REVIEW
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Dual-action therapeutics of *Ganoderma* spores: integrating bioactivity and delivery for acute lung injury treatment

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Competing interests

The authors declare no conflicts of interest.

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Abbreviations

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; GLS, *Ganoderma lucidum* spores; COPD, chronic obstructive pulmonary disease; COX-2, cyclooxygenase; COVID-19, novel coronavirus disease; DDS, drug delivery system; DPI, dry powder inhaler; E/E-SBGS ,ethanol/ethanol extract; EPR, permeability and retention; Gal, galactose; Glc, glucose; GLP4, Ganoderma lucidum activated peptide; GLSP, *Ganoderma lucidum* spore polysaccharides; IEC-6, rat intestinal crypt epithelial cells; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-18, interleukin-18; iNOS, nitric oxide synthase; LPS, lipopolysaccharide; LTBI, spontaneous latent tuberculosis infection; mTOR, target of rapamycin; NLRP3, Nucleotide-binding oligomerization domain-like receptor protein 3; NMR, nuclear magnetic resonance; ROS, reactive oxygen species; SBGS, broken wall spores of Ganoderma lucidum; TCM, traditional Chinese medicine; TNF-α, tumor necrosis factor α.

Citation

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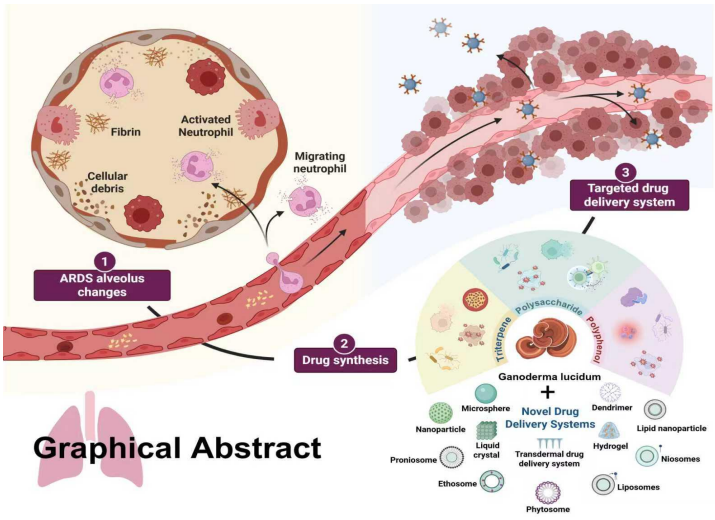
Abstract

Acute lung injury (ALI) is a critical condition characterized by the destruction of the alveolar-capillary barrier, infiltration of inflammatory cells, and an imbalance in oxidative stress, with a mortality rate reaching 30%-40%. Acute respiratory distress syndrome (ARDS) represents a severe form of ALI. The pathogenesis of ALI is complex, involving a cytokine storm, excessive production of reactive oxygen species (ROS), and endothelial cell damage. Current treatments, such as mechanical ventilation and glucocorticoids, offer limited efficacy and significant side effects, highlighting the urgent need for the development of novel, targeted treatment strategies. Recent studies have demonstrated that bioactive components of *Ganoderma lucidum* spores (GLS), particularly *Ganoderma lucidum* polysaccharides and triterpenes, possess significant pharmacological properties such as antioxidant, anti-inflammatory, and immunomodulatory activities.In recent years, natural drug delivery systems have gained considerable attention due to their biocompatibility and functional diversity. Among these, GLS have emerged as promising carriers for the treatment of ALI, owing to their unique physicochemical properties and biological activity.The *Ganoderma lucidum* spore delivery system provides a dual-treatment paradigm, functioning simultaneously as a natural carrier and an active ingredient, which enhances its efficacy and safety for ALI therapy. Compared to synthetic materials, it boasts superior biodegradability, lower immunogenicity, and reduced preparation costs, making it well-suited for industrial-scale production. Furthermore, its clinical translational potential has been demonstrated in tumor adjuvant therapy, where it has shown promise in enhancing immune function and improving the quality of life for chemotherapy patients.This article reviews the potential applications and mechanisms of GLS as drug delivery carriers in the treatment of ALI.

Keywords: *Ganoderma lucidum* spores, drug delivery system, acute lung injury, inflammatory response, therapeutic mechanism



Graphical Abstract

Highlights

1. Dual-action therapeutic platform: *Ganoderma lucidum* spores function synergistically as both a natural drug delivery carrier and an active pharmaceutical ingredient, offering a combined bioactivity-and-delivery strategy for treating acute lung injury (ALI).
2. Superior carrier properties: Compared to synthetic materials like PLGA, the spore-based system demonstrates better biodegradability, lower immunogenicity, ideal particle size for lung deposition, and potential for cost-effective industrial-scale production.
3. Multi-mechanistic efficacy: The bioactive components (e.g., polysaccharides, triterpenes) within the spores exert anti-inflammatory, antioxidant, and immunomodulatory effects by regulating key pathways such as NLRP3 inflammasome and PI3K/Akt/mTOR, addressing the complex pathogenesis of ALI.

Medical history of objective

Ganoderma lucidum (Lingzhi), the source of *Ganoderma* spores, was first recorded in the "Shennong Ben Cao Jing" (Shennong's Classic of Materia Medica, approximately 206 BCE - 220 CE). Later, in Li Shizhen's "Ben Cao Gang Mu" (Compendium of Materia Medica, completed in 1578 CE), the medicinal properties of *Ganoderma lucidum* were further detailed, noting its effects of "relieving cough and asthma, tonifying the lung and kidney" (This TCM approach combines symptom relief (easing cough/asthma by clearing lung pathways) with root-cause treatment (strengthening lung-kidney synergy to improve respiratory vitality), reflecting TCM's holistic "treat both branch and root"philosophy.). In modern times, pharmacological studies have confirmed that Ganoderma spores, as the reproductive organ of *Ganoderma lucidum*, inherit its traditional medicinal value and exhibit significant anti-inflammatory, antioxidant, and immunomodulatory activities, laying the foundation for their development as a novel drug delivery system.

Introduction

Acute lung injury (ALI) and its severe form, acute respiratory distress syndrome (ARDS), are common and life-threatening clinical conditions, characterized by the destruction of the alveolar-capillary barrier, inflammatory storms, and oxidative stress imbalances. These conditions have a mortality rate of 30%-40% [1, 2]. Despite the widespread use of traditional therapies such as mechanical ventilation and glucocorticoids, their efficacy is limited and they often lead to systemic side effects [3]. The core issue lies in the inefficiency of drug delivery,when administered orally or via injection, less than 5% of the drug reaches the lungs [1].

