Antifungal Nanomaterials: Current Progress and Future Directions

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ABSTRACT

Fungal infection poses a severe threat to human health worldwide resulting in a serious problem in clinic. Due to the limited arsenal of existing antifungal drugs, the nanomaterials were thus regarded as the candidate for developing new antifungal drugs. On the one hand, the antifungal nanomaterials are divided into inorganic nanomaterials, organic nanomaterials, and hybrid nanomaterials, among which inorganic nanoparticles include metal and semiconducting categories. On the other hand, they can also be divided into inorganic particles, organic structures, and mixed nanostructures. Currently various directions for the research and development of antifungal nanomaterials are undergoing. To improve the antifungal effect, the chemical modification of nanomaterials and combination with the available drugs are two strategies widely used. In addition, optimizing the synthetic process of nanomaterials is also a major method to broaden their antifungal application. This review focuses on the current research progress and cutting-edge technologies of antifungal nanomaterials in the field of pharmacodynamics, synthesis and combination of drugs. The nanomaterial will provide a promising and broadly effective antifungal strategy and represent a potentially repositionable candidate for the treatment of fungal infections.

Keywords: antifungal, nanomaterial, nanoparticle

INTRODUCTION

Compared with antibiotics, the number of antifungal agents is very limited. At present, there are approximately 80 kinds of antifungal drugs used in clinic.[1] The three major classes of clinical antifungal agents are ergosterol synthesis inhibitors (azoles, allylamines amines, morpholines), fungal cell membrane lysis agents (polyenes), and β-glucan prochloroglycan synthesis inhibitors (echinomycins). In addition, the pyrimidine analogues interfere with the synthesis of fungal nucleic acid and are widely used as adjuvant drugs,[2,3]; however, with the abuse of antibiotics and antifungal drugs, antifungal resistance has emerged as a serious problem in clinic for treatment. For example, *Candida albicans* resistance has been on the rise in recent years. According to research, *C. albicans* resistance increased from 36.36% in 2003 to 63.98% in 2005.[4] The problem of fungal resistance greatly increases the difficulty of clinical treatment and seriously threatens human health. Besides, the incidence of invasive fungal infections has reached more than one in 100,000 (Pfaller et al. 2006),[5] and the fatality rate has reached 20% to 40% (*C. albicans*), 20% to 70% (*Aspergillus fumigatus*), and 50% to 90% (*Cryptococcus neoformans*), respectively (Butts and Krysan 2012).[2,6] Therefore, it is extremely urgent and important to seek new treatment strategies and develop new therapeutic drugs. For the latter, one of the challenges is to develop fungus-specific drugs.[7]

With the development of nanomaterial-related technology, the specific surface area and unique physical
properties such as light and electricity can be developed as an ideal antifungal target. Many nano-antifungal materials include metallic nano-antifungal agents like nano-Ag, photocatalytic nano-antifungal agents like nano-TiO₂, and composite nano-antifungal agents have been experimentally proven to be effectively antifungal.

**COMMON ANTIFUNGAL NANOMATERIALS**

Currently, the common antifungal nanomaterials include three categories: inorganic nanoparticles, organic nanostructures, and hybrid nanostructures. Inorganic nanoparticles mainly contain metal nanoparticles and semiconductor nano-ions with photocatalytic effect.

**Silver Nanoparticles**

Silver nanoparticles are inorganic nanomaterials that have been studied extensively. Usually the silver nanoparticles with different concentrations can be incubated with fungus like *Trichosporon asahii* or *Ascomycetes* or *Aspergillus* in YPD-agar plate for different time periods. Then the fungistatic effect was evaluated by minimum inhibitory concentration. The antifungal effect of silver nanoparticles was then examined by the scanning electron microscope and transmission electron microscope or evaluated by minimum inhibitory concentration.

