, dextranase, mannase, lysozyme, and protease	*Saprolegnia parasitica*	Degrade cell walls	[29]

2.2 Natural enzymes derived from plants

Most plants suppress fungal growth through intrinsic enzymes such as antioxidase, chitinase, and POD [21,22]. Among them, POD is the most common. WP19, a basic heme-POD stemming from wheat grains, can inhibit hyphae formation but not the growth of *B. cinerea*, *Fusarium culmorum*, and *Trichoderma viride* [23]. Latex is a mixture that contains secondary metabolites and proteins. Oliveira *et al.* purified a class III POD which has a high affinity for guaiacol and H_2O_2 and shows kinetic parameters consistent with other PODs. The class III POD inhibits the conidia germination of *Fusarium oxysporum* and *Fusarium solani* through the production of reactive oxygen species (ROS), finally resulting in fungal death [24]. Not only that, legumes also can secrete POD [25]. The chitinases contained in certain plants destroy fungal cell walls through the exploitation of their chitinolytic potential, and have been reported in plants such as oats, *Arabidopsis*, and *Agaricus blazei* [21]. Recently, researchers found cysteine peptidase in *Calotropis procera*, which facilitates membrane permeabilization, leakage of cellular content, and the production of ROS toward *F. oxysporum* [26].

2.3 Other organisms

In addition, certain insects damage fungal pathogens based on the catalysis of β-1,3-glucanase. There is great competition between termites and wood-rot fungi for common habitats and food sources. Their saliva can protect termites from fungal attack due to its β-1,3-glucanase activity [27,28]. Similarly, enzymes resist fungus that has been found in fertilization envelopes (FEs). The FE derived from fish eggs possesses the functions of multiple enzymes such as cellulase, chitinase, β-1,3-glucanase, lichenase, xylanase, dextranase, mannase, lysozyme, and protease. They can inhibit the growth of *Saprolegnia parasitica* and achieve the lysis of fungal cell walls, and even induce fungus death [29].

2.4 Natural enzymes existing in immune cells

The immune cells use a number of response mechanisms to deal with fungal infections such as the formation of mature phagolysosomes, cytokine release, activating the adaptive immune system, and antimicrobial peptides.

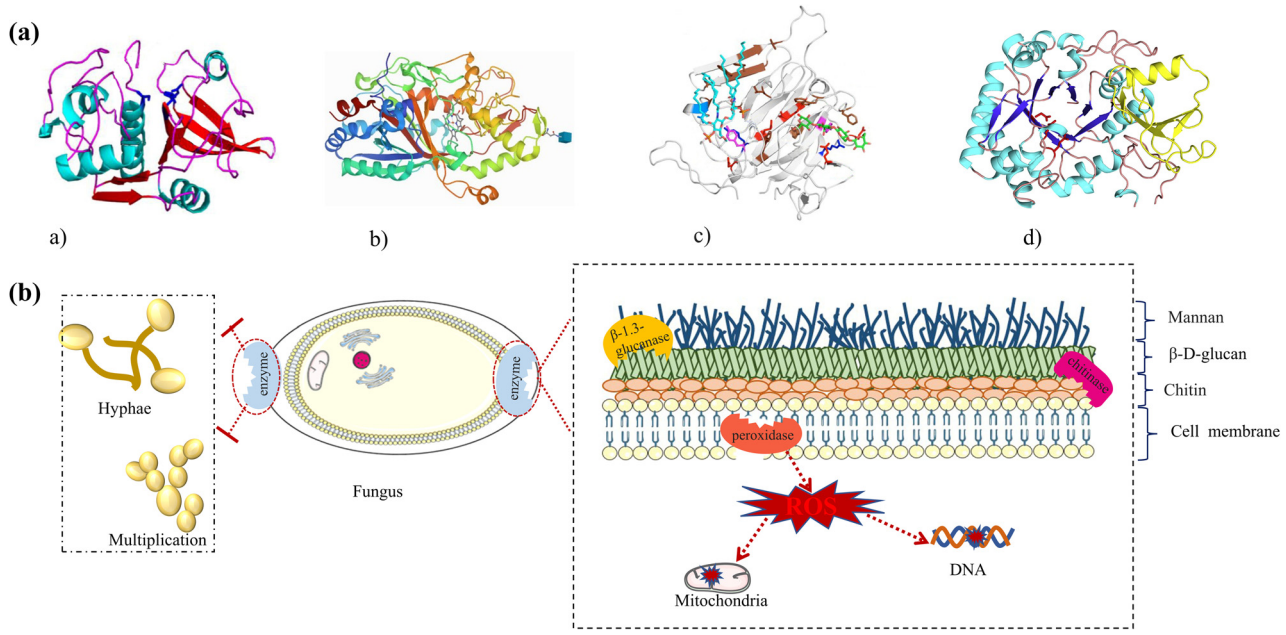

Figure 2: (a) (Schematic of three-dimensional models of cysteine peptidases (a) [26], POD (PDB ID: 3afv) (b) [81], (c) β-1,3-glucanase [82], and chitinase (d) [83]). (b) The mechanisms of the action of natural enzymes against fungus. (1) Fungal cell wall degradation by the interaction of enzymes and cell wall components (chitin and β-1,3-glucan). (2) ROS information and cell membrane damage. (3) Inhibition of hyphal formation and cell multiplication. All images were reproduced with permission.

The process of surveillance and elimination of fungal pathogens in mammals depend heavily on phagocytosis by immune cells, especially macrophages, dendritic cells, and neutrophils [30]. Macrophages perform phagocytosis on fungal cells and form mature phagolysosomes *via* a series of cascade reactions mediated by Rab (Ras-like proteins in brain) GTPases and calcium ions [31]. In phagolysosomes, pathogens including fungi are killed by the production of ROS and acidification *via* NADPH oxidase, POD, and vacuolar H^+ ATPase. Similarly, neutrophils induce fungi death, relying heavily on the production of ROS by the catalysis of NADPH oxidase and myeloperoxidase [32,33]. In short, the natural enzymes of immune cells such as NADPH oxidases, POD, and vacuolar H^+ ATPase play important role in preventing fungal infections in mammals.

