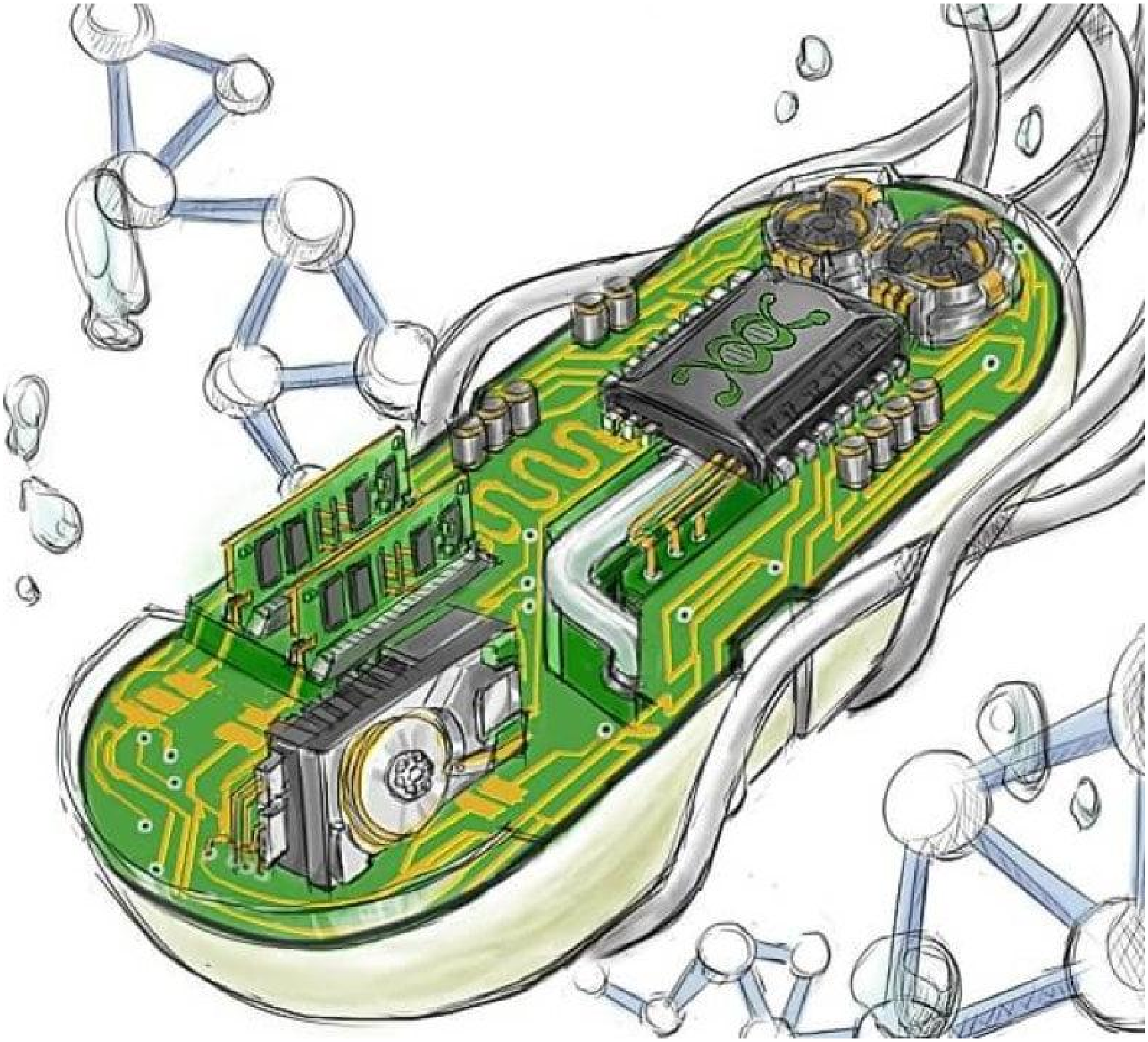
Fungal engineered living materials and the application of bacteria on producing functional mycelium materials: A review



Chen Hou

Fungal engineered living materials and the application of bacteria on producing functional mycelium materials: A review

A literature review by

Chen Hou, student number 0834661
Faculty of Science, Utrecht Universit, Utrecht, the Netherlands
Contact: c.hou1@students.uu.nl

Supervisor and Examiner

Prof. dr. H.A.B. Wösten
Faculty of Science, Molecular Microbiology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, the Netherlands
Contact: H.A.B.Wösten@uu.nl

Second reviewer

Dr. L.G. (Luis) Lugones
Faculty of Science, Molecular Microbiology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, the Netherlands
Contact: l.g.lugones@uu.nl

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Abstract

To address the environmental challenges posed by the linear economy of "produce, use, and dispose," various industries are transitioning toward bio-based and circular economy strategies. In this context, mycelium materials have emerged as a promising class of circular and sustainable materials, gaining significant research interest and commercialization potential across construction, packaging, and fashion sectors. The asexual growth of fungal mycelium offers an energy-efficient biomanufacturing method, where the mycelium colonizes and decomposes organic substrates, assembling into extensive networks that can be tailored to produce either dense, flexible mycelium-based leather-like materials or lightweight, insulating, soundproof, and fire-resistant composites to meet diverse application needs. At the end of their lifecycle, these materials are fully biodegradable, embodying a cradle-to-cradle recycling model. As such, they are widely recognized as sustainable alternatives to traditional energy-intensive building materials, plastics, textiles, and leather. However, current production processes involve heat treatment and drying after typically several weeks of growth, which kills the living fungal organisms. This practice inevitably limits the inherent biological functionalities of fungi, such as self-healing, self-regeneration, and environmental sensing and response.

Recent research has increasingly explored fungi as promising microbial platforms for the development of Engineered Living Materials (ELMs). This review broadens the perspective on the use of filamentous fungi for ELM production by summarizing significant advancements and pioneering efforts in the field. The potential of fungal ELMs for the development of biosensors, smart wearable devices, robotic skins, intelligent self-cleaning surfaces, and self-healing materials is highlighted. Additionally, the ability of fungal mycelium materials to facilitate the in situ production of large-scale, living comstruction materials is emphasized, offering promising prospects for the growing field of engineered living materials. However, limitations in the genetic editing of filamentous fungi have been identified in current fungal ELM research. In response, the discussion then shifts to the potential of using engineered bacteria to regulate mycelium growth and functionalize mycelium materials, presenting a viable new strategy for the production of multifunctional, large-scale fungal ELMs. Finally, future research opportunities and challenges in this emerging field are outlined, aiming to inspire further innovation and exploration in the development of fungal-based ELMs.

Keywords: Mycelium materials, Engineering living materials, Filamentous fungi

Layman's Summary

To address the environmental challenges posed by the current way of producing, using, and throwing away products, various industries are transitioning toward bio-based and circular economy strategies. In this context, fungal materials have emerged as a promising class of circular and sustainable materials, gaining significant research interest and commercialization potential across construction, packaging, and fashion sectors.