Recent advancements in nanotechnology and biomaterials have driven the development of novel drug delivery systems (DDS), which aim to improve drug targeting, enhance sustained release, and increase bioavailability. These new strategies hold significant potential for the treatment of ALI [4].

Traditional Chinese medicine (TCM) has a history of thousands of years, and its abundant active components exhibit unique pharmacological effects in the treatment of various diseases [5]. In recent years, with the development of nanotechnology and drug delivery systems, the active components of TCM have not only attracted attention as therapeutic agents but also been explored as drug delivery carriers to address key issues in modern drug delivery, such as low solubility, low bioavailability, and insufficient targeting ability [6]. Compared with traditional synthetic carriers, natural carriers derived from TCM (e.g., *Ganoderma lucidum* spores (GLS), liposomes, and polysaccharide nanoparticles) are highly favored due to their excellent biocompatibility, low toxicity, and multi-target regulatory capacity [7].

As a core component of TCM, GLS are not only rich in active substances such as polysaccharides and triterpenes and possess multiple pharmacological effects including anti-inflammatory, antioxidant, and immunomodulatory activities, but also emerge as a promising "natural carrier-active component" dual therapeutic platform, attributed to their unique micro-nano structure (with a diameter of 2–5 μm, which can reach 100–200 nm after wall-breaking), high drug-loading capacity, and environmental responsiveness [8].

The unique advantages of TCM active components lie in their diverse chemical structures and pharmacological properties. For instance, triterpenes and polysaccharides in GLS exhibit immunomodulatory and anti-tumor activities, while their nano-scale structure enables natural penetration of biological barriers [9]. Studies have shown that *Ganoderma lucidum* spore polysaccharides (GLSP) can alleviate lung tissue damage by inhibiting the release of pro-inflammatory factors such as TNF-α and IL-1β, and activating the Keap1-Nrf2/ARE pathway to enhance the activity of antioxidant enzymes. In addition, targeting ligands (e.g., RGD peptides, mannose) can be modified on their surface, and they can be combined with magnetic or pH-sensitive materials to achieve precise delivery to inflammatory sites and intelligent drug release [10]. These natural carriers can not only accumulate in diseased tissues through passive targeting (e.g., enhanced permeability and retention effect, EPR effect) but also achieve precise delivery via active targeting (e.g., ligand-receptor recognition) [5].

As an innovative bioactive carrier, GLS presents unique advantages over traditional synthetic carriers in treating ALI. These advantages stem from the synergistic therapeutic effects of triterpenoids and endogenous active ingredients such as β-D-glucan, which regulate macrophage polarization through the PI3K/Akt/mTOR signaling pathway and inhibit caspase-1 activation mediated by NLRP3 inflammasomes. This forms a multi-dimensional anti-inflammatory regulatory network [11, 12].

In terms of drug delivery characteristics, the GLS microparticle system optimized by ultrasonic atomization has a mass median aerodynamic diameter (MMAD) of 2.8 ± 0.3 μm and a geometric standard deviation (GSD) of ≤1.5. This system achieves an alveolar deposition efficiency of 68.7 ± 4.2% in an in vitro bionic lung model, which is a 57.9% improvement over the Poly (lactic-co-glycolic acid) (PLGA) carrier ($P < 0.01$). These precise lung biodistribution characteristics minimize the risk of systemic exposure [13].

Importantly, the industrial preparation of GLS integrates green processes such as hot water gradient extraction and low-temperature ultrasonic wall breaking. This approach not only maintains the stability of the active ingredients but also boasts a significantly better environmental friendliness index compared to the PLGA synthesis process, which requires the use of organic solvents. This provides an industrialization pathway that meets ICH Q1A standards for clinical transformation [13]. The natural carrier system's integration of biotherapeutic functions, precise delivery characteristics, and environmentally friendly manufacturing offers a promising new paradigm for the local and efficient treatment of respiratory diseases like ALI.

Despite its promising prospects, the *Ganoderma lucidum* spore delivery system faces challenges. Wall breaking technology can affect drug loading stability, the in vivo metabolic dynamics are unclear, and long-term safety requires further verification. Future research could incorporate advanced nanoengineering strategies, such as reactive oxygen-sensitive drug loading designs and gene editing vectors, alongside multi-omics analysis, to optimize its targeting and controlled release capabilities. This would advance the treatment of ALI from "extensive intervention" to a new stage of "precision regulation."

ALI and acute respiratory distress syndrome

ALI and its more severe form, ARDS, are nonspecific pulmonary

inflammatory responses triggered by trauma, infections, or the inhalation of harmful substances. These conditions manifest as progressive hypoxemia and pulmonary edema, resulting from the destruction of the alveolar-capillary barrier [14]. From a pathophysiological perspective, ALI is characterized by pulmonary oxygenation dysfunction ($PaO_2/FiO_2 \leq 300$ mmHg), whereas ARDS represents a more severe stage ($PaO_2/FiO_2 \leq 200$ mmHg). Both conditions are essentially different severities of the same disease spectrum [15]. As a pulmonary manifestation of systemic inflammatory response, ALI/ARDS is clinically marked by bilateral pulmonary infiltrates, decreased lung compliance, and refractory hypoxemia. These conditions predominantly affect previously healthy young and middle-aged individuals, causing tens of thousands of deaths annually worldwide [16] (Figure 1). Notably, the incidence of COVID-19-induced ALI/ARDS has increased dramatically. The distinct pathological mechanisms characteristic of viral pneumonia, particularly cytokine storms and microthrombosis, have substantially complicated clinical management, highlighting the persistent public health threat posed by this disease [17].