2.5 Natural enzymes in practical applications

It is known that natural enzymes have been used in biomedical research and clinical applications, that is, in the treatment of gastrointestinal disease, leukemia, viral diseases, *etc*. [34]. For example, amylase has been widely used as an oral medicine for the treatment of functional dyspepsia, and the compound digestive enzyme capsules (H20051951) have been authorized to sell in the market of China [35]. Although the treatment of fungal infections based on enzymatic therapy has not been used clinically, some enzymes are applied in scientific research and industry. For example, the ability of zymolyase derived from *Arthrobacter gaminerie* to lyse fungal cell walls has been applied in scientific studies. The β-glucanase that hydrolyzes glucan has been applied in the winery [36].

3 Antifungal activity and potential applications of nanomaterials with enzyme-like activity

Whether chitinase, POD or others, enzymes have a great effect on fungal growth. However, the high cost and poor stability of natural enzymes limit their applications [37]. Based on this, much effort has been devoted to the design of artificial enzymes as an alternative to natural enzymes to defend against fungal invasion. A nanozyme is a kind of nanomaterial with enzyme-like activities, which has the advantages of tunable catalytic activity, easy large-scale production, and low cost compared to natural enzymes and traditional artificial enzymes [7]. So far, nanozymes have exhibited hundreds of catalytic activities such as oxidoreductase-like, hydrolase-like, isomerase-

like, and lyase-like activities [38]. Due to their characteristics like mimicking enzyme activity and wide-spectrum antifungal activity, nanozymes have emerged as new antifungal agents. The current studies of nanozymes regarding fungus are summarized in Table 2.

3.1 The effect of enzyme-mimicking nanozymes on fungal activities

3.1.1 Nanozymes with POD activity against fungus

Nanozymes have the inherent characteristics of nanomaterials as well as natural enzymatic activities and have great potential to be used in various fields. Many nanomaterials (ZnO, Fe_3O_4) [39,40] show enzyme-like activity and can catalyze H_2O_2 to produce hydroxyl radicals (˙OH) to achieve the effect of sterilization. Nanozymes with strong antifungal activity show different enzymatic activity, among which POD-like activity is the most common. Fe_3O_4 nanozymes combined with H_2O_2 can kill fungus depending on POD-like enzyme activity. Compared to the effect of H_2O_2 or nanozyme alone, their combination could improve the inhibition rate to 75.70% [41]. Similarly, snowball-like hybrid nanostructures (NSBs) constituted by *Viburnum opulus* extraction and Cu^{2+} ions also exhibited catalytic activity. NSBs resulted in the inactivation of *Candida albicans* and *Candida glabrata* through the action of various radicals and Cu^{1+}. Compared to the minimum inhibitory concentration of NSBs against *Escherichia coli* and *Staphylococcus aureus*, that of NSB against fungus could be lower, at 10 μg/mL [42]. Consistent with NSBs, norepinephrine nanoflowers (neNFs) with POD-like activity also rely on a Fenton-like reaction to induce the death of *C. albicans*. In the presence of H_2O_2, 1 mg/mL neNFs could kill 91, 94, and 82% of *C. albicans*, *E. coli*, and *S. aureus*, respectively [43]. In general, nanozymes with POD-like activity are promising wide spectrum antibacterial agents for killing the representative pathogens of fungal, Gram-positive, and Gram-negative bacteria.

3.1.2 Fungus resistance by nanozymes with multi-enzyme-like activities

It is known that immune cells induce fungi death, relying heavily on the production of ROS mediated by the catalysis of NADPH oxidase and myeloperoxidase. In natural enzymes, POD and oxidase (OXD) can generate ROS in the presence of H_2O_2 or O_2, and scavenging ROS and converting superoxide into H_2O_2 and O_2 are related to superoxide dismutase (SOD) and catalase (CAT). It has been reported that nanozymes with multi-enzyme-like activities induce fungal death relying on the generation of ROS. The Ce-metal-organic framework (Ce-MOF) nanozyme inhibited 93.3–99.3% of the growth of fungi, and caused the deformation of hyphae and conidiophores by damaging the fungal cell membrane and generating oxidative stress in the fungal cell due to its multi-enzymatic activities (CAT, SOD, and POD) [44]. Ji *et al.* synthesized glucose oxidase-modified magnetic NPs (GMNPs) with POD-like activities that could catalyze the oxidation of glucose to generate H_2O_2, which can be catalyzed by magnetic NPs (MNPs) to generate ROS, resulting in cell death [45]. Antonoglou *et al.* reported that Cu–Fe NPs synthesized by a reproducible wet chemical approach showed strong antifungal activity. The IC_{50} value of Cu–Fe NPs toward *Saccharomyces cerevisiae* was 34.34 ± 2.32, 38.4 ± 2.27, and 50.3 ± 1.72 μg/mL for 5, 10, and 24 h exposure, respectively. The results show that Cu–Fe NP induces fungus inactivation related to time and concentration. The Cu–Fe NPs can enter the fungal cells and copper and iron released from it can react with O_2-producing ROS (O^{2-}), which can be catalyzed by SOD to generate H_2O_2. The copper and iron further converted H_2O_2 to ROS leading to fungal death through Fenton and Haber–Weiss chemistry. These reports show that nanozymes with OXD-like, SOD-like, and POD-like activities may perform cascade catalysis to convert O_2 into toxic radicals with strong antifungal activity [46].

3.1.3 Nanozymes with other enzyme-like activities against fungi

In addition, there are some nanozymes with enzymatic activities that target fungal cell walls. Xiao *et al.* prepared Fe_3O_4 MNPs to destroy the cell walls of yeast cells depending on their zymolyase-like lytic activity [47]. Piętka-Ottlik *et al.* prepared nanoemulsions with glutathione POD-like activity which showed high antifungal activity. The nanoemulsion could inhibit biofilm formation at the level of 86.2%, and controlled the adhesion rate to 35.7–57.4% by destroying the morphology of fungal cells through deformation and wrinkling [48].