The production of fungal materials relies on the natural growth of the root system of fungi which are called mycelium. It can be tailored to produce either dense, flexible mycelium-based leather-like materials or lightweight, insulating, soundproof, and fire-resistant composites to meet diverse application needs. At the end of their lifecycle, these materials are fully biodegradable, embodying a cradle-to-cradle recycling model. As such, they are widely recognized as sustainable alternatives to traditional energy-intensive building materials, plastics, textiles, and leather. However, current production processes involve heat treatment to kill the living fungal organisms. This practice inevitably limits the inherent biological functionalities of fungi, such as self-healing, self-regeneration, and sensing and response.

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Introduction

The continuous growth of the global population and industrialization has led to the excessive exploitation of non-renewable natural resources. Under the current linear economic model of "produce, use, and discard," industrial materials such as traditional construction materials and petroleum-based plastics are typically unsustainable (Bitting et al., 2022; van den Brandhof & Wösten, 2022) . Their production and disposal consume significant amounts of energy and pollute the environment. As non-renewable natural resources are depleted and global pollution intensifies, the recognition of the necessity for sustainable development is increasing. Consequently, public interest in green energy and biobased materials is rising (An et al., 2023). Seeking sustainable and renewable alternatives for materials and enhancing the recycling of waste residues instead of discarding is crucial for human society to transition towards a circular economy (Girometta et al., 2019).

Nature serves as a vast source of inspiration; over 4.5 billion years of evolution have resulted in life systems that exist sustainably and creatively through elegant and intelligent means (An et al., 2023). Fungi, as an integral part of nature, serve as essential organic decomposers in ecosystems. They release a diverse array of enzymes, not only participating in the breakdown and recycling of complex biological polymers in plant and animal tissues but also obtaining the carbon and nitrogen nutrients needed for their own growth (El-Enshasy, 2007). Within the fungal kingdom, single-celled yeasts play an indispensable role in the fermentation processes of bread, beer, and wine, impacting human society for millennia (Meyer et al., 2020). Multicellular filamentous fungi, which have hyphal structures, are used industrially to produce various enzymes, antibiotics, and small molecule compounds like organic acids (El-Enshasy, 2007). For instance, citric acid produced by fermenting *Aspergillus niger* surpassed the extraction of citric acid from citrus fruits nearly a century ago (Cairns, Nai, Meyer, & biotechnology, 2018).

In recent years, the utilization of filamentous fungi colonizing organic substrates to produce sustainable biomaterials has garnered extensive attention and research from material scientists, biologists, and designers. The key to fungi being used as biomaterials lies in their hyphal structures, known as mycelium. Mycelium is a dense and intricate fibrous network composed of hyphae (Islam et al., 2017). This mycelial network, with its large surface area-to-volume ratio, enhances the ability to decompose substrates and absorb nutrients, enabling effective colonization and penetration of organic materials (Wösten, 2019). The vegetative growth of fungal mycelium can be harnessed as an energy-efficient method of biological manufacturing, forming the basis for the transition from the microscopic structure of mycelium to its macroscopic application as a material. Mycelium-based materials exhibit excellent properties such as sound absorption, thermal insulation, and fire resistance. At the end of their lifecycle, these materials are fully biodegradable, enabling a cradle-to-cradle recycling model. They are recognized for their potential to serve as sustainable alternatives to traditional building materials, plastics, textiles, and leathers, which are typically energy-intensive to produce (Jones, Mautner, Luenco, Bismarck, & John, 2020).

Mycelium-based materials are generally categorized into two types. Composite mycelium materials consists of mycelium integrated with organic substrates, while pure mycelium materials comprise solely mycelial biomass. Both types are produced through the vegetative growth of fungi, albeit under distinct conditions (Manan, Ullah, Ul-Islam, Atta, & Yang, 2021). Mycelium composites are formed through the colonization and consolidation of organic substrates by fungi. These substrates are usually low-cost lignocellulosic agricultural or forestry by-products or wastes, such as hemp shives, straw, rice husks, cotton stalks, and sawdust (Jones et al., 2020). Consequently, the production of mycelium composites facilitates the recycling of local agricultural waste. During the colonization process, the mycelial network acts as a binder, integrating the dispersed substrate particles. As the mycelium spreads and infiltrates, the substrate is gradually degraded and converted into mycelial biomass. By drying or heat-inactivating the fungi before complete substrate degradation, a mycelium composite is obtained (Meyer et al., 2020).

Mycelium composites exhibit excellent thermal insulation properties, with a thermal conductivity ranging from 0.04 to 0.08 W/m·K. This performance is comparable to that of traditional insulation materials, such as glass wool (13–100 kg/m^3, 0.03–0.045 W/m·K) and expanded polystyrene (EPS, 15–35 kg/m^3, 0.035–0.04 W/m·K) (Hung Anh & Pásztory, 2021; Jones et al., 2020). The low density (57–99 kg/m^3) and the substrate contribute to the excellent insulation properties of mycelium composites. The low density is attributed to the porous, loose substrate, which contains a significant amount of air, a substance with a very low thermal conductivity of 26.2 × 10−3 W/m·K at 0.1 Mpa (Jones et al., 2020). Additionally, substrates used in mycelium composites, such as hemp (0.04-0.05 W/m·K) and rice straw (0.046–0.056 W/m·K), are inherently good natural insulators (Hung Anh & Pásztory, 2021). Moreover, mycelium composites are effective acoustic insulation materials, capable of achieving a noise absorption rate of 70-75% or higher for perceivable road noise (Pelletier, Holt, Wanjura, Bayer, & McIntyre, 2013). Mycelium materials also exhibit good fire resistance. However, their typical foam-like mechanical properties and high-water absorption limit their application in structural building materials. Currently, they are primarily used in insulation layers, door panels, furniture, and packaging (Jones et al., 2020).

Pure mycelium materials are cultivated through either liquid or solid-state fermentation processes. Liquid fermentation can be further classified into static and dynamic methods. In static fermentation, mycelium skin is harvested from the surface of the liquid medium. In dynamic fermentation, dispersed mycelial pellets are filtered and pressed together to form the material. For solid-state fermentation, mycelium is obtained by peeling off the fungal skin from the air interface of the solid substrate. One of the fields that pure mycelium material has been used and commercialized is fashion industry, as the pure mycelium presents a sustainable alternative to traditional leather. The soft and flexible mycelium material can be processed and tailored in many of the same ways as animal leather, and thus used to make clothes, shoes and bags (Meyer et al., 2020). In the construction industry, pure mycelium foam can replace conventional sound-absorbing materials, offering superior sound absorption properties compared to commonly used ceiling tiles and cork in road noise attenuation tests. Additionally, it can function as a structural foam as a scaffold for bioartificial organs (Pelletier et al., 2019). Moreover, pure mycelium can be processed into healthy meat alternatives, which are low in saturated fats and rich in fiber and amino acids (Meyer et al., 2020).