At present, it is believed that the mechanism of the antibacterial activity of silver nanoparticles includes binding sulfhydryl groups to inactivate enzymes and inhibiting normal cell oxidation inactivation of enzymes by mercapto, which blocks the normal process of cell oxidation; combining with DNA to inhibit its replication and affect the normal proliferation of cells; binding to surface proteins alters ion channel permeability and damages cell walls; generating reactive oxygen species that are toxic to cells. The production of reactive oxygen species toxic to cells. However, the cytotoxicity of silver nanoparticles cannot be ignored. It generates toxic and side effects on the host cells, which is usually attributed to the production of reactive oxygen species. Furthermore, the toxicity is closely related to particle size, sedimentation, and synthetic pathway. Studies have shown that silver nanoparticles act on a variety of cellular targets, causing mitochondrial damage, leading to inflammation, free radical production and cell membrane damage, and ultimately cell death. In addition, there is research showing that the cytotoxicity of silver nanoparticles is closely related to the release of Ag ions. After entering the cell, the silver nanoparticles are degraded in the cell, releasing Ag ions that interfere with normal mitochondrial function and inducing cell apoptosis. When developing antifungal silver nanoparticles, we need to consider safety, reduce their cytotoxicity, and achieve more precise targeting of drug delivery, such as by adding some capping agents and combining with other drugs. The size of nanoparticles also needs to be considered, as smaller nanoparticles can easily move around in the body, deposit in target organs, penetrate cell membranes, and stay in mitochondria.

**Semiconductor Nanoparticles**

Semiconductor nanoparticles are usually photocatalyt-ic due to their specific optical and electrical characteristics. They can be irradiated by a beam of certain wavelength to exert their efficacy in the indicated parts. This will significantly reduce the toxic and side effects of drugs on other tissues and organs and finally improve the duration of effective drug concentration to achieve accurate drug delivery. The representative materials are titanium, zinc, and cadmium oxide nanoparticles, some of which have good tissue compatibility, durability, and high temperature resistance. This provides the possibility for improvement of medical practices such as organ implantation.

The application of nanomaterials in antifungal purpose is also being developed. For example, nanometer SnCl₄/SiO₂ can be used as a mild and economical catalyst for the synthesis of benzimidazole derivatives with antifungal activity. Compared with the traditional synthesis process, nanomaterials as catalysts have the advantage of environmental friendliness and mild reaction conditions.

**Organic Nanoparticles**

Organic nanomaterials, including solid lipid nanoparticles, liposomes, vesicles, micelles, and nano-emulsions, are usually loaded with antifungal drugs to form an efficient targeted drug delivery system. By virtue of their unique properties, such as small size, large surface area, high concentration loading, and the interaction with different phases on the interface, organic nanomaterials improve the selectivity and effectiveness of drugs and reduce the toxicity of drugs, so as to reduce the dose of drugs, enhance their therapeutic effect, and improve their safety in clinical practice. For example, the topical gel of solid lipid nanoparticles loaded with the famous antifungal agent fluconazole can improve its efficiency in the treatment of pityriasis versicolor. And a new chitosan nanocapsule drug delivery system containing ticonazole or econazole nitrate can solve the problem of low water solubility of ticonazole and econazole in the treatment of vaginal candidiasis.

Daniel Brustolin Ludwig et al. made a chitosan-coated poly acid nanoparticle containing Amphotericin B, which is demonstrated to be an potential carrier of AmB by them; Nasseri et al. showed that *Zataria multiflora* essential oil–loaded solid lipid nanoparticles are useful in controlling fungal pathogens. Due to the high affinity for lipid binding, the lipid system carrying amphotericin B has also been extensively studied. Different antifungal organic nano-systems have been continuously manufactured and further improved to improve their safety and economy.
Hybrid Nanomaterials
Most of the single nano-antibacterial particles have some limitations, and their therapeutic effects are often not satisfactory. The cytotoxicity of many nanomaterials, including silver nanoparticles, needs further study. Therefore, the study of composite nano-antifungal agents is particularly important. Nowadays, the research of composite nanomaterials has taken a great step forward. We can see, for example, the research on TiO2 supporting other metals as catalysts to improve its antifungal performance,[23] and a series of numerous drug-loaded nanomaterials are constantly emerging. The coupling between different nanomaterials and antifungal agents expands the research scope of antifungal nanomaterials.