3.2 Potential applications of nanozymes

3.2.1 Disinfection

Generally speaking, nosocomial infections that harm human health result from polluted environments, including

Table 2: Summary of the current researches of nanozymes in antifungal activity

Nanomaterials	Enzyme-like activity	Antifungal mechanism	Target	Application	References
Fe_3O_4@MoS_2-Ag	POD	·OH, hyperthermia, Ag^+	*Candida albicans*	Disinfection	[49]
GMNP	Glucose oxidase, POD	ROS	*C. albicans*	Disinfection	[45]
Fe_3O_4 MNPs	Zymolyase	Destroy cell walls	*C. albicans*	Industry	[47]
N, F co-doped TiO_2	POD	·OH	*Fusarium oxysporum*	Agriculture	[58]
Ce-MOF	POD, CAT, SOD	·OH	*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *C. albicans*, *Rhodotorula glutinis*	Inhibit biofilms formation	[44]
Nanosnowball ($Cu_3(PO_4)_2{\cdot}3H_2O$)	POD	·OH, Cu^{1+}	*C. albicans*, *Candida glabrata*	Antimicrobial agent	[42]
Nanoflowers (green tea extraction-Cu^{2+})	POD	·OH, Cu^{1+}	*C. albicans*	Cosmetics	[70]
Norepinephrine nanoflower (neNF)	POD	ROS, Cu^{1+}	*C. albicans*		[43]
CuTlm ($[Cu_8Tlm_{16}]{\cdot}24H_2O$)	SOD		*Candida parapsilosis*, *Candid atropicalis*, *C. albicans*		[87]
Nanoemulsions (Ebselen)	Glutathione peroxide		*C. albicans*	Inhibit biofilms formation	[48]
Nitrogen-iodine doped carbon dots (I-CDs)	POD	·OH	*C. albicans*	Disinfection	[71]
CoZnO/MoS_2-30	POD	·OH, $\cdot O_2^-$	*A. flavus*	Broad-spectrum bactericidal agents	[84]

contamination of air, water, medical apparatus, instruments, *etc*. Abdelhamid *et al*. isolated opportunistic pathogenic fungi from the outside air of a hospital. A Ce-MOF nanozyme showed wide-spectrum antifungal activity toward *C. albicans*, black *Aspergillus*, *etc*. Not only that, the virulence of the fungus (hyphae and adhesion) was also destroyed by the Ce-MOF nanozyme [44]. Han *et al*. constructed Fe_3O_4@MoS_2-Ag nanozymes by a simple hydrothermal method and *in-situ* light deposition of Ag NPs (Figure 3). The disinfection process of Fe_3O_4@MoS_2-Ag was determined by its photothermal properties and enzymatic activity. With irradiation at 808 nm near infrared (NIR) , local hyperthermia assisted Ag^+ leakage from the nanozyme surface. Local hyperthermia together with ·OH, and Ag^+ can attack the cell membranes of fungi and can induce 80% *C. albicans* death [49]. Similarly, an N-carbon nanozyme combined with NIR had a great antifungal effect on *C. albicans* and *Trichoderma rubrium* through the conversion of light energy into heat energy, leading to fungus death [50]. Fe_3O_4 nanozymes can reduce the concentration of H_2O_2 needed for disinfection and improve inhibition rates toward fungus. The NSBs are able to kill opportunistic pathogens such as *C. albicans* and *C. glabrata via* damaging cell membrane and oxidative stress [42]. Based on these results, nanozymes are expected to serve as disinfectants with strong antifungal ability for application in air and equipment disinfection. However, the nanozyme is mostly applied *in vitro* and its safety needs to be identified [49]. For instance, the residue (metal ions) applied *in vitro* may not affect their use effect due to the superficial contact with humans and the short time exposed in humans [51]. Once nanozymes enter the *in vivo* system, the location, biodegradability, and metabolizability will determine their toxicity.

3.2.2 Nanozymes that inhibit biofilm formation

It is well known that it is difficult to kill fungi due to their tough cell walls. In particular, fungi can form biofilms, secrete invasive enzymes, and form hyphae to achieve escape from immune cells, which brings great difficulties in the treatment of fungal infections. The adherence of fungi to biological or non-biological surfaces is the first step in the formation of a biofilm, and is also a necessary prerequisite for mucosal colonization and infection [52,53]. Studies have found that nanozymes can inhibit the prerequisites for fungal biofilm formation: adhesion and colonization [44]. The researchers found that nanoemulsions can scavenge biofilms by inhibiting the proliferation of hyphae, yeast, and pseudohyphae. At the same time, a

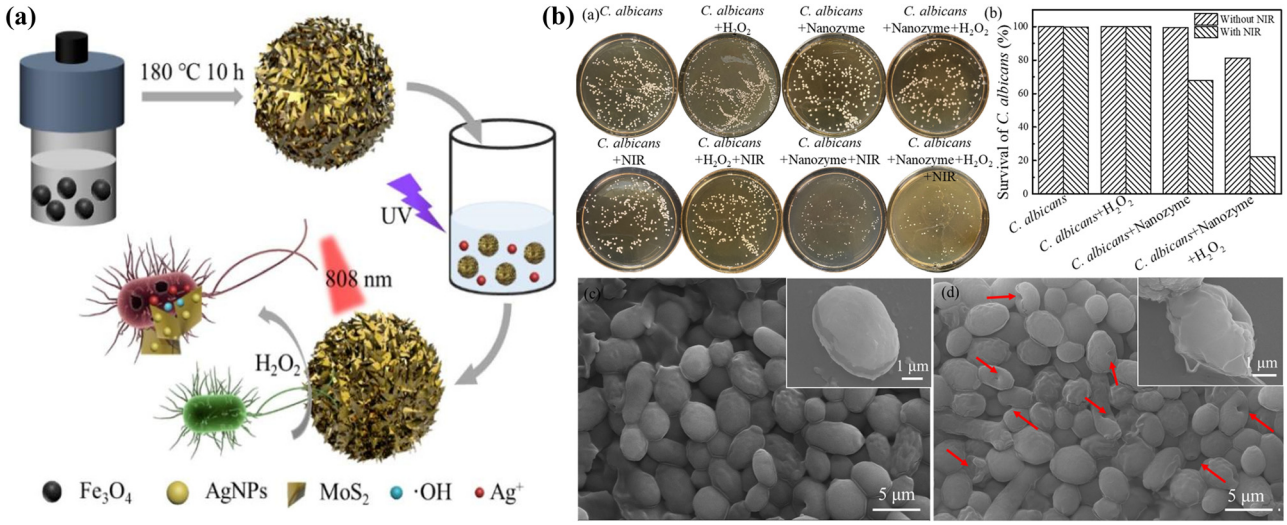

Figure 3: (a) Schematic of the preparation of Fe_3O_4@MoS_2-Ag for antibacterial or antifungal applications. (b) Fe_3O_4@MoS_2-Ag nanozyme for antifungal assay assessed by colony imaging (a), survival assay (b), and scanning electron microscopic (SEM) imaging (c and d) [49]. All images were reproduced with permission.