Mycelium materials, known for their multifunctionality, lightweight properties, and biodegradability, have entered the commercial market and continue to evolve. Companies such as Ecovative Design, Grown, Mycelium Materials Europe, Mogu and LoopBiotech have commercially developed mycelium composite materials for applications in packaging, furniture, insulation, acoustic tiles, and coffins (Elsacker, Søndergaard, Van Wylick, Peeters, & De Laet, 2021). Bolt Threads, through its Mylo brand, works on the production and research of mycelium-based leather. Currently, fungal-based materials and products undergo inactivation treatments before leaving the factory, rendering them inert. This inert treatment of fungal materials ensures their stability and biosafety during use, enhancing their structural functionality and public acceptance. Nonetheless, this practice inevitably limits the biological functionalities inherent to fungi, such as self-healing, self-production, and environmental sensing and response. Retaining some degree of biological activity in mycelium materials could leverage the inherent biological functions or enable engineering modifications, such as genetic editing, to create more versatile and functional mycelium materials (McBee et al., 2022).

Living mycelium materials endow them not only with biological functionalities but also with structural functionalities at the macroscopic scale. This dual functionality overlay holds promise and unique advantages for mycelium materials to emerge as Engineered Living Materials (ELMs). ELMs represent a new class of materials developed on the latest advancements in synthetic biology and materials science. They consist of living matter or cells embedded within a matrix, possessing the capabilities of self-repair, self-generation, environmental sensing, and programmable biological functionalities (K. Li et al., 2023; McBee et al., 2022). Currently, ELMs exhibit significant potential in applications such as green energy production, self-healing concrete, disease treatment, and the manufacturing of advanced smart materials (An et al., 2023). Fungi-derived ELMs, as a new generation of macroscopic, economical, and low-energy sustainable smart materials, are poised to promote the development of a sustainable economy and society.

This review discusses the state-of-the-art fungal ELMs, establishing a conceptual framework to explore the potential applications of fungi in this emerging field. Initially, the review introduces the definitions and classifications of ELMs, along with their development prospects and limitations, with a particular focus on microbial ELMs. This sets the foundation for discussing the suitability of fungi for producing ELMs and the unique potential of fungal ELMs. Subsequently, the review enumerates research cases from the past decade on fungal-based ELMs, with a focus on summarizing and analyzing the current state of research in various application domains. Notably, this paper also discusses the potential of using engineered bacteria to regulate fungal mycelium material production and functionalize mycelium materials to create multifunctional living materials with macroscopic structures, based on the interactions between bacteria and fungi. Finally, the review outlines the challenges, knowledge gaps, and future prospects related to fungal mycelium ELMs.

Engineering living materials

ELMs represent an innovative class of intelligent materials that draw inspiration from and incorporate living systems, positioned at the intersection of synthetic biology and materials science (Srubar, 2021). Compared with other biomaterials, ELMs dedicate to the use of living cells as nanomaterials synthesis factories. These factories can continuously absorb and utilize nutrients and energy from the environment to synthesis and self-assemble hierarchical structures, and maintain and regulate structures by sensing environmental change (Nguyen, Courchesne, Duraj-Thatte, Praveschotinunt, & Joshi, 2018). The term "engineered" in ELMs refers to the manipulation of biological activities to direct the creation of specific materials or to customize material properties. This includes a wide range of approaches, from genetic editing of living cells and alteration of chemical or material properties to spatial or mechanical engineering for cellular confinement (Elsacker, Zhang, & Dade-Robertson, 2023; Nguyen et al., 2018). ELMs not only have conventional material properties such as structural, electrical, or chemical characteristics but also exhibit biological functionalities, including self-organization, self-healing capabilities, and the ability to respond to environmental stimuli by executing corresponding engineering commands (Chen, Zhong, & Lu, 2015; Srubar, 2021). This new generation of sustainable and smart material hold the potential for applications from medicine and electronics to construction, enabling micro- to macro-scale implementations (Srubar, 2021).

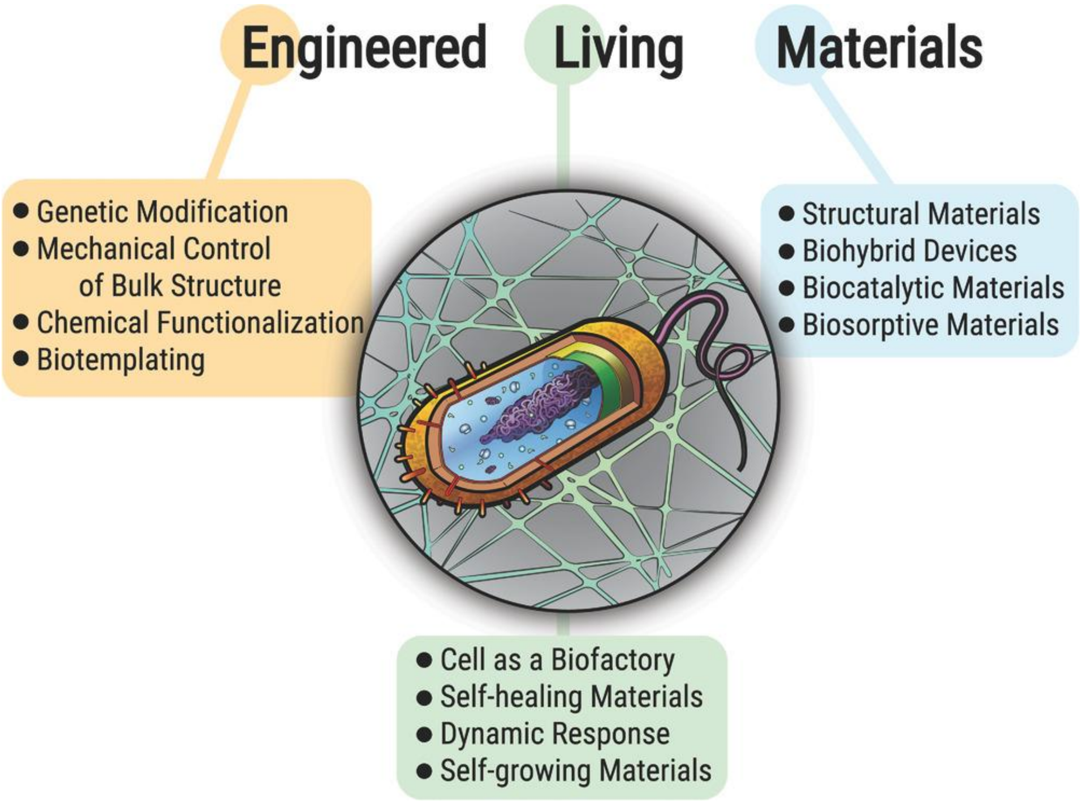

Figure 1. Properties of ELMs. ELMs are a new generation of sensing, self-sustaining, and adaptive smart materials based on bioengineering, materials science, and living systems, These materials hold promise for innovative

applications in structural engineering, medical devices, bio-based materials and environmental sustainability. These materials hold promise for innovative applications in structural engineering, medical devices, bio-based materials and environmental sustainability. The figure is adapted from (Nguyen et al., 2018).