IMPROVEMENT OF ANTIFUNGAL NANOMATERIALS

Improving the Function of Antibacterial Nanomaterials Through Modification
Specific modifications to nanomaterials can improve their antifungal properties. Montazeri et al.[24] performed amino functionalization on silica nanomaterials, and verified that the modification had a beneficial effect on the antifungal efficacy of econazole through control experiments and the effect of this modification on the antifungal efficacy of econazole was verified by controlled experiments.[25] The scientists made the pH-sensitive coatings or simply binding agents to titanium dioxide nanotubes via these bonds, pH-triggered release retains more agents in the neutral environment, while accelerating release in the acidified environment.[26] Zhuk et al.[27] reported that coatings obtained by direct assembly of tannic acid and cationic antibiotics (tobramycin, gentamicin, and polymyxin B) prevented drug release for up to 35 days in a neutral environment and reduced the burst release at lower pH, greatly prolonging the duration of the antibacterial effect.

Enhancing Efficacy by Coupling With Other Drugs
Combining with nanomaterials is a promising way to explore possible strategies to combat drug resistance when antifungal drugs are limited. The highly specific surface area and special physical properties of nanomaterials make it possible to significantly improve the efficacy of nanomaterials when used in combination with traditional antifungal drugs. Muhammad Asim Hussain et al.[25] compared the polar antibacterial effects of nystatin myosin and ketoconazole with their silver nanoparticles and found that the silver nanoparticles enhanced their antibacterial effects and significantly increased the percentage of fungal inhibition (90%–100%). Benjamin Horev et al.[28] used nanoparticle materials to carry the farnesol to disrupt oral biofilm virulence. The effects of nanoparticle-mediated farnesol delivery on the onset of carious lesions were striking. Both the number and severity of carious lesions (Ds level) were significantly reduced in farnesol-loaded nanoparticle-treated animals compared with nanoparticle controls. In sharp contrast, free farnesol showed no effect on either incidence or severity of lesions compared with vehicle control, which established that drug efficacy of farnesol is improved through delivery using pH-responsive polymer nanoparticle carriers.[28]

Improving Synthesis Pathway of Antibacterial Nanomaterials
It would be a novel idea to investigate the new biosynthesis methods by combining with bionics, which can not only reduce costs and achieve environmental friendliness, but also improve the antifungal properties of nanomaterials in all aspects and reduce the toxic and side effects.[8] Many different types of biological templates are applied in the synthesis of nanomaterials. Because microorganisms contain metal-binding peptides, and some of them have the ability to reduce metal ions, they can be used as a tool to prepare metal nanoparticles. For example, Pseudomonas has been used in the synthesis of silver nanoparticles, and Erythrococcus has been used in the synthesis of gold nanoparticles. In addition, Lactobacillus, Actinomycetes can also be used in synthesis.[29]

OTHER APPLICATIONS OF NANOMATERIALS IN THE CLINICAL FIELD
Nanomaterials are also widely used in other clinical fields. For example, in cancer treatment, medical personnel can use the photosensitive properties of nanoparticles to target drug delivery, enhance local drug concentrations, and improve prognosis.[30] In addition, nanomaterials can be used as probes to detect early DNA damage in patients with cancer.[31] In addition, given the ability of nanomaterials to cross the blood-brain barrier, some scholars have suggested that nanomaterials may play an important role in the treatment of neurological diseases.[32] The development of new nanomaterials is also expected to improve existing drug delivery methods, improve drug utilization, and reduce their toxic and side effects.[33]

DISCUSSION
With the increasing incidence of fungal resistance, the limited arsenal of antifungal agents available is not able to meet the clinical requirement. Commonly used antifungal drugs include azole, polyene, and echinomycin. Most of the new drug research is based on these substances for structural modification to improve efficacy and safety, but further efforts are needed to develop new targets and new compounds.[7] Drug resistance is
becoming a problem for existing antifungal drugs. Many kinds of azoles and polyechinoderms have some resistance.[34] Due to the high cost and time-consuming process of developing a chemosynthetic new drug, the application of nanomaterials to the antifungal drugs might provide a bright future.