biofilm reduction from 46.6 to 86.2% was confirmed by crystal violet staining in the nanoemulsions treatment [48]. Many chronic diseases are related to fungal infections involving biofilm formation, such as gum infections [52], urinary tract infections, and respiratory tract infections [53]. Fungal biofilms play an important role in the formation and development of dental caries and periodontal disease. Fungi utilize polysaccharides and other nutrients to form biofilms, metabolize acid, and corrode the surface of teeth, resulting in periodontal tissue infection [54]. Pulp infection is a common clinical oral disease mainly resulting from *Enterococcus faecalis* and *C. albicans* [55]. Recently, researchers found that nano-silver particles inhibit *C. albicans* growth by generating ROS and limiting glucose uptake. When combined with glucose metabolism inhibitors (BrPA), they will produce a synergistic antifungal effect [56]. Glucose oxidase (GOX), as an intrinsic biocatalyst, is an oxidoreductase which effectively catalyzes the oxidation of glucose and converts it into H_2O_2 and gluconic acid. Inspired by this, Ji et al. developed GMNP, which catalyzes the degradation of biofilms and effectively kills fungi through a cascade of reactions mediated by the catalytic activity of GOX and POD (Figure 4) [45]. At the same time, experiments conducted *in vitro* determined that GMNP has good biocompatibility and blood compatibility. These studies show that nanozymes can be expected to be introduced into oral applications due to their potential in removing biofilms. Meanwhile, they also may be applied in pulp materials and catheters (central venous catheters, urinary catheters, *etc.*) to prevent biofilms formation. However, studies on their applications *in vivo* are rarely reported and it is necessary to evaluate the biological safety of nanozymes *in vivo*.

3.2.3 Potential applications in industry and agriculture

Proteins existing in the cytoplasm of *S. cerevisiae* are valuable in applications in the biotechnology and pharmaceutical industries. Fe_3O_4 MNPs with zymolyase-like activity showed lytic activity toward yeast cells and provided an alternative for industrial-scale cell disruption [47]. Laccase stems from fungi living in streams play an important role in various applications such as food processing, wastewater treatment, and biosensors. Nanocopper oxide (nanoCuO) caused serious deformation of fungal hyphae whether from clean or metal-polluted streams. Simultaneously, nanoCuO could regulate laccase activity in a concentration-dependent manner. The study can explain the relation between nanoCuO and laccase, which has potential application in wastewater treatment [57]. Fungal invasions in agricultural plants (*e.g.*, tomato, melon) lead to huge losses of fruits and vegetables. N, F co-doped TiO_2 gathered on the surface of *F. oxysporum*, causes destruction of cell wall and promotes the leakage of intracellular contents from cytoplasm (Figure 5). This provides a potential application in agriculture due to NPs' ability to stop the invasion of fruits by fungi with retained immunity [58]. Nanozymes, as a novel pesticide, can protect crops and fruits from fungus invasion and provide a convenient and low-cost method to break fungal cell walls. The emergence of