Pathophysiological mechanism of lung injury

The pathophysiological mechanism of lung injury involves complex interactions between inflammatory responses, oxidative stress, and cell death pathways. The core event is initiated by increased vascular permeability, mediated by proinflammatory cytokines (such as TNF-α and IL-1), which directly lead to alveolar fluid accumulation and pulmonary edema formation [18]. On the oxidative stress level, the excessive generation of reactive oxygen species (ROS) and NO, triggered by hypoxia or reperfusion injury, exacerbates tissue damage through lipid peroxidation and DNA damage [19]. Concurrently, the programmed cell death (apoptosis) and non-programmed cell death (necrosis) of alveolar epithelial and endothelial cells exhibit a dynamic imbalance. Apoptosis is characterized by cell shrinkage and chromatin condensation, whereas necrosis involves the release of damage-associated molecular patterns (DAMPs) through membrane rupture. These processes collectively drive the local inflammatory cascade [18]. Notably, the intracellular components released by dead cells activate immune effector cells, such as macrophages, forming a vicious cycle of "cell death-inflammatory activation." This not only expands the extent of lung parenchymal damage but may also lead to systemic inflammatory response syndrome (SIRS) [18, 20].

Current commonly used clinical treatment methods and Limitations

In the management of ALI, lung protective ventilation and conservative fluid management strategies are essential. For patients with severe ARDS, high-frequency oscillatory ventilation and consideration for extracorporeal membrane oxygenation (ECMO) are recommended. To date, no drug therapy has demonstrated significant benefit in large clinical trials [16]. Drug treatment for ALI remains unsatisfactory, primarily because drugs cannot be specifically targeted to the lungs. Effective drug delivery to the deep alveolar regions via inhalation is crucial for treating ALI. However, traditional inhalable carriers (such as lactose and mannitol) are generally inert. Therefore, the development of new pharmacologically active carriers for pulmonary delivery could produce synergistic effects in treating ALI [17]. Current clinical treatments, such as mechanical ventilation and glucocorticoids, have limited efficacy and significant systemic side effects. A core challenge in treatment is the low efficiency of drug delivery—when administered orally or by injection, less than 5% of the drug reaches the lungs, making it difficult to achieve therapeutic concentrations [17].

Main pharmacologically active ingredients and extraction methods of GLS

Ganoderma lucidum has a broad range of therapeutic effects, including sedative, hypnotic, neuroprotective, anti-inflammatory, analgesic, anti-epileptic, and anti-depressant properties [21], and is also used to treat insomnia [22]. As an important component of TCM, there are more than 80 species of *Ganoderma* worldwide [23]. However, only two species are listed in the Chinese Pharmacopoeia, namely *G. lucidum* (GL, "Hongzhi" or "Red *Ganoderma*") and *G. sinense* (GS, "Zizhi" or "Purple *Ganoderma*") [24, 25]. *Ganoderma* has a long history of application in enhancing immunity, anti-tumor effects, regulating blood pressure, and blood glucose, with a wide range of indications [10, 26]. These two *Ganoderma* species are considered to have the same therapeutic effects [25]. However, there are actually differences

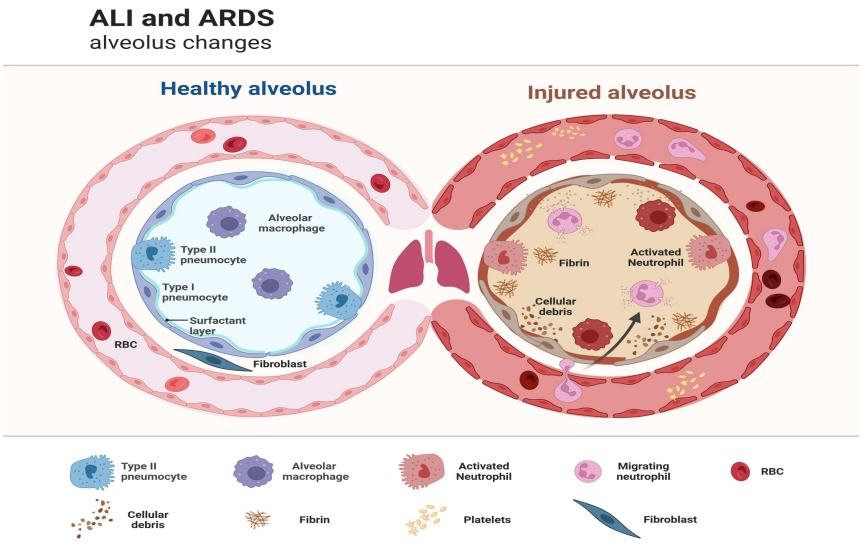


Figure 1 Alveolar pathological changes in ALI and ARDS.
Destruction of alveolar structure and activation of the inflammatory cascade. In healthy alveoli, type I and type II cells maintain gas exchange and immune regulation. In diseased states, macrophage activation, type II alveolar cell proliferation, surfactant layer disruption, epithelial shedding, neutrophil infiltration, and fibrin deposition compromise the endothelial barrier. ROS and pro-inflammatory factors exacerbate inflammation. The figure was created by BioRender (biorender.com). ROS, reactive oxygen species; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; RBC, red blood cell.

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between *G. lucidum* (GL) and *G. sinense* (GS) in terms of chemistry and partial pharmacological studies. The contents of triterpenes [27–30], nucleosides and nucleobases [31], and sterols [32] in GL are significantly higher than those in GS. Additionally, the anti-proliferative activity of GL extract against tumor cells is stronger than that of GS [28, 33]. The active components of *Ganoderma* fruiting bodies, *Ganoderma* mycelia, and *Ganoderma* spore powder are similar [34], but they differ in the enrichment of specific components. *Ganoderma* fruiting bodies are the classical source of polysaccharides and triterpenoids. *Ganoderma* mycelia are rich in *Ganoderma* polysaccharides, with extremely low triterpene content, and mycelial polysaccharides exhibit higher activity than extracellular polysaccharides [35–37]. In contrast, *Ganoderma* spore powder is abundant in lipophilic components such as *Ganoderma* triterpenes, fatty acids, and trace elements, and it also contains unique components (e.g., volatile oils). Polysaccharides exhibit various biological activities, including immunomodulation [38], anti-tumor effects [39], regulation of intestinal flora, and antioxidant properties [40], significantly enhancing the immunomodulatory [41], antioxidant [42], anti-tumor [43], and antibacterial properties [38] of *Ganoderma lucidum*. The triterpenoid content is responsible for its anti-tumor, anti-inflammatory [44], antioxidant, anti-hepatitis [45], antimalarial, hypoglycemic, antibacterial, and anti-inflammatory activities [46, 47]. The volatile oil components exhibit activities of inhibiting tumor cell proliferation and scavenging free radicals [34]. Polyphenols contribute to their antioxidant, antibacterial, anti-inflammatory properties, and anti-tyrosinase activity [48, 49] (Figure 2).