Since 2016, the number of publications with the keyword "engineered living materials" in Web of Science and Google Scholar has increased significantly (Lantada, Korvink, & Islam, 2022). Most of the literature categorizes ELMs into biological ELMs (bio-ELMs) and hybrid living materials (HLMs) based on the matrix into which the active biological component is embedded (Lantada et al., 2022). The former are also referred to as self-organizing living materials, it is produced by “bottom-top” strategy; the living part can self-organize the production of the high order scaffolds (Elsacker et al., 2023). Functionalized bio-ELMs are often grown in situ using model strains such as *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, *Sporosarcina, Saccharomyces,* which are easy to genetically edit (Srubar, 2021), and reflecting the results of the genetic modifications into the material while self-manufacturing and assembling (Gilbert & Ellis, 2019; K. Li et al., 2023).

The HLMs are normally produced by “top-bottom” approach, embedding and precisely integrating live cells into artificially synthesized matrix. One of the advantage of this production strategy is its adaptability to additive manufacturing technologies, such as the use of advanced 3D printing techniques to generate structurally complex and large-scale ELMs, thus enabling the use of smart living materials in macroscopic applications beyond tissue engineering (Gantenbein et al., 2023; Srubar, 2021). A typical example is the development of self-healing concrete based on microbial mineralization capacity. Additionally, in HLMs, engineered cells are confined in low porosity multilayer structural materials, thus reducing the risk of engineered cells escaping into the environment and avoiding biosafety issues (Guo et al., 2020). However, it cannot be ignored that the use of exogenous scaffolds increases manpower and manufacturing costs. More importantly, the physical barrier provided by exogenous scaffolds somewhat restricts the diffusion of macromolecules, thereby often imposes limitations on the cells' ability to perceive the external environment and maintain viability (Guo et al., 2020; K. Li et al., 2023).

Even existing bio-ELMs often cannot be entirely composed of active biological entities. For example, materials like bacterial cellulose, predominantly produced using model strains such as *E. coli* and *B. subtilis* as primary chassis, entail a cellulose network as a structural component, a byproduct of bacterial activity rather than the active bacteria themselves (Elsacker et al., 2023). In contrast, fungal mycelium, whether in the form of pure mycelium materials or mycelial networks colonizing substrates, can serve as structural scaffolds at the macroscopic scale. Moreover, mycelial cells themselves as active components can be functionalized and engineered.

Lantada et al. (2022) proposed a more comprehensive classification system for ELMs, which is more aligned with biological taxonomy than with materials science. They posited that the essence and core of ELMs lie in their living components. Consequently, they classified ELMs into five domains and eight kingdoms based on the used living cells (Figure 2). Archaeal-, bacterial-, and eukaryotic-derived ELMs are the 3 basic ELMs domains. ELMs employing synthetic cells (artificial cells) form the fourth domain. Additionally, the cross-domain refers to ELMs that utilize a combination of different cell types (Lantada et al., 2022).

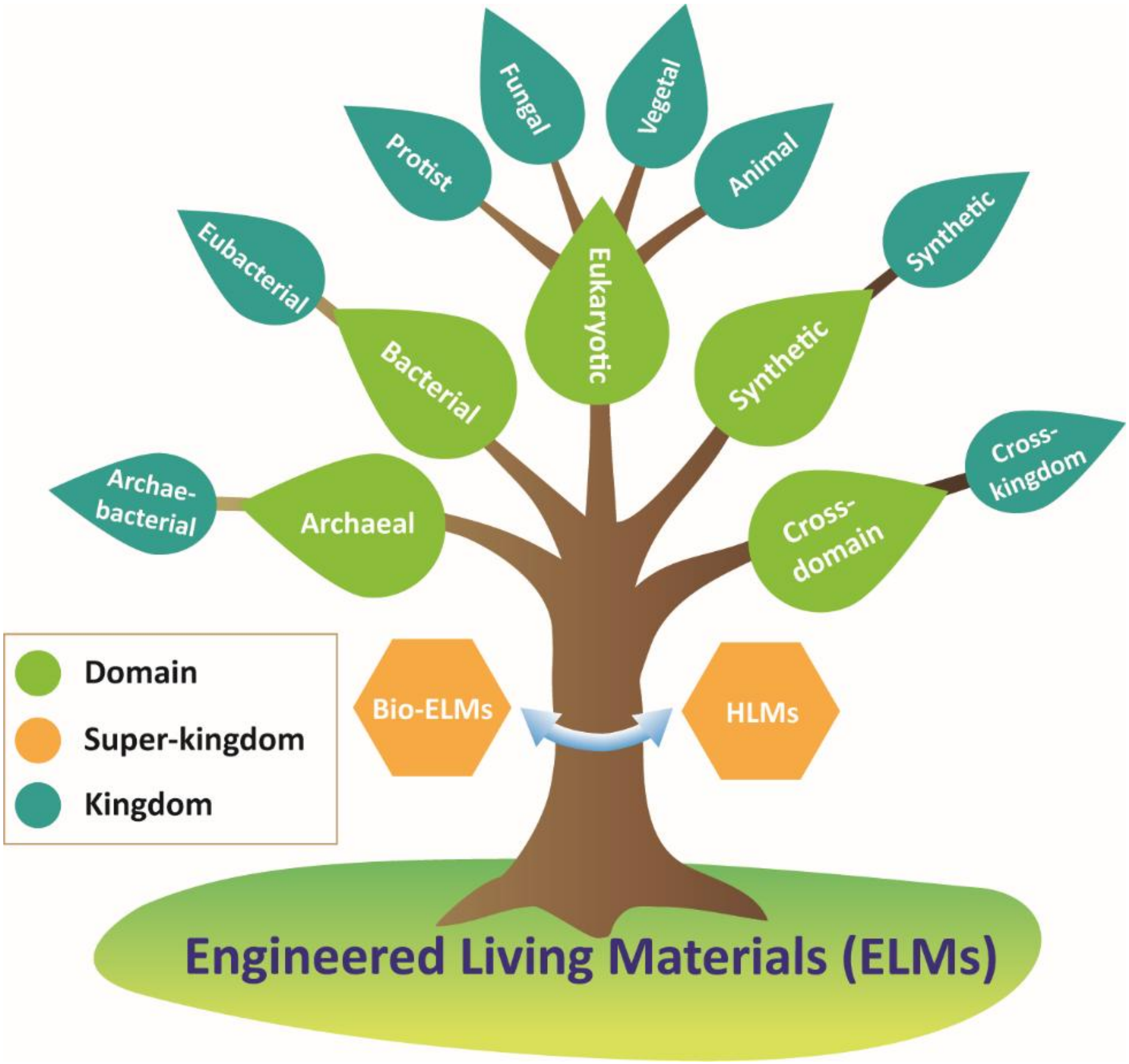

Figure 2. The taxonomy of ELMs includes the domains, super-kingdoms, and kingdoms of ELMs. The figure is from (Lantada et al., 2022).

Although various living cells can be utilized for ELMs, the exploration of plant and mammalian cells in the ELMs field is relatively limited due to their higher environmental requirements and stricter regulatory constraints (Srubar, 2021). In contrast, bacteria are the most used organisms in ELMs production since their rapid proliferation and genetic tractability (Branda, Vik, Friedman, & Kolter, 2005; Nguyen et al., 2018). Much of ELM's pioneering work has focused on *E. coli* biofilm engineering (Gilbert & Ellis, 2019), and several reviews have comprehensively summarized the key advancements and innovative developments in bacterial ELMs, including model biological systems such as bacterial biofilm amyloids and bacterial cellulose (BC) (Gilbert & Ellis, 2019; Nguyen et al., 2018).