The inorganic antifungal nanomaterials have made some progress in research and development and it is still necessary to explore the application of hybrid nanomaterials with larger space. In addition, a large number of studies have been carried out to verify the antifungal effect of materials, while the research on the mechanism is somewhat insufficient. Many studies have verified the antifungal effects of many nanomaterials, such as nanoparticles of Ag and ZnO.[11,35] In addition, the antifungal effects of many organic nanoparticles, such as curcumin and chitosan, and semiconductor nanoparticles, such as silicon SiCl4, have also been demonstrated.[36,37] At present, the mechanism of metal nanoparticles has been studied deeply, especially ZnO and Ag nanoparticles.[38–40] The development of new therapeutic targets and new mechanism to inhibit the growth of fungi is also ongoing.

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References

1. Li M-h, Tan Q-m. Clinical application of antifungal drugs [in Chinese]. People’s Military Doctor. 2007;1:32–34.
2. Zhang Y, Chen S-m, Guo S-y, Hou W-t, Jiang Y-y, An M-m. Advances in research on new antifungal drugs [in Chinese]. The Fungus Sinica. 2019;038:1253–1263.
3. Campoy S, Adrio JL. Antifungals. Biochem Pharmacol. 2016;133:86–96.
4. Wang Y, Huang H, Li J-f, Wen X-d, Jiang Y-y. Advances in the study of fungal drug resistance [in Chinese]. Pharmaceutical Care and Research. 2009;3:13–17.
5. Pfaller MA, Pappas PG, Wingard JR. Invasive fungal pathogena: current epidemiological trends. Clin Infect Dis. 2006;43(suppl 1):S3–S14.
6. Butts A, Krysan DJ. Antifungal drug discovery: something old and something new. PLoS Pathog. 2012;8(9):e1002870.
7. Liu Y, Yan L, Jiangu Y-y. Research progress of new antifungal drugs [in Chinese]. Chinese Journal of Mycology. 2018;13:45–53.
8. Miao Q, Cao Y-b, Zhang S-q, Lin H, Jiang Y-y. Progress in the study of anti-fungal activities of nanomaterials and their mechanisms [in Chinese]. Chinese Journal of Mycology. 2012;007:111–115.
9. Xia ZK, Ma QH, Li SY, et al. The antifungal effect of silver nanoparticles on trichosporon asahii. J MicrobIol Immunol Infect. 2014;49:182–188.
10. Vitiello G, Silvestri B, Luciani G. Learning from nature: bioinspired strategies towards antimicrobial nanostructured systems. Curr Top Med Chem. 2018;18:22–41.
11. Siddiqi KS, Husen A, Rao RAK. A review on biosynthesis of silver nanoparticles and their biocidal properties. J Nanobiotechnology. 2018;16:14.
12. AshaRani PV, Kaur Mun GL, Prakash Hande M, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano. 2009;3:279–290.
13. Eom HJ, Choi J. P38 mapk activation. DNA damage, cell cycle arrest and apoptosis as mechanisms of toxicity of silver nanoparticles in jurkat t cells. Environ Sci Technol. 2010;44:8337–8342.
14. Piao MJ, Kang KA, Lee IK, et al. Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis. Toxicol Lett. 2011;201:0–100.
15. Singh RP, Ramarao P. Cellular uptake, intracellular trafficking and cytotoxicity of silver nanoparticles. Toxicol Lett. 2012;213:249–259.
16. Nel A, Xia T, Mdler L, Li N. Toxic potential of materials at the nano level. Science. 311, 622–627.
17. Zamani L, Faghih Z, Zomorodian K, Mirjalili B, Jalilian A, Khabnadideh S. Nano-SnCl4:SIO2, an efficient catalyst for synthesis of benzimidazole drivatives as antifungal and cytotoxic agents. Res Pharm Sci. 2019;14:496–503.
18. El-Housiny S, Shams Eldeen MA, El-Attar YA, et al. Fluconazole-loaded solid lipid nanoparticles topical gel for treatment of pityriasis versicolor: formulation and clinical study. Drug Deliv. 2018;25:78–90.
19. Calvo NL, Sreekumar S, Svetaz LA, Lamas MC, Leonard D. Design and characterization of chitosan nanof ormulations for the delivery of antifungal agents. Int J Mol Sci. 2019;20:3686.
20. Ludwig DB, Camargo LEAD, Khalil NM, Auler ME, Mainardes RM. Antifungal activity of chitosan-coated poly(lactic-co-glycolic) acid nanoparticles containing amphotericin b. Mycopathologia. 2018;183:659–668.
21. Nassiri M, Golmohammadzadeh S, Arouiee H, Jaafari MR, Neamati H. Antifungal activity of zataria multiflora essential oil-loaded solid lipid nanoparticles in-vitro condition. Iranian J Basic Med Sci. 2016;19:1231–1237.
22. Faustino C, Pinheiro L. Lipid systems for the delivery of amphotericin B in antifungal therapy. Pharmaceutica. 2020;12:29.
23. Yu KP, Huang YT, Yang SC. The antifungal efficacy of nano-metals supported tio2 and ozone on the resistant aspergillus niger spore. J Hazard Mater. 2013;261:155–162.
24. Montazeri M, Razzagh-Abyaneh M, Nasrollahi SA, Mai-bach H, Nafisi S. Enhanced topical econazole antifungal efficacy by amine-functionalized silica nanoparticles. Bulletin of Materials Science. 2020;43:13.
25. Hussain MA, Ahmed D, Anwar A, et al. Combination therapy of clinically approved antifungal drugs is enhanced by conjugation with silver nanoparticles. Int Microbiol. 2018;22:239–246.
26. Li Y, Yang Y, Li R, Teng X, Qin Y. Enhanced antibacterial properties of orthopedic implants by titanium nanotube surface modification: a review of current techniques. Int J Nanomedicine. 2019;14:7217–7236.
27. Zhuk I, Jariwala F, Attygalle AB, Wu Y, Libera MR, Sukhishvili SA. Self-defensive layer-by-layer films with bacteria-triggered antibiotic release. ACS Nano. 2014;8:7733–7745.
28. Horve B, Klein MI, Hwang G, et al. Ph-activated nanoparticles for controlled topical delivery of farnesol to
disrupt oral biofilm virulence. *ACS Nano*. 2015;9:2390–2404.