Polysaccharides are the primary bioactive components in GLS, and GLSP is obtained via hot water extraction. GLSP consists of three monosaccharides: arabinose (Ara), glucose (Glc), and galactose (Gal). 1D and 2D NMR data reveal that GLSP is primarily composed of two polysaccharides—β-glucan and arabinogalactan. Arabinogalactan has a galactan backbone with arabinofuranose (Araf) in the side chain. β-glucan is the dominant polysaccharide in GLS. Molecular weight analysis shows that GLSP induces IEC-6 cell proliferation in a concentration-dependent manner. Additionally, GLSP has strong anti-inflammatory properties, inhibiting the excessive production of NO and pro-inflammatory cytokines such as LPS (lipopolysaccharide) -induced interleukin-6 (IL-6) and interleukin-1β (IL-1β) [50]. Meanwhile, to investigate the chemical components of volatile oils in the *Ganoderma* fermentation broth, the steam distillation method was adopted for extraction, and GC-MS was used to analyze the extracted volatile oils. Three volatile oil components with relatively high levels (1-propanol, 2-hexyl-1-decanol, and benzaldehyde) were identified [34].

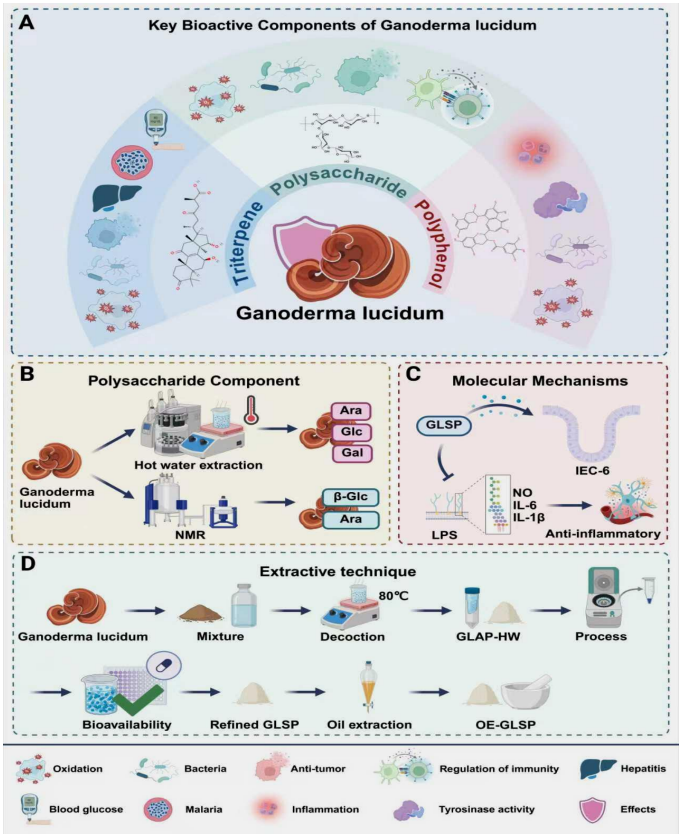

Figure 2 Main bioactive components of *Ganoderma lucidum* and their related properties.
(A) Key bioactive–components of *Ganoderma lucidum*, include triterpenoids with anti-inflammatory, hepatoprotective, and anti-tumor effects; polysaccharides with immunomodulatory functions, and polyphenol. (B) Polysaccharides are the primary bioactive components of *Ganoderma lucidum* spores. GLSP can be extracted using hot water and is primarily composed of three monosaccharides: Ara, Glc, and Gal. Its molecular structure is identified using NMR technology, and optimizing the extraction process directly influences the bioavailability of the polysaccharides. (C) GLSP exerts anti-inflammatory effects by inhibiting inflammatory factors (NO, IL-6, IL-1β), with its activity verified in IEC-6. (D) *Ganoderma lucidum* spores are extracted through traditional hot water extraction (solid-liquid ratio 1:20, decocted at 80 °C for 4 hours), yielding crude polysaccharide GLAP-HW. After concentration and freeze-drying, further acid-heat treatment refines the polysaccharide to GLSP, reducing its molecular weight and significantly improving water solubility and bioavailability. OE-GLSP retains substantial biological activity and effectively reduces serum AST, ALT levels, and the release of inflammatory factors (IL-1β, IL-18, TNF-α). GLSP, *Ganoderma lucidum* spore polysaccharide; NMR, nuclear magnetic resonance; Ara, arabinose; Glc, glucose; Gal, galactose; LPS, lipopolysaccharide; IL-6, interleukin-6; IL-1β, interleukin-1β; IEC-6, intestinal epithelial cells; OE-GLSP, GLSP extracted after oil extraction.

In the traditional extraction method, GLS are mixed with purified water at a specific material-liquid ratio and boiled at 80 °C for 4 hours. The supernatant is then concentrated and freeze-dried to obtain the crude polysaccharide of GLS, known as GLAP-HW [51]. GLSP is extracted through acid-heat treatment, concentration, and centrifugation. This process significantly reduces the molecular weight of the polysaccharide while increasing its water solubility and bioavailability [52]. GLSP extracted after oil extraction (OE-GLSP) retains a large amount of bioactive substances, demonstrating certain biological activity [53]. Compared to the 50% ethanol model group (MG), GLSP obtained from spore germ layer damage significantly reduced serum AST and ALT levels ($P < 0.0001$) and the release of inflammatory factors, including IL-1β, IL-18, and TNF-α ($P < 0.0001$) [54]. An ultrasound-assisted process was employed to extract triterpenes from GLSP, with the process optimized using response surface methodology (RSM) [55].

Concept and importance of drug delivery system

Before the advent of controlled drug delivery, all medications were traditionally produced and stored in pill or capsule forms. In the 20th century, advanced coating technologies were introduced. For example, Malm et al. pioneered the use of polyacetate cellulose phthalate for enteric coating, which dissolves at the weakly alkaline pH found in the small intestine, making it ideal for controlled release in the digestive tract [56]. In the 1960s, liposomes, a type of lipid vesicle, were discovered, marking the beginning of nanotechnology in drug delivery. Polymer-drug conjugates and liposomes ushered in the era of nanocarriers, which continued to evolve with the advent of third-generation drug delivery systems. Modern DDS feature advanced properties such as smaller particle sizes, enhanced permeability, improved solubility, higher efficacy, specific site targeting, greater stability, reduced toxicity, and sustained drug release. These advancements significantly improve the therapeutic performance of agents compared to traditional dosage forms. Erythrocyte membrane-camouflaged nanoparticles represent a new class of DDS that showcases these advancements [57] (Figure 3).