BC is a prime example of a bio-ELM produced using a "bottom-up" strategy. Its superior flexibility and hydrophilicity have led to commercial applications in biomedicine and skincare, and it also shows potential as a material for electronic products (Nguyen et al., 2018). Moreover, some BC-producing bacteria can be gene edited to tailor the properties and functionalities of BC materials (Cameron, Bashor, & Collins, 2014; Walker, Goosens, Das, Graham, & Ellis, 2019). This use of genetic modification further enhances the versatility and applicability of BC in various domains.

Additionally, bacterial biomineralization has been applied in the development of HLMs (Schaffner, Rühs, Coulter, Kilcher, & Studart, 2017). A notable example is the use of bacteria in the production of self-healing concrete. Bacterial-based self-healing concrete has already reached commercialization. The Dutch company Basilisk Self-healing Concrete has developed three repair technologies: Self-Healing Repair Mortar and a liquid repair system for existing concrete cracks, and a self-healing admixture for new concrete. These technologies can successfully repair cracks up to 1 mm wide ("Basilisk Self-Healing Concrete," 2021).

Fungal ELMs

Compared to bacteria, the genetic information available for fungi is relatively limited. The application of biotechnology in ELMs relies heavily on the availability of comprehensive biological data on relevant species. As a result, research on fungal-based ELMs has been relatively scarce (Van Wylick et al., 2021). However, recent advancements and commercialization of fungal mycelium materials as insulation, soundproofing materials, and leather alternatives have highlighted the potential of filamentous fungi in ELM applications. The presence of chitin in the cell walls of mycelium confers excellent tensile strength to these materials. The continuous extension and expansion of the multicellular mycelial network can fill voids, enabling the material to self-assemble, self-repair, and sense and adapt to environmental changes. This capacity allows it to survive in harsh conditions such as low nutrient availability, dryness, and extreme temperatures (Elsacker et al., 2023).

In comparison to other microorganisms, the most significant advantage of filamentous fungi in the development of ELMs is that these biological functions are inherent to the mycelial cells themselves, rather than being inert by-products of their metabolic activities. Consequently, the mycelial network that exhibits these biological functions also serves as the structural scaffold of the material, addressing a critical gap in the ELMs field where the living cells provides both macroscopic structure and biological functionality.

In this section, the author consolidates nearly a decade of research on fungal-based ELMs into Table 1. The table encompasses 17 studies, each examining various capabilities of fungi such as sensing, regeneration, biomineralization, and structural integrity. These studies span multiple applications of multifunctional fungal ELMs, including fungal sensors and electronic components, self-cleaning packaging materials, and self-healing construction materials.

Table 1. This table compiles research cases on fungal-based ELMs from the past ten years. The classification of Kingdom and Super-kingdom follows the taxonomy proposed by Lantada et al(Lantada et al., 2022). The table includes the fungal species used in each study, the primary function of active fungal cells within the materials, the potential applications of these materials, and whether genetic engineering techniques were employed.

Described ELMs	Kingdom	Super-kingdom	Species	Funcation	Application	Gene editing
Smart self-cleaning surfaces(Gerber, Koehler, Grass, & Stark, 2012a, 2012b)	Fungi	HLM	*Penicillium roqueforti*	Self-regeneration	Smart materials packaging, indoor surfaces, and in biotechnology	No
Mycelium Architecture functionalised with nanoparticles and polymers (Adamatzky, Ayres, Belotti, & Wosten, 2019)	Fungi	HLMs	*Pleurotus djamor*	Sensing and responding, Structural support	Construction materials	No
Applying pH-regulated mutant fungal strains to self-healing concrete(Menon et al., 2019)	Fungi	HLMs	*Aspergillus nidulans*	Self-regeneration, biomineralization	Construction materials	Yes
Reactive fungal	Fungi	HLMs	*Pleurotus ostreatus*	Sensing and	Smart wearables	No

wearable(Adamatzky, Nikolaidou, Gandia, Chiolerio, & Dehshibi, 2021)				responding		
Programmable ELMs grown from co-culturing engineered yeast and BC-producted bacteria (Gilbert et al., 2021)	Cross	Bio-ELMs	*Saccharomyces cerevisiae*, *Komagataeibacter rhaeticus*	Sensing and responding	Biosensor	Yes
Producing living and multifunctional mycelium composites for large-scale application using robotic abrasive wire-cutting (Elsacker et al., 2021).	Fungi	HLMs	*Trametes versicolor, Ganoderma resinaceum*	Self-regeneration	Construction materials	No
Directed assembly of genetically engineered yeast cells into living functional materials via ultrahigh-affinity protein interactions (Yi et al., 2022)	Fungi	HLMs	*S. cerevisiae*	Self-assembly	Chemical separation and biomedical applications	Yes
self-healing of mycelium composites(Ng, Barati, & Karana, 2022)	Fungi	HLMs	*Ganoderma Strains, Schizophyllum commune*	Self-regeneration	Material driven design	No
Enhancement of fungal concrete self-healing by immobilization of fungi with calcium alginate beads (Khushnood, Ali, Faraz Bhatti, & Ahmed Khan, 2022)	Fungi	HLMs	*Rhizopus oryzae, Trichoderma longibrachiatum*	Self-regeneration, biomineralization	Construction materials	No
Concrete bioprotection coating based on fungal biomineralisation(Zhao, Dyer, Csetenyi, Jones, & Gadd, 2022)	Fungi	HLMs	*Neurospora crassa*	Self-regeneration, biomineralization	Construction materials	No
Fungal–bacterial ELMs(McBee et al., 2022)	Cross	HLMs	*Ganoderma sp., Pantoea agglomerans*	Fungi: structural function, Engineered bacteria: sensing and responding	Construction materials	Yes
Electrical response of fungi to changing moisture content (Phillips, Gandia, & Adamatzky, 2023)	Fungi	HLMs	*P. ostreatus*	Sensing and responsing	Smart buildings, wearables and biosensors	No
Engineered living mucelium materials capable of tunable self-dyeing (K. Li et al., 2023)	Fungi	Bio-ELMs	*Aspergillus niger*	Sensing and responding, regeneration	Fabrics, packaging and biosensors	Yes
Fungi-mediated self-healing concrete (Van Wylick, Brouwers, Rahier, Peeters, & De Laet, 2024; Van Wylick, De Laet, Peeters, & Rahier, 2023)	Fungi	HLMs	*Trichoderma reesei, N. crassa*	Self-regeneration, biomineralization	Construction materials	No
3D printed living mycelium materials(Gantenbein et al., 2023)	Fungi	HLMs	*Ganoderma lucidum*	Self-regeneration, Sensing and responding	Robotic' skin	No
Levaging phase separating ability of fungal mycelium to	Fungi	HLMs	*G. lucidum*	Self-regeneration	Mycelium composites with	No

produce living mycelium materials with improved properties(H. Wang et al., 2024)					improved mechanical properties and hydrophobicity	

Sensing oriented fungal ELMs

Fungi possess sensory capabilities that are almost equivalent to those of humans (Bahn & Mühlschlegel, 2006). Numerous studies on fungal sensory abilities over the years have demonstrated that fungi can perceive a variety of physical stimuli, including light, electrical field changes, gravity, and tension, as well as chemical stimuli such as gaseous substances. These stimuli elicit corresponding electrical responses in fungi (Adamatzky, 2018a, 2018b; Adamatzky, Gandia, & Chiolerio, 2021; Adamatzky, Nikolaidou, et al., 2021; Bahn & Mühlschlegel, 2006). It has been suggested that fungi might use these electrical responses as a form of communication and information processing (Adamatzky, 2022).