29. Park TJ, Lee KG, Lee SY. Advances in microbial biosynthesis of metal nanoparticles. *Appl Microbiol Biotechnol*. 2016;100:521–534.

30. Liu Y, Xu Y, Zhang Z, et al. A simple, yet multifunctional, nanoformulation for eradicating tumors and preventing recurrence with safely low administration dose. *Nano Lett*. 2019;19:5515–5523.

31. Lee SH, Jun BH. Silver nanoparticles: synthesis and application for nanomedicine. *Int J Mol Sci*. 2019;20:865.

32. Furtado D, Björn malm M, Ayton S, Bush Al, Kempe K, Caruso F. Overcoming the blood-brain barrier: the role of nanomaterials in treating neurological diseases. *Adv Mater*. 2018;30:e1801362.

33. Patra JK, Das G, Fraceto LF, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnology*. 2018;16:71.

34. Perlin DS, Rautemaa-Richardson R, Alastruey-Izquierdo A. The global problem of antifungal resistance: prevalence, mechanisms, and management. *Lancet Infect Dis*. 2017;17:e383–e392.

35. Miri A, Mahdinejad N, Ebrahimy O, Khatami M, Sarani M. Zinc oxide nanoparticles: Biosynthesis, characterization, antifungal and cytotoxic activity. *Mater Sci Eng C Mater Biol Appl*. 2019;104:109981.

36. Bhawana, Basniwal RK, Buttar HS, Jain VK, Jain N. Curcumin nanoparticles: preparation, characterization, and antimicrobial study. *J Agric Food Chem*. 2011;59:2056–2061.

37. Mousavi SA, Ghotaslou R, Kordi S, et al. Antibacterial and antifungal effects of chitosan nanoparticles on tissue conditioners of complete dentures. *Int J Biol Macromol*. 2018;118:881–885.

38. Li J, Sang H, Guo H, et al. Antifungal mechanisms of ZnO and Ag nanoparticles to Sclerotinia homoeocarpa. *Nano technology*. 2017;28:155101.

39. Kumari M, Giri VP, Pandey S, et al. An insight into the mechanism of antifungal activity of biogenic nanoparticles than their chemical counterparts. *Pestic Biochem Physiol*. 2019;157:45–52.

40. Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. *Int J Mol Sci*. 2016;17:1534.