Spores, particularly those from *Ganoderma lucidum*, offer unique advantages as drug delivery systems in treating ALI. Their core value lies in their alignment with clinical needs, offering multiple benefits such as thermal stability, tolerance, multifunctionality, biocompatibility, ease of preparation, and economic viability.

Firstly, their ideal particle size (2–5 μm) and density enable efficient aerosolization and deep lung deposition upon inhalation, which is critical for treating ALI [13, 17]. After wall-breaking, the resulting nanoparticles (100–200 nm) can further facilitate cellular uptake and targeting. Secondly, unlike inert synthetic carriers, GLS possess inherent bioactivity. Their core components, such as GLSP and triterpenes, exhibit potent anti-inflammatory, antioxidant, and immunomodulatory effects. This creates a synergistic "carrier-and-drug" dual-function platform, where the carrier itself contributes to the therapeutic outcome [8, 50]. Thirdly, their natural polysaccharide-based structure provides excellent biocompatibility and lower immunogenicity compared to some synthetic polymers like PLGA [8, 17]. The surface of spore-derived particles can be further chemically or physically modified (e.g., with targeting ligands or responsive materials) to achieve active targeting or controlled drug release [10, 58]. Finally, as a natural product derived from traditional cultivation, GLS offer advantages in scalability and potential cost-effectiveness for large-scale production. Their relative stability also simplifies storage and transportation.

Application of GLS as drug delivery carriers

The application of GLS as drug delivery carriers has recently shown promise in the field of tumor treatment. This includes combining active targeting and passive targeting mechanisms for more precise drug delivery, as well as breakthroughs in basic research on their applications [59] (Figure 4). These findings suggest that similar methods could be employed for drug combination treatments in acute lung injury.

GLSP delivery system: active targeting and immune-mediated mechanisms

In active targeting, ligands such as antibodies or peptides are engineered to selectively bind overexpressed receptors on pathological cells. This modification enhances targeting efficiency. For example, in a dual-ligand system composed of mannose and hyaluronic acid, the surface of nanoparticles loaded with GLSP can be modified with mannose (targeting M2-type tumor-associated macrophages) and

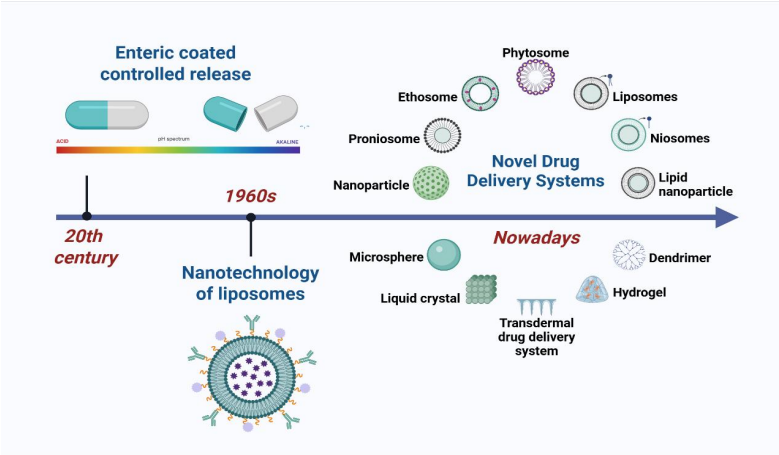

Figure 3 Overview of the development of drug delivery systems.

In the 20th century, with advancements in enteric coating technology, polyacetate cellulose phthalate material developed by Malm et al. enabled pH-dependent dissolution (such as in the weakly alkaline environment of the small intestine), facilitating the development of enteric controlled release systems. In the 1960s, liposomes (lipid vesicles) were introduced as the first nanocarriers, laying the foundation for nanotechnology alongside polymer-drug conjugates. Since then, drug delivery systems have evolved into their third generation—modern systems characterized by controlled release technologies, including micronization (smaller particle size), enhanced permeability and solubility, targeted delivery, improved stability, and reduced toxicity. The figure also illustrates various dosage forms that emerged in the late 20th century, such as microspheres, liquid crystals, hydrogels, and transdermal delivery systems, underscoring the technological leap in drug delivery from basic sustained release to precise targeting. The figure was created by BioRender (biorender.com).

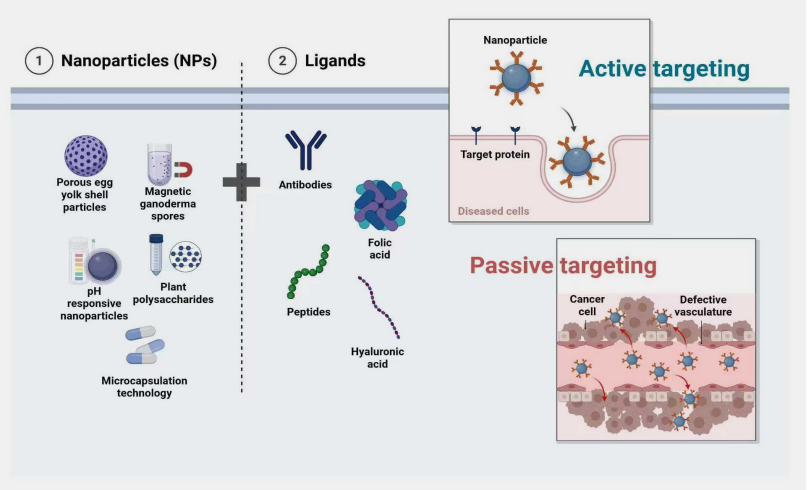

Figure 4 Passive-active synergistic targeted drug delivery system based on nanoparticles.
Nanoparticles, including porous egg yolk shell particles, magnetic *ganoderma* spores, pH-responsive nanoparticles, plant polysaccharides, and microcapsulation technology, enable drug loading and delivery through both physical and chemical properties. Ligands, such as antibodies, hyaluronic acid, peptides, and folic acid, modify the surface of nanoparticles to enhance their targeting recognition ability. Passive targeting: relies on the EPR effect to accumulate nanoparticles in tumor tissues while preserving the integrity of endothelial barriers in healthy tissues, limiting nonspecific distribution. Active targeting: ligand-receptor interactions trigger endocytosis, facilitating intracellular delivery and enabling precise targeting of diseased cells. The figure was created by BioRender (biorender.com). EPR, enhanced permeability and retention.

hyaluronic acid (targeting the CD44 receptor). This modification enhances the active recognition of both tumor cells and immune cells. Moreover, immune cell-mediated targeting exploits the natural homing ability of immune cells such as macrophages or neutrophils. GLS components (e.g., polysaccharides) can activate these immune cells to deliver drugs to sites of inflammation or tumors. For instance, macrophages loaded with magnetic nanoparticles can be guided to tumor areas using an external magnetic field, facilitating precision treatment through targeted drug release. GLS-derived delivery systems can also incorporate environmental response mechanisms (e.g., pH, enzyme, or temperature-sensitive materials) to achieve intelligent drug release. For example, combining *Ganoderma lucidum* polysaccharides with pH-sensitive polymers can facilitate the rapid release of drugs in the acidic environment of tumors, minimizing toxicity to normal tissues [59].