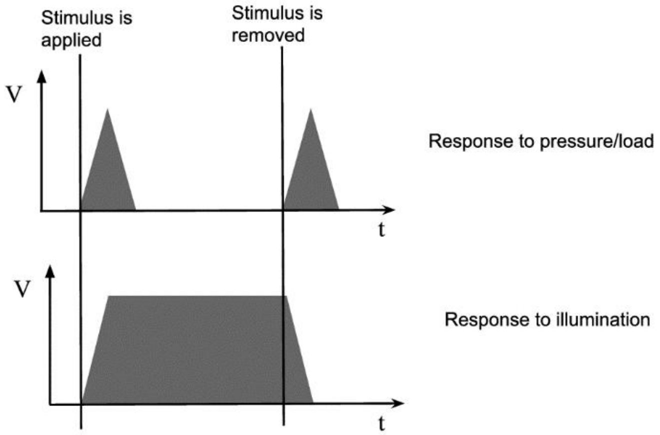

Figure 3. The different electrical response of fungal skin to mechanical load and optical stimulations. The image is from (Adamatzky, Gandia, et al., 2021).

Adamatzky et al. (2021) demonstrated that a thin layer of homogeneous living pure mycelium of *G. resinaceum* can sense and respond to both tactile and optical stimuli, with distinct electrical responses to each type of stimulation. Figure 3 illustrates that the fungal skin exhibited two transient potential spikes at the initiation and cessation of mechanical stimulation. In contrast, light stimulation induced a gradual increase in potential, which was sustained until the light source was turned off.(Adamatzky, Gandia, et al., 2021). Another study demonstrated that hemp fabric colonized by the fungus *P. Ostreatus* exhibited distinct responses to chemical stimuli, such as ethanol, and to mechanical stress from weight. Notably, the areas of the fabric distant from the point of ethanol application reacted simultaneously with those near the application site. This suggests that the response of the fungal fabric to chemical stimuli is likely due to the electrical reactions and signal transmission between cells caused by damage to the cell wall pulses, rather than the diffusion or evaporation of ethanol within the fabric. This finding is consistent with previous studies on the application of physical, chemical, and thermal stimuli to fungal mushroom fruiting bodies, where not only the stimulated fruiting body showed spike reactions, but other fruiting bodies in the cluster also responded (Adamatzky, 2018a).

This series of studies demonstrates that fungi can respond to external environmental stimuli by generating electrical signals, which can be involved in transmission, information processing, decision-making, and reporting. Based on these findings, researchers have hypothesized that fungi use electrical reactions as a form of communication and information processing, akin to a language. They conducted an analysis of the spiking electrical responses of four fungal species, focusing on the complexity of the language and information content. The results showed that the distribution of word lengths in fungal "language," as indicated by spike groupings, mirrors that of human language (Adamatzky, 2022). Although the analysis and decoding of fungal electrical language are still in the early stages, ongoing research in fungal sensing continues to advance our understanding. For example, studies are exploring how to infer the weight, location, and direction of movement of a load applied to fungal skin based on its electrical response patterns, as well as how to map the spectrum of light applied to the skin from patterns of electrical activity (Adamatzky, Gandia, et al., 2021). These lines of research pave the way for the development of fungal sensors, electronic skins, and large-scale intelligent active wearable devices.

The aforementioned studies focus on the innate sensing capabilities of fungi, but the genetic editability of certain model fungi provides opportunities for engineering enhanced fungal sensing abilities. Li et al. (2023) developed a self-regulating, color-changing mycelial living membrane material based on engineered *A. niger*. Through techniques like gene knockout and inducible gene expression, the researchers inserted a xylose-activated promoter sequence upstream of the genes involved in melanin synthesis in *A. niger*. This engineering enabled the living membrane material to sense varying concentrations of xylose and display corresponding color intensities. This innovation could be further developed into a potential biosensor for detecting xylose levels in industrial papermaking wastewater. Notably, these living materials maintained viability, self-regeneration, and genetic stability even after three months of storage (K. Li et al., 2023). This research introduces a novel, engineerable fungal chassis for ELMs and offers a new bioengineering approach for developing fungal sensors with precise signal response capabilities.

Self- healing oriented fungal ELMs

In addition to utilizing the sensory capabilities of fungi, another significant opportunity for developing fungal ELMs lies in exploiting the regenerative and self-repairing abilities of fungi (Elsacker et al., 2023).

Fungal-based self-healing concretes

Cracks in concrete provide pathways for water and gases to penetrate deeper into the structure, accelerating aging and compromising the stability and durability of the concrete. The development of self-healing concrete can effectively reduce the labor and high costs associated with the maintenance and repair of concrete structures. Currently, self-healing concrete can achieve this functionality through three primary mechanisms: autogenous healing, the embedment of polymeric materials, and microbially induced carbonate precipitation (MICP) (Seifan, Samani, & Berenjian, 2016). The MICP solution, which is more sustainable and environmentally friendly, relies on microbial activity to induce the formation of calcium carbonate ($CaCO_3$). Since $CaCO_3$ closely resembles the composition of concrete, it is often regarded as an ideal candidate for filling cracks in concrete (Menon et al., 2019).

Microbially Induced Carbonate Precipitation (MICP) includes several metabolic pathways, such as urea hydrolysis (Luo et al., 2018; Zhao et al., 2022), organic acid oxidation (Jonkers, 2011), and nitrate reduction(Erşan, Belie, & Boon, 2015). Among these, urea hydrolysis is the most efficient pathway for producing calcium carbonate, making it particularly suitable for application in self-healing concrete (Zhao et al., 2022). Urease, an enzyme found widely in both bacteria and fungi, catalyzes the hydrolysis of urea into carbonate and ammonium, which raises the pH of the surrounding environment. This pH increase indirectly leads to the precipitation of calcium as calcium carbonate (Q. Li, Csetenyi, & Gadd, 2014; Luo et al., 2018).