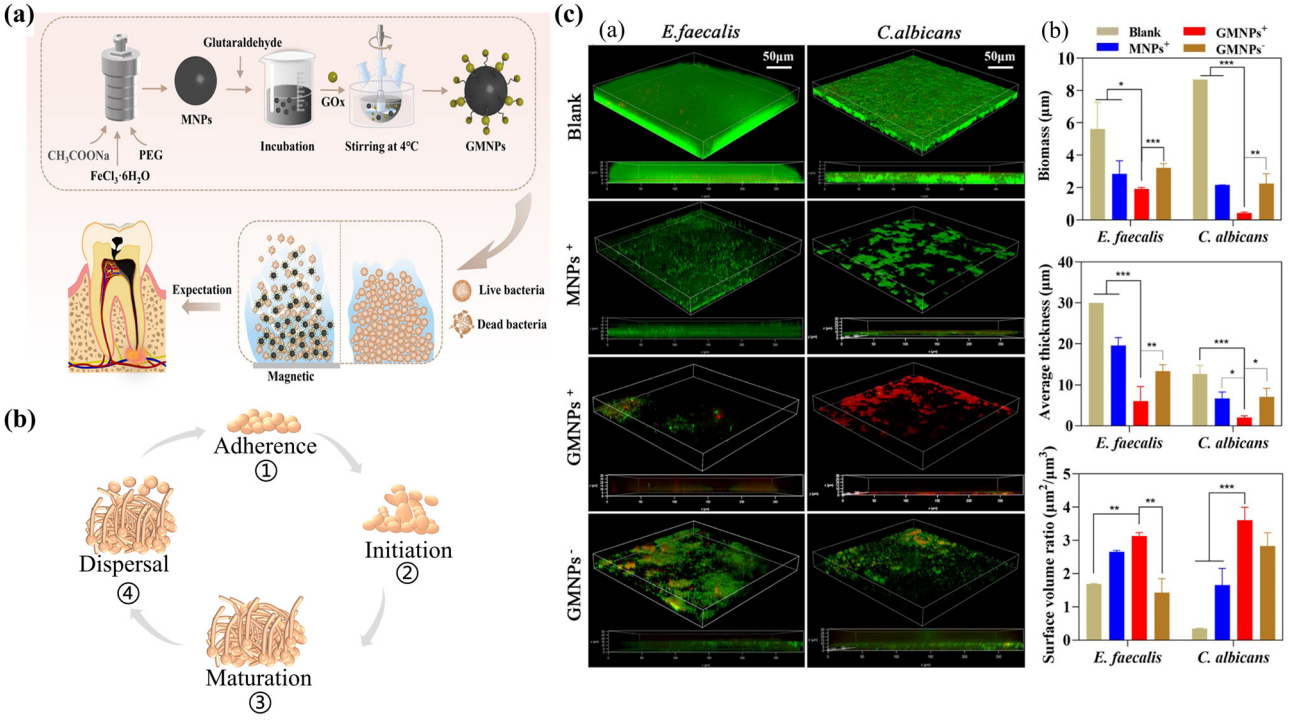

Figure 4: (a) Schematic of the preparation of GMNPs and their application in pulp infections. (b) Schematic of *Candida albicans* biofilm life cycle. (c) (Antibiofilm effect of GMNPs evaluated by three-dimensional imaging (a) and biomass, thickness, and volume images (b) [45]). All images were reproduced with permission.

nanozymes seems to be beneficial to agriculture and industry, but some problems have not been resolved. For example, industry should consider efficiency when nanozymes are applied in digesting the cell walls of yeast on a large scale. The problem that whether nanozymes affect the growth of crops and fruits should be evaluated. These issues need to be addressed for future applications of nanozymes.

4 The mechanism of nanozymes' action against fungus

Nanozymes are nanomaterials with intrinsic enzyme-like activity, which catalyze the substrates of natural enzymes and obey the same reaction kinetics. The enzyme-like activity of a nanozyme comes from its special nanostructure, without the introduction of additional catalytic groups. Recently, more and more nanomaterials with enzyme-like activity have been reported, which can be roughly divided into four types: oxidoreductase, hydrolase, lyase, and isomerase, of which oxidoreductase-mimicking activity is the most important, including SOD, CAT, POD, and OXD [38]. Consistent with the antifungal mechanisms of natural enzymes, nanozymes heavily catalyze the corresponding substrate to produce ROS to combat fungal invasion. Although ROS have important biological functions in humans, excessive ROS can cause death or apoptosis of normal cells [59,60].

4.1 Activities of nanozymes involved in ROS regulation

ROS are intermediate products that mainly include superoxide anion (O^{2-}), hydroxyl radical ($^{\cdot}OH$), H_2O_2, and singlet oxygen ($^1O_2$). Nanozymes with multiple enzyme activities have been designed to regulate ROS levels, in order to protect normal cells or disrupt tumor cells [61–63]. Generally, nanomaterials with POD and OXD activities generate ROS in the presence of H_2O_2 or O_2, and scavenging ROS and converting superoxide into H_2O_2 and O_2 are related to SOD (Figure 6) [64]. It must be noted that these activities are dependent on pH, that is, the optimal pH for POD and OXD falls into the range of acidic pH (3–6), while those for SOD or CAT is around physiological (neutral) pH. This dependence on pH suggests that the local microenvironment needs to be considered when using nanozymes for antifungal treatment, as increasing ROS is required to kill fungal cells, but

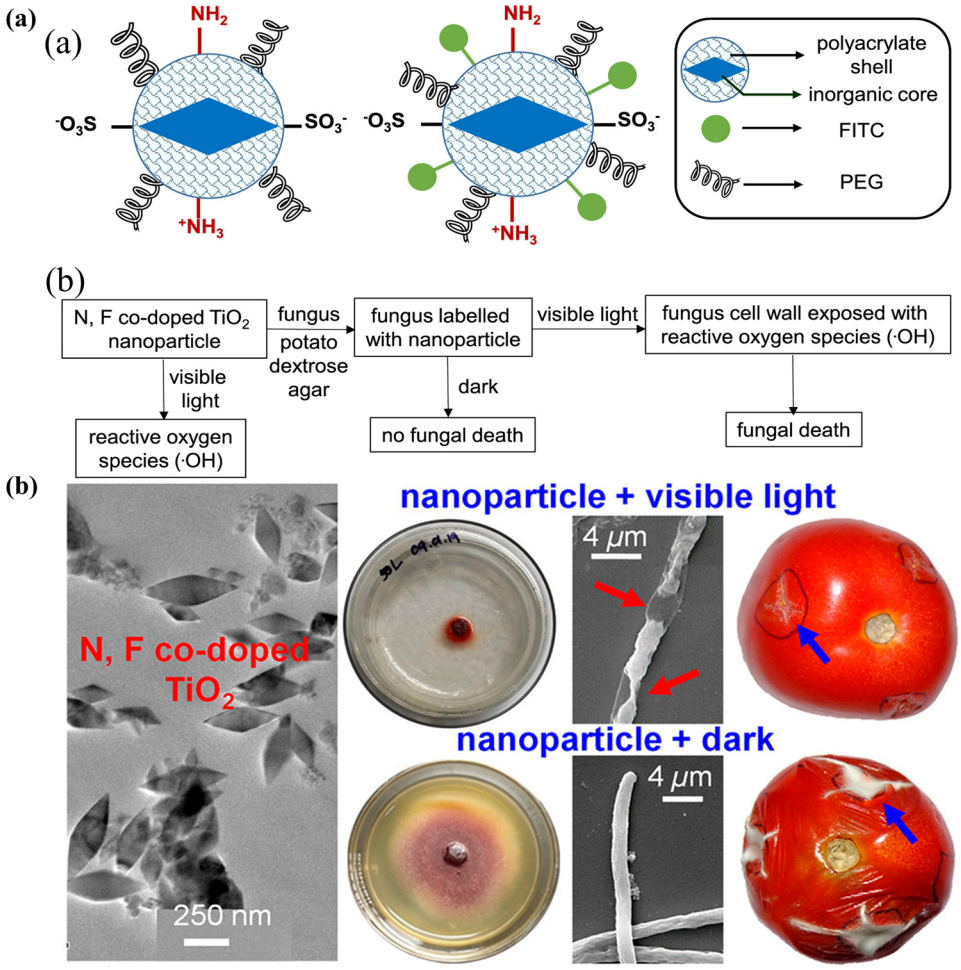

Figure 5: (a) (Schematic of the chemical structure of N, F co-doped TiO_2 (a) and the research method for the study of the effects of NPs on fungus (b)). (b) The antifungal application of N, F co-doped TiO_2 in agriculture evaluated by SEM images, fungal colony images and experiments *in vivo* [58]. All images reproduced with permission.