Ganoderma lucidum spore nanocarriers: enhancing drug delivery efficiency through passive targeting mechanisms

Passive targeting relies on the physicochemical properties of the carrier,such as particle size, surface charge and the pathological characteristics of the tumor microenvironment, such as vascular leakage and lack of lymphatic drainage. This mechanism enhances drug accumulation at the tumor site through the enhanced permeation and retention (EPR) effect. GLS naturally possess a nanoscale structure (approximately 5–8 μm in diameter). After wall disruption, the nanoparticles (including polysaccharides or lipid components) can be further reduced to sizes ranging from 100–200 nm, allowing them to pass through tumor blood vessel leakages and meet lung deposition requirements, thus improving drug delivery efficiency [17].

Porous *Ganoderma lucidum* polysaccharide carriers: overcoming drug delivery bottlenecks through multi-dimensional technology

In 2018, Zheng Xing et al. used three-needle electrospray technology to engineer porous yolk-shell particles loaded with *Ganoderma lucidum* polysaccharides. These particles targeted oxidative stress, reducing oxidative damage and improving cell viability, particularly for lung delivery in chronic lung diseases [60]. In 2013, Sahoo B et al. studied magnetic *Ganoderma lucidum* spore (mGLS) as targeted drug delivery carriers. By integrating magnetic materials, these spores could be precisely guided to tumor sites, increasing local drug concentrations while minimizing impact on healthy tissues. This technique improved the therapeutic effects of the drug and reduced side effects [61]. Additionally, *Ganoderma lucidum* polysaccharides have been used to develop pH-responsive nanoparticle drug delivery systems that release drugs in specific acid-base environments, aligning with the acidic characteristics of tumors. This system improves both the efficacy and safety of treatment [62].

Microencapsulation, a common coating technology, can encapsulate the active ingredients of GLS in tiny capsules. This isolates the active components from the adverse external environment while allowing for slow release, thereby enhancing bioavailability. Studies have shown that microencapsulation significantly improves the oxidative stability of Ganoderma lucidum spore oil and extends its shelf life [62].

Importance of drug delivery systems in pulmonary treatment

Pulmonary drug delivery systems (PDDS) offer multiple advantages and provide innovative solutions for the treatment of respiratory diseases. These systems primarily help in avoiding the first-pass effect, reducing systemic side effects, addressing chronic inflammation, improving permeability and absorption efficiency, and advancing delivery technologies.

Unlike oral drug administration, pulmonary drug delivery bypasses the liver, preventing drug metabolism before reaching the bloodstream [63]. This enhances bioavailability, especially for treatments requiring high drug concentrations for efficacy [64]. This is particularly important for drugs that need to maintain therapeutic levels without being rapidly metabolized.

Pulmonary drug delivery systems can deliver drugs directly to the lungs, thus reducing systemic side effects. Many drugs cause adverse reactions when circulating systemically, but local delivery minimizes these risks. For instance, pulmonary antibiotic delivery can achieve high concentrations in the lungs with relatively minimal systemic impact, thereby reducing the risk of side effects in patients [65, 66].

For lung diseases caused by chronic inflammation, such as chronic obstructive pulmonary disease (COPD), pulmonary drug delivery systems offer effective treatment strategies. Nanoparticle-based delivery systems, in particular, have shown significant therapeutic effects. These systems can regulate drug release rates, target the inflammation site, and enhance therapeutic outcomes [67].

The design of pulmonary drug delivery systems also takes into account the permeability and absorption efficiency of the drug. By optimizing the physicochemical properties of drugs, such as particle size and surface characteristics, the absorption rate in the lungs can be enhanced. This modification improves lung absorption, which, in turn, increases the therapeutic effectiveness [68, 69].

Recent advances in nanotechnology and biomaterials have led to the development of innovative drug delivery systems. New delivery devices, such as dry powder inhalers (DPIs) and metered-dose inhalers (MDIs), have improved patient compliance and enhanced lung drug deposition [68]. These technologies enable more efficient and precise drug delivery, which significantly improves treatment outcomes [68] (Table 1).

Experimental models of GLS drug delivery system

Before conducting drug evaluation, establishing relevant disease models is crucial. These models simulate specific lung diseases, such as COPD, asthma, and pneumonia, providing a preliminary assessment of drug efficacy [64]. The construction of experimental models for the *Ganoderma lucidum* spore drug delivery system encompasses three major categories: in vitro, animal, and disease models, and evaluates its delivery efficacy and therapeutic mechanisms at multiple levels.

In vitro models

In vitro experimental models are used to simulate pulmonary drug delivery mechanisms, including drug inhalation, deposition, and biological effects. Recent advances have introduced several human lung cell culture models, ranging from nasal/tracheal, bronchial, to alveolar barrier cultures, and evolving from 2D monolayer to more complex 3D co-culture systems. Notable research includes Chortarea and Beyeler's work, where recombinant human bronchial tissues from asthma patients and primary human bronchial epithelial cells from COPD patients were used to explore the correlation between long-term exposure to multi-wall carbon nanotubes and adverse effects [70, 71]. K.A. Foster et al. utilized the A549 human lung adenocarcinoma cell line to establish an in vitro model of type II alveolar epithelium, studying the role of cell metabolism and macromolecular processing in lung epithelium drug delivery mechanisms [72]. Jiaqi Zhu's team developed a mouse lung tissue-organ macrophage model to assess whether mesenchymal stem cells could alleviate LPS-induced acute lung injury [73].