Bacteria-mediated self-healing concrete technology has already reached commercialization. The Dutch company Basilisk Self-Healing Concrete has developed technologies that can successfully repair cracks up to 1 mm wide ("Basilisk Self-Healing Concrete," 2021). Recent groundbreaking research has extended the development of bacterial-based self-healing concrete by exploring the potential of fungal spores for microbial-induced self-repair in concrete. The feasibility of using filamentous fungi such as *A. nidulans*, *T. reesei*, *Fusarium oxysporum*, and *R. oryzae* for producing self-healing concrete through MICP has been demonstrated (Khushnood et al., 2022; Luo et al., 2018; Menon et al., 2019; Van Wylick et al., 2024; Van Wylick et al., 2023; Zhang, Fan, Li, Samia, & Yu, 2021; Zhao et al., 2022).

Compared to bacteria, fungi exhibit superior survival capabilities under the harsh conditions of concrete, including high pH, dryness, and fluctuating temperatures (Zhao et al., 2022). Luo et al. (2018) reported that *Trichoderma reesei* spores could germinate into hyphae even under extreme pH conditions of 13.0 and grew equally well with or without the presence of concrete. Menon et al. (2019) engineered an alkalinity-mimicking mutant of *A. nidulans* that remains in a state of alkaline gene activation and acidic gene repression regardless of extracellular pH changes. This mutant strain grows normally on high-pH concrete slabs, mimicking wild-type gene expression under alkaline conditions (Menon et al., 2019). Additionally, the three-dimensional mycelial network of filamentous fungi offers abundant nucleation sites for biomineral formation (Jin, Yu, & Shui, 2018).

Fungi also surpass bacteria in their ability to promote calcium carbonate precipitation. Unlike bacteria, which rely solely on the MICP process, fungi can induce calcium carbonate precipitation through both biomineralization and organomineralization. The chitin in fungal cell walls has a strong affinity for binding calcium ions. This allows both living and dead fungal biomass to sequester calcium ions, which then react with carbonate ions from urea hydrolysis to form calcium carbonate, nucleating and precipitating on the hyphae (Menon et al., 2019). This capability enables filamentous fungi to fill larger cracks in concrete. For example, encapsulating *R. oryzae* in calcium alginate beads allowed the fungi to fill concrete cracks up to 1.3 mm wide (Khushnood et al., 2022) , surpassing the 1 mm limit achieved by bacterial-based self-healing technologies ("Basilisk Self-Healing Concrete," 2021).

The strategies for applying MICP (Microbially Induced Calcium Carbonate Precipitation) to concrete can be divided into two main approaches: incorporating microorganisms into the concrete matrix and surface coating techniques.

The first approach involves encapsulating fungal spores within the concrete. These spores remain dormant until cracks form, at which point they become active and repair the cracks through biomineralization (Van Wylick et al., 2024; Van Wylick et al., 2023). The challenges here include developing effective encapsulation techniques, ensuring spore viability within the concrete, and minimizing the impact of healing agents on the concrete's mechanical properties (Erşan et al., 2015; J. Y. Wang, Snoeck, Van Vlierberghe, Verstraete, & De Belie, 2014). A spore encapsulation method using sodium alginate and calcium lactate, previously reported for bacterial-based self-healing concrete (Fahimizadeh, Diane Abeyratne, Mae, Singh, & Pasbakhsh, 2020), has been adapted for fungal spores by Khushnood et al. (2022) and Van Wylick et al. (2024).

Khushnood et al. (2022) focused on the healing capabilities of *R. oryzae* and *T. longibrachiatum* spores encapsulated in calcium alginate beads (CAB) within concrete. Their research, conducted in a moist environment to maintain spore viability, demonstrated that *R. oryzae* could heal cracks up to 0.6 mm wide when directly mixed into concrete, and up to 1.3 mm wide when encapsulated. Additionally, the mechanical properties and microstructure of the concrete improved significantly with the encapsulated fungi (Khushnood et al., 2022). Van Wylick et al. (2024), on the other hand, aimed to optimize the encapsulation technique and assess its feasibility for fungal spores. Their studies showed that spores of *T. reesei* and *N. crassa* survived encapsulation, though spore viability decreased significantly after drying and was completely lost under conditions mimicking those found in concrete. They also quantified biomass formation and calcium carbonate precipitation under various conditions, providing valuable insights into optimizing the encapsulation process for fungal spores in self-healing concrete (Van Wylick et al., 2024; Van Wylick et al., 2023).

The second strategy for applying fungal MICP to concrete is surface coating. This approach serves as a preventive measure, applied before cracks form, and acts as a barrier to liquids and gases (Gadd & Dyer, 2017; Zhao et al., 2022). Research has explored the feasibility of using urease-positive filamentous fungi like *N. crassa* to colonize porous building materials such as mortar and cement, forming a protective hyphal-mineral layer that prevents crack formation. Filamentous fungi can express hydrophobins, making the hyphal network hydrophobic (Khushnood et al., 2022; Sunde, Kwan, Templeton, Beever, & Mackay, 2008). This dense network, combined with biomineralized calcium carbonate, fills surface pores and physically blocks moisture infiltration, thereby enhancing the hydrophobicity and durability of the material (Zhao et al., 2022). Compared to incorporating microbially induced healing agents into the concrete matrix, this coating approach presents fewer challenges for fungal survival and does not negatively impact the concrete's mechanical properties. However, further research is needed to optimize key factors such as pH, urea, and calcium concentrations, as well as to assess the effects of environmental conditions like temperature fluctuations and weathering. Additionally, the long-term effectiveness and economic feasibility of this microbial mineralization-based coating technology require further exploration.

In all the studies on fungal- induced self-healing concrete, the fungi used belong to the Ascomycota phylum. However, no research has yet explored the potential of Basidiomycetes in self-healing concrete applications. Basidiomycetes, such as *P. ostreatus*, *G. resinaceum*, and *Trametes* species, are widely used in producing mycelium materials, renowned for their high colonization rates and robust hyphal networks. Some Basidiomycetes are also known to thrive in harsh environments with high levels of oxidative stress (Van Wylick et al., 2021). Moreover, several studies have demonstrated that active leather-like mycelial materials (Elsacker et al., 2023) and mycelium composites (McBee et al., 2022; Ng et al., 2022) possess great regenerative and self-healing abilities. Given these properties, exploring the feasibility of Basidiomycetes in promoting biomineralization and calcium carbonate precipitation within concrete environments could be an intriguing research direction. This exploration could potentially unlock new possibilities for more resilient and sustainable self-healing concrete technologies.

Self-cleaning surface

Gerber et al. (2012) have developed an adaptive self-cleaning antimicrobial surface using the regenerative capabilities of fungi. This two-dimensional self-cleaning surface consists of three material layers. The base layer is a polyvinyl chloride (PVC) film that provides structural support. The middle layer, which is the living component, contains fungal spores embedded in agar to sustain the fungi. The top layer is a nano-porous polymer with dual functions: it protects the living layer from external influences and its nano-porous structure allows for nutrient transport while preventing the escape of fungal spores and hyphae into the environment (Gerber et al., 2012a, 2012b).