excessive ROS may damage host cells. Furthermore, one single nanozyme may perform multiple enzyme-like activities which favor cascade reactions to generate ROS by consuming a certain substrate. For instance, nanozymes with bifunctional glucose oxidase (GOX)-like and POD-like activities can generate H_2O_2 by glucose oxidation and then convert H_2O_2 into ·OH radicals [65], which not only generates toxic radicals, but also consumes nutrients for cell growth, leading to the effect of "killing two birds with one stone." Besides directly generating or scavenging ROS, nanozymes that can consume glutathione (GSH) may indirectly increase ROS by breaking the redox balance [11], which is effective for antifungal treatment. In addition, the activities of nanozymes can be regulated by physical signals, such as infrared light-induced photothermal effect [66,67] or chemical activators/inhibitors [68]. Collectively, the activities of nanozymes for ROS regulation can be tuned for antifungal application through multiple strategies.

4.2 The antifungal mechanism of nanozymes based on the ROS pathway

Many nanomaterials [39,40] showed strong enzyme-like activity and catalyze H_2O_2 to produce toxic radicals to achieve a sterilization effect. As a strong oxidant, H_2O_2 can kill pathogens such as *S. aureus*, *E. coli*, and *C. albicans*. Fe_3O_4 nanozymes combined with H_2O_2 can kill fungi by the generation of ROS. However, the low combination probability of nanozymes and fungi and the long distance between fungi and the generated ROS limit the antifungal effect of nanozymes [69]. Baldemir *et al.* found that nanoflowers with POD-like activity destroyed fungal cell membranes, depending on electrostatic interaction and ·OH [70]. To better trap fungi, Mukherjee's team designed a kind of TiO_2 NP co-doped with nitrogen and fluorine, which could attach to the fungus surface and induce fungal death *via* the interaction of ROS (·OH) with

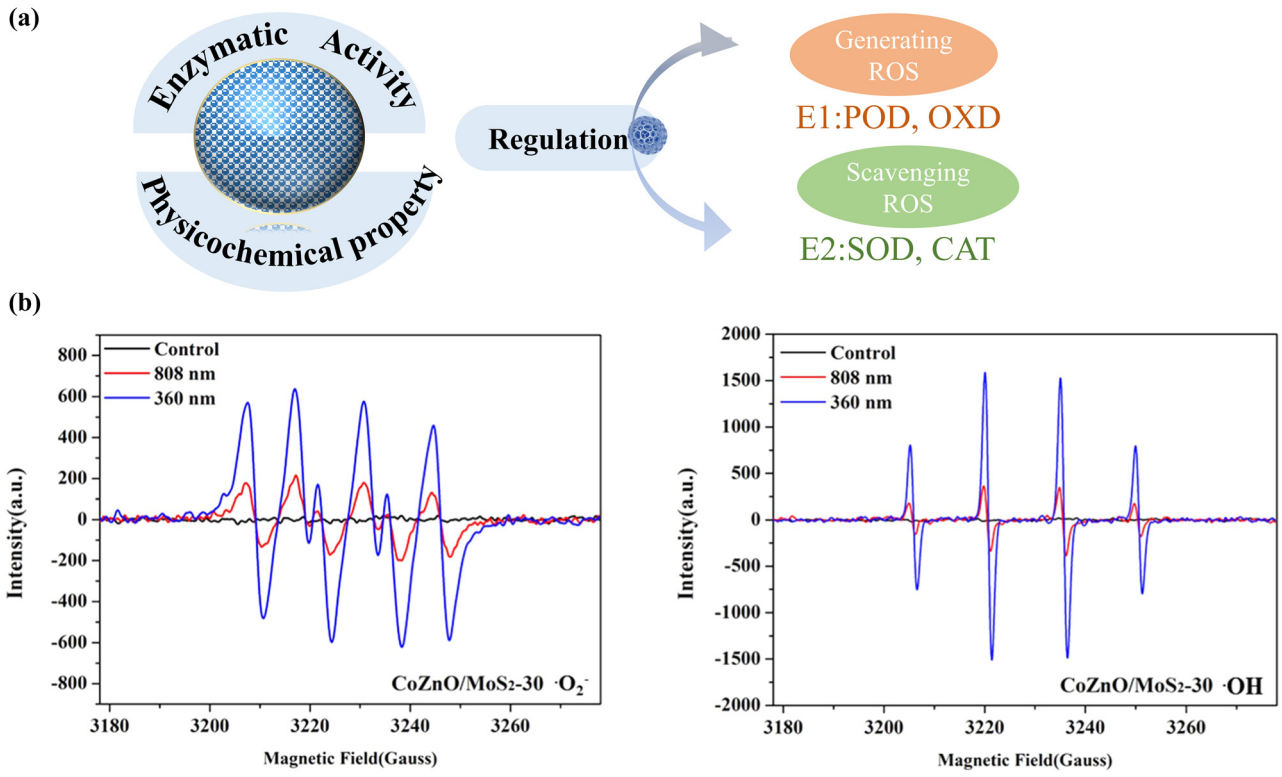

Figure 6: (a) Schematic of the regulation of ROS levels by nanozyme. (b) Electron spin resonance spectra of both superoxide radicals and hydroxyl radicals during POD-like catalysis of CoZnO/MoS_2 under photoirradiation at 808 or 360 nm [84]. All images were reproduced with permission.

the chitin or glucan in the fungal cell walls [58]. These ·OH can cleave to the glycosidic linkages of chitin or glucan, causing damage to the cell wall structure and leakage of intracellular contents. Nitrogen–iodine-doped carbon dots with POD-like activity can enhance the photocatalytic inactivation of *C. albicans* depending on ROS (·OH) through the gradual enhancement of POD activity under the visible light irradiation [71]. Nanozymes have the unique properties of nanomaterials as well as the catalytic functions of enzymes. The Fe_3O_4@MoS_2-Ag nanozyme captures fungal cells due to its rough surface and then induces fungus death *via* attacking the fungal cell membrane and further damaging DNA by its enzymatic activity and photothermal properties.