These in vitro models use cell culture technology to examine the effects of drugs on lung epithelial cells or other related cell lines. The advantage of in vitro models lies in their precise control of experimental conditions, allowing for the evaluation of drug effects a the cellular level [74]. For instance, studies have shown that *Ganoderma lucidum* significantly inhibited inflammatory cytokine levels in the serum of nude mice and suppressed macrophage RAW264.7 activation and inflammatory mediator expression (IL-1β, TNF-α, iNOS, COX-2) in vitro [75]. Compared to traditional synthetic carriers, such as poly(lactic-co-glycolic acid) copolymers, GLS offer better biodegradability, lower immunogenicity, and are well-suited for lung deposition with a particle size (usually 2-5 μm) that enhances delivery efficiency [17] (Figure 5). The aforementioned model has demonstrated efficacy in investigating the cellular-level effects of drugs; however, it still has notable limitations. In contrast to the detection of multiple cytokines (e.g., IL-8, IL-33, GM-CSF) in BALF from patients with ARDS, LPS-induced stimulation only elicits a limited set of factors such as TNF-α and IL-6. Consequently, this model exhibits constraints in simulating cellular inflammation [76].

In vivo models

Animal models serve as essential tools for studying lung diseases and treatment effects. By selecting appropriate models, the pathological characteristics of human lung diseases can be replicated. These include species like mice, rats, rabbits, and pigs, which share similar physiological and pathological traits with humans. Animal models are instrumental not only in evaluating drug efficacy but also in exploring disease pathogenesis [77]. For example, the spontaneous latent tuberculosis infection (LTBI) mouse model, which mimics human LTBI, is invaluable for drug and vaccine development in tuberculosis research.

In the field of acute lung injury, Jin Li et al. successfully established an LPS-induced acute lung injury model in C57BL/6 mice, using obacunone (OB) injections to explore the protective effects of OB on LPS-induced injury [78]. For pulmonary fibrosis, Benedikt Jaeger's team developed a specific pulmonary fibrosis humanized mouse model (C57BL/6J) based on human ABC cells, which promotes the progression of pulmonary fibrosis [79]. Additionally, Mecozzi et al. verified the efficacy of anti-fibrotic treatments in a bleomycin-induced pulmonary fibrosis mouse model using micro-radiation computed tomography [80].

Studies show that GLSP significantly inhibits the growth of various tumor models, including sarcoma S180, Lewis lung cancer, liver cancer H22, and colon cancer C26. In vivo experiments have demonstrated that *Ganoderma lucidum* polysaccharides (GLP) enhances the synthesis of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione S-transferase (GST), while reducing glutathione levels, thus protecting the vascular endothelium [81]. Moreover, *Ganoderma lucidum* activated peptide (GLP4), an activated peptide from *Ganoderma lucidum*, has shown potential in alleviating lung injury caused by cadmium exposure by inhibiting NLRP3 inflammasome activation, reducing cell counts and inflammatory marker levels in BALF, and alleviating lung tissue damage and inflammation [82].

A water-soluble polysaccharide named GSG, extracted from GLS, induces TNF-α and IL-6 secretion via MAPKs- and Syk-dependent

Table 1 The importance and core advantages of pulmonary drug delivery systems

Advantages	Specific characteristics and mechanisms	Examples/Applications	References
Avoid first-pass effect	Deliver drugs directly to the lungs, bypass liver metabolism, and significantly improve bioavailability	Drugs requiring high concentrations (e.g., anti-inflammatory drugs, targeted therapeutic drugs)	[62, 63]
Reduce systemic side effects	Local delivery reduces systemic drug exposure and adverse reactions (such as high lung concentrations of antibiotics and low systemic toxicity)	Antibiotics for lung infections	[64, 65]
Address chronic inflammation	Nanoparticle delivery systems can regulate drug release, target inflammatory sites, and enhance efficacy	Inflammation regulation in chronic obstructive pulmonary disease (COPD), asthma	[66]
Optimize permeability and absorption efficiency	Improve lung deposition and absorption by adjusting drug particle size and surface properties (such as hydrophilicity)	Dry powder inhalers (DPIs) to optimize drug distribution in the lungs	[67, 68]
Innovative delivery technology advancement	Combining nanotechnology, biomaterials, and new devices (such as MDI, DPI) to improve drug delivery accuracy and patient compliance	Smart responsive nanocarriers, biomimetic delivery systems	[67]

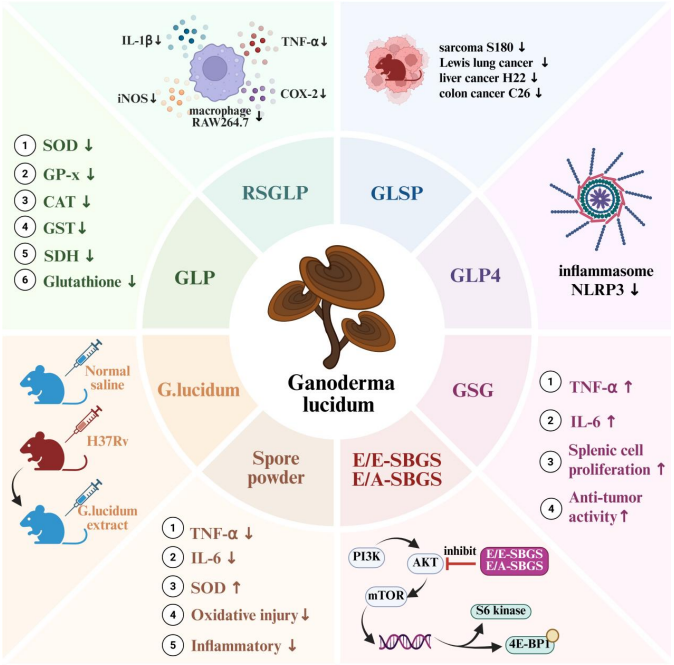

Figure 5 Molecular mechanism network of multi-pathway regulation of anti-tumor and inflammatory effects by active ingredients of *Ganoderma lucidum* spores.