The mechanism of fungal action in this self-cleaning surface is similar to that of fungi encapsulated in concrete. Fungi are confined to a two-dimensional planar structure with limited nutrients. In the absence of a nutrient source, they can survive as spores for several months. When nutrients such as food spills or bacterial contamination come into contact with the surface, the fungi become metabolically active. They consume the nutrients through the cover layer and proliferate within the living layer, releasing secondary metabolites like penicillin to inhibit bacterial contamination. Once the nutrients are depleted, the fungi revert to a dormant state, awaiting the next opportunity for nutrient intake (Gerber et al., 2012a). This self-cleaning surface, based on the regenerative and metabolic activities of fungi, presents an innovative approach for applications such as food packaging and indoor surfaces like dining tables.

Amplifying the structural functions of mycelum materials with additive manufacturing techniques

While we have primarily focused on the biological functions of fungi in discussing research on fungal Engineered Living Materials (ELMs) over the past decade, nearly every study involving filamentous fungi highlights their unique advantage in ELMs—the structural support provided by the mycelial network. However, without human intervention, it is challenging for fungal materials to form complex, specific three-dimensional structures. Therefore, the further development and production of fungal materials are closely linked to additive manufacturing technologies, such as 3D printing.

The ability to 3D print living inks into intricate geometries can improve the structural support capacity of mycelial materials, enabling the creation of specific structures that combine biological functions to meet particular application requirements. Gantenbein and colleagues described a 3D printing method for fungal materials using hydrogel loaded with *G. lucidum* as bio-ink. They successfully printed a robotic gripper and a spherical robotic skin that exhibited mechanical robustness, self-cleaning properties, and the ability to self-repair after damage (Gantenbein et al., 2023). However, several challenges must be addressed to realize the potential of 3D printing complex, large-scale mycelium composites: (1) The substrate must possess sufficient fluidity to be smoothly extruded from the nozzle. (2) Simultaneously, the substrate must also exhibit adequate load-bearing and anti-deformation properties to ensure that it adheres to the layer above without collapsing or deforming under pressure. (3) Maintaining a sterile environment throughout the entire production process presents significant difficulties (Elsacker et al., 2021).

Elsacker et al. (2021) developed a pipeline that integrates biological and digital fabrication techniques for the production of large-scale mycelium material blocks. As illustrated in Figure 4, this study involves the layered production of square mycelium-based materials. Multiple layers of mycelium composites are stacked and then self-welded into a single, cohesive material through the natural growth of the mycelium. Subsequently, these mycelium blocks are cut into specific structures using digitally controlled robotic wire-cutting machine (Elsacker et al., 2021). This approach parallels the modular production strategy employed by McBee et al. (2022). Both studies utilize the biological welding capability of living mycelium to bridge gaps and join modular mycelium blocks, achieving material scales that would be impossible to reach using molds alone. This demonstrates the potential for constructing complex shapes at an architectural scale using only mycelium, without the need for mortar, adhesives, or metal fasteners. Moreover, it highlights the applicability of modular mycelium production in the construction of large-scale biobased structures.

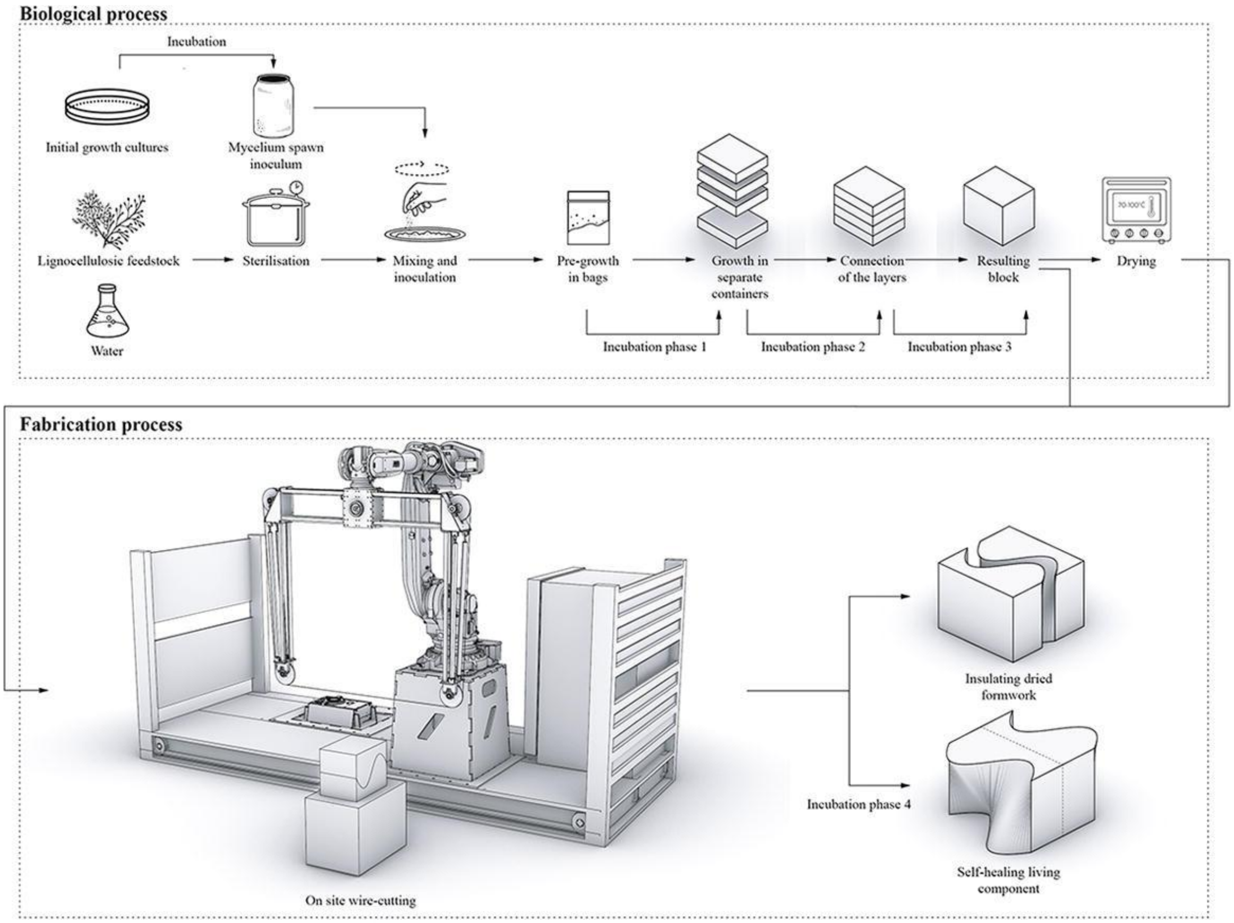

Figure 4. Workflow including biological and digital fabrication process for the production of large-scale mycelium material blocks. The image is from (Elsacker et al., 2021).

From the summarized research cases on fungal-based ELMs, it is evident that, apart from a few ELMs produced by model fungi such as yeast (Yi et al., 2022) and *A. niger* (K. Li et al., 2023), most research and development of filamentous fungi-based ELMs rely more on their inherent biological processes initiated by physical interventions and environmental responses, such as molds, growth substrates, and post-processing methods (Elsacker et al., 2023). Unlike most bacteria-based ELMs, which heavily depend on synthetic biology techniques such as genetic editing for material functionalization. The development of genome engineering tools is less advanced in