4.3 An antifungal nanozyme mechanisms based on non-ROS pathways

Nanozymes not only show ROS-dependent but also ROS-independent pathways for killing fungi (Figure 6). For example, Xiao *et al.* prepared Fe_3O_4 MNPs by the co-precipitation method, which showed strong lytic activity like that of zymolyase on yeast cell walls [47]. NanoCuO can destroy the morphology of fungi and inactivate laccases [57]. Of course, some transition metal oxide NPs are considered mimics of halogenating enzymes, the antifungal activity of which is based on hypohalous acids derived from halides, H_2O_2, or ·OH [72] (Figure 7).

In short, the antifungal mechanisms of nanozymes against fungal pathogens can be divided into two pathways: ROS-dependent and ROS-independent pathways. However, the exact ways of some nanozymes that work depending on the ROS-independent pathway have not been completely explained. For example, the nanoemulsions with strong antifungal activity do not have an exact explanation about the way their action directly targets the components of fungal cells or indirectly depends on the products produced by the catalysis of glutathione peroxidase (GSH-Px) [48].

5 The features and disadvantages of nanozymes in antifungal activity

At present, fungal infections are largely treated with polyenes, pyrimidines, pyrroles, allylamines, and echinocandins.

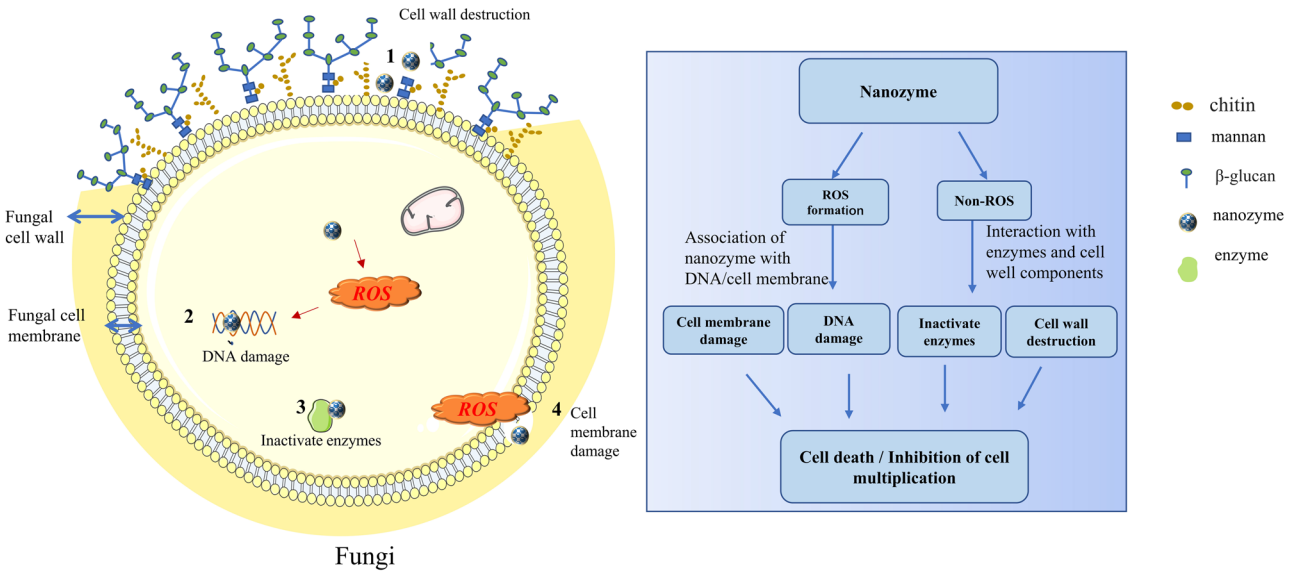

Figure 7: Schematic of nanozymes' mechanisms of action.

Although the drugs are effective, they have potentially fatal side effects such as liver and kidney toxicity. For example, amphotericin B can interact with mammalian cholesterol, damaging the cell membranes of normal cells [73,74]. The development of nanomaterials provides an opportunity to create new antifungal drugs. The antifungal activity of nanomaterials such as Ag NPs and Au NPs has been reported. Taking Ag NPs as an example, Ag NPs have a great effect on pathogens, namely *C. albicans*, *C. glabrata*, *Candida krusei*, and *Candida pseudotropicalis*, in the concentration range of 20–40 μg/mL [75]. Although Ag NPs have a great effect on fungus, they also exhibit high toxicity toward normal cells due to the excessive generation of ROS [76]. A natural enzyme can effectively degrade the fungal cell walls or damage cell membranes through enzymatic activity with high selectivity, such as that of chitinase, POD, and β-1,3-glucanase. More importantly, such an enzyme has enormous potential application in killing fungus due to the lack of chitin or glucan in humans. For example, zymolyase is a mixture extracted from the culture of *Arthrobacter luteus*, which has strong lytic activity toward fungal cell walls. Therefore, it is an alternative treatment for fungal infections *via* enzymatic activity. However, natural enzymes have the disadvantages of high cost, difficulty in mass production, and poor stability, which greatly limit their practical application [7].