Ganoderma lucidum extract and its components exhibit multiple bioactive activities, including anti-inflammatory, antioxidant, anti-tumor, and immunomodulatory effects. The extract reduces inflammation by inhibiting pro-inflammatory factors and NLRP3 inflammasomes while upregulating antioxidant enzymes to mitigate oxidative damage and protect tissues. In terms of anti-tumor effects, *Ganoderma lucidum* inhibits the growth of various cancer cells while enhancing spleen cell proliferation and anti-tumor activity. Additionally, *Ganoderma lucidum* spore powder components (E/E-SBGS, E/A-SBGS) inhibit the PI3K/AKT/mTOR signaling pathway, synergistically reducing inflammatory factors (TNF-α, IL-6), enhancing antioxidant capacity, and alleviating inflammatory responses. The figure was created by BioRender (biorender.com). E/E-SBGS, ethanol/ethanol extract; E/A-SBGS, ethanol/water extract; TNF-α, tumor necrosis factor α; IL-6, interleukin-6; SOD, superoxide dismutase; GLP, *Ganoderma lucidum* polysaccharides; GPx, glutathione peroxidase; CAT, catalase; GST, glutathione S-transferase; SDH, mitochondrial succinate dehydrogenase; RSGLP, *Ganoderma* spore germ layer removed spores; IL-1β, interleukin-1β; iNOS, nitric oxide synthase; COX-2, cyclooxygenase; GLSP, *Ganoderma lucidum* spore polysaccharides; GLP4, *Ganoderma lucidum* activated peptide; NLRP3, nucleotide-binding oligomerization domain-like receptor protein 3; GSG, *Ganoderma lucidum* water-soluble polysaccharides.

pathways in mouse resident peritoneal macrophages. Dectin-1 partially mediates its biological activity, and GSG has demonstrated, antitumor activity against Lewis lung cancer [83]. Furthermore ethanol/ethanol extract (E/E-SBGS) and ethanol/water extract (E/A-SBGS) from broken-wall GLS have shown dose-dependent inhibition of the Akt/mTOR pathway in tumor cells, suggesting their potential role in lung cancer therapy [84].

Ganoderma lucidum extract has shown protective effects in a new LTBI mouse model by reducing *Mycobacterium tuberculosis* H37Rv replication, indicating its potential in tuberculosis prevention [85] (Figure 5). Admittedly, animal models have contributed to the research on drug mechanisms; however, due to differences in physiological structures between humans and animals, these models cannot fully demonstrate the complexity of real pathological lesions. For instance, although mouse models can simulate early ALI, they fail to replicate the microthrombosis and fibrotic remodeling observed in the progressive stage of human ARDS [86]. Furthermore, most of the relevant experiments reported so far are limited to the analysis of cytokines and histology, lacking studies on blood gas analysis, lung compliance, and other parameters. As a result, there remains a gap in the connection with clinical practice.

Safety evaluation of GLS drug delivery system

Studies have shown that plant spores exhibit excellent degradability in the body without inducing significant toxic reactions, making them safer and more reliable for biomedical applications [87]. Proper handling and storage conditions are crucial to minimize oxidation and acidic degradation during processing, thereby enhancing the safety and stability of GLS [88]. Acute and chronic toxicity tests are conducted to assess the potential risks posed by GLS to organisms [88]. Oral administration of E/E-SBGS and SBGS has been shown to significantly inhibit tumor volume and weight in mice without gross toxicity [84].

However, to advance the clinical translation of GLS, it is clearly insufficient for its safety profile to be limited to "no acute toxicity"; further in-depth investigations are still required in the context of clinical complexity. Although GLS are claimed to have "low immunogenicity", β-glucan in their cell walls can be recognized by Dectin-1. Following repeated exposure, the proportion of neutrophils in BALF increases persistently, indicating a potential risk of low-grade inflammation [89]. Additionally, existing studies have confirmed that alveolar macrophages can phagocytose fungal spores but cannot achieve complete degradation; thus, further research is needed to determine whether GLS can be safely degraded in the human body [90]. Moreover, it remains to be verified whether potential batch-to-batch variations in natural products may affect the final efficacy.

Summary and outlook

GLS, as natural micron-sized particles, have become a research focus for drug delivery systems due to their unique structural properties, such as porosity and high drug loading capacity, and their potential biological activities, including anti-inflammatory and antioxidant effects. This article reviews the application and mechanisms of GLS as drug delivery carriers in the treatment of ALI. Studies suggest that GLS can load therapeutic drugs (e.g., anti-inflammatory molecules or

growth factors) through surface modification or encapsulation and achieve precise lung delivery via inhalation or intravenous injection. Their natural polysaccharide components enhance drug retention in the lungs and reduce oxidative stress and inflammation by modulating macrophage polarization and inhibiting key inflammatory factors such as NLRP3, IL-1β, TNF-α, iNOS, and COX-2.

Compared to traditional synthetic carriers, such as polylactic acid-co-glycolic acid copolymers, GLS offer better biodegradability, lower immunogenicity, and a particle size (2-5 μm) ideal for lung deposition, which improves delivery efficiency. The *Ganoderma lucidum* spore delivery system provides a dual treatment paradigm of "natural carrier-active ingredient" for ALI treatment, offering both efficiency and safety. Its clinical application potential has been validated in adjunct tumor therapy, improving immune function and quality of life in chemotherapy patients.

However, challenges remain in optimizing spore wall breaking technology, drug loading stability, and in vivo metabolic kinetics. In future studies, isotope tracing can be employed, where GLSP is labeled with ^{13}C or ^{2}H and combined with the dual platforms of MALDI-MSI and LC-MS/MS, enabling simultaneous quantification of in situ distribution and plasma drug concentration [91]. Alternatively, magnetic particle imaging (MPI) can be adopted: GLS are loaded with superparamagnetic iron oxide nanoparticles, and real-time scanning allows for multiple samplings in animal models to support further research [92]. Additionally, in the design of responsive materials, such as ROS-sensitive drug delivery systems, polythiocopper nanoparticles can cleave thiocopper bonds in a high-level ROS environment, thereby accumulating at pulmonary inflammatory sites and releasing their carried payloads. This approach enhances the targeting ability and controlled release performance of GLS [93]. Additionally, exploring composite applications with other natural active ingredients (e.g., cyclodextrin frameworks) could help address the complex pathological microenvironment of ALI and provide new strategies for precision treatment. The in vivo drug release kinetics and long-term safety need further validation in animal models, with considerations for potential allergies.

In conclusion, the *Ganoderma lucidum* spore drug delivery system holds significant promise for the treatment of ALI. Its synergistic multi-mechanism approach and natural safety profile provide a compelling direction for the development of highly effective and low-toxic lung-targeted drugs. With further advances in materials science and pharmacology, this field is poised to usher in a new stage for precision ALI treatment.

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