Compared with natural enzymes, nanozymes similarly depend on catalytic defense against fungal pathogens, but nanozymes have the characteristics of easy preparation, low cost, and high stability [77]. These features greatly resolve the problems confronted by natural enzymes (Table 3). The above study showed that Fe_3O_4 MNPs mimicking zymolyase-like activity can destroy the cell wall of *S. cerevisiae*. Certain enzymes can damage cell membranes and the integrity of fungal cells through high levels of ROS, depending on their intrinsic enzymatic activity. But natural enzymes cannot regulate the levels of ROS. Once the production of ROS exceeds the antioxidant capacity of cellular antioxidants in biological systems, it will damage normal cells. Different from natural enzymes, nanozymes are able to regulate the production of ROS through pH, temperature, morphology, size, and so on [38]. A nanozyme, as a novel artificial enzyme, generates ROS to destroy the integrity of cell membranes and remove the matrix components of biofilms. The process whereby immune cells eliminate fungus starts with the fungus extending its pseudopod and wrapping around the immune cells. In this process, rough surfaces (such as pollen) are more adherent than smooth ones. Inspired by the innate immune cells, the rough surface of Fe_3O_4@MoS_2-Ag more easily traps fungi [49]. Similarly, nanoflowers shorten the distance between the particles and the fungus through electrostatic action, and then kill stubborn fungi by the Fenton reaction. Generally speaking, fungi can achieve escape from immune cells by their virulence, such as using hyphae and invasion enzymes [42]. Nanozymes can effectively and efficiently kill fungi and inhibit hyphae formation to avoid escape. Like antifungal drugs, nanozymes can be wide-spectrum antifungal agents, which can kill various fungi and bacteria such as *C. albicans*, *Aspergillus flavus*, *Aspergillus niger*, *E. coli*, and *S. aureus* [44]. More importantly, the fungi have a lower possibility of forming drug-resistant strains

Table 3: Comparison among antifungal drugs, natural enzymes, nanomaterials, and nanozymes in antifungal activity

	Advantages	Disadvantages	References
Antifungal drugs	Broad spectrum antifungal activity (polyenes)	Development of drug resistance	[4,78,88]
	Long biological half-life	Liver and kidney toxicity	
	Good penetration into the central nervous system (CNS) (Azoles)	High price	
	Antibiofilm activity (Echinocandins)	Lengthy treatment	
Natural enzymes	High selectivity	High cost	[7]
	High catalytic activity	Difficulty in mass production	
	Good biocompatibility	Poor stability	
	Exact mechanisms		
Nanozymes	Easy preparation	Poor biocompatibility	[7,44,49]
	Low cost	Ambiguous mechanism	
	Strong adsorption (electrostatic, tough surface)	limited types of nanozymes	
	Tunable enzymatic activity	few applications *in vivo*	
	Broad spectrum antifungal activity		
	Low possibility for drug resistance		
	Antibiofilm activity		
Nanomaterials	Broad spectrum antifungal activity	High toxicity	[75,76]
	Easy preparation	Unable to regulate the level of ROS	
		Few applications *in vivo*	

due to the definite composition of nanozymes. Collectively, compared to other antifungal strategies, such as antibiotics and antimicrobial peptide, nanozymes provide robust antifungal action with low drug resistance.

6 The perspectives and challenges of using nanozymes to combat fungi

Since Fe_3O_4 NPs with POD-like activity were first found in 2007, hundreds of nanozymes have been investigated in various fields such as biosensing, theranostics, and antimicrobials [7]. However, antifungal applications of nanozymes are in an exploratory stage, and some problems urgently need to be solved.

(1) The exact mechanism of nanozymes against fungal pathogens is not still completely understood. More comprehensive and in-depth research on nanozymes should be conducted to make them more easily acceptable by the public.

(2) The toxicity of nanozymes has not been completely evaluated. As nanozymes are nanomaterials with enzyme-like activity, the toxicity may come from two aspects: the enzyme-like activity and the components of nanomaterials. In antifungal treatment depending on ROS, OXD, or POD-like activities are preferred to boost ROS level in order to kill fungi rapidly and effectively. However, it is well known that ROS is a double-edged sword, that is, high ROS levels damage host cells. Besides toxic ROS, the components of nanozymes may also cause toxicity. Once nanozymes enter the *in vivo* system, the location, biodegradability, and metabolizability will determine their toxicity. For instance, iron oxide nanozymes can be eventually degraded into iron ions once they enter acidic microenvironments and the iron ions are able to be metabolized through iron-related signal pathways, therefore showing low toxicity. In contrast, if the component of nanozymes cannot be metabolized, it will accumulate and cause persistent damage to the host system. To avoid toxicity, targeting the modification of nanozymes comprising biocompatible materials may be an effective approach that can specifically kill fungi. Taken together, toxicity is a critical issue for using nanozymes in antifungal treatment, and needs to be systematically evaluated with multiple models and minimized for the host system.

(3) Based on the discovery of fluconazole-resistant *C. albicans* [78], the problem of drug resistance needs to be solved urgently. The antifungal action of nanozymes mainly concentrates on the production of ROS. However, it is unknown whether fungi will be resistant to nanozymes, and the problem should be explored in future.

(4) Disease related to fungi is usually associated with virulence factors (hyphae, biofilms, and invasion enzymes), and hyphae formation seems to be

connected with immune escape. At present, reports about the inhibition of virulence factors by nanozymes are lacking. Nanozymes facilitating the maturation of immune cells based on ROS have been reported [79]. Therefore, designing the nanozyme that can promote immune cells maturation seems to be a potential direction.

(5) Nanozymes are expected to be applied in biomedicine research and clinical practice with the following advantages: high biocompatibility, biodegradability, and selectivity.
   (a) To improve the biosafety of nanozymes, it is possible to use biocompatible or biodegradable components existing in human body to synthesize or modify nanozymes, such as amino acids, peptides, nucleic acids, or polysaccharides.
   (b) To improve antibacterial efficacy, more enzyme-like activities of nanozymes need to be designed and realized by mimicking the active center of natural enzymes. Besides those activities for ROS regulation, nanozymes with protease-like, lysozyme-like, nuclease-like, or lipase-like activities that can degrade proteins, polysaccharides, nucleic acids, or lipids may facilitate antifungal efficiency.
   (c) At present, nanozymes are mostly designed for oxidoreductase-mimicking activities. The designs of nanozymes directly targeting the components of the fungal cell wall are few. To improve selectivity, the nanozymes can be designed or modified to specifically bind to the surface of fungal cell walls. For example, if a nanozyme has an active site similar to the natural enzyme of β-1,3 glucanase, it may directly target fungal cell walls [80].

Nanozymes represent a new but rapidly developing cross-disciplinary field, the application of which has just begun. In addition, the antifungal activity of natural enzymes provides a way to design nanozymes. With the deepening of research and the transfer of knowledge from basic research to clinical practice, we believe that nanozymes will become new antifungal agents to prevent and treat fungal infections to improve the quality of human health and life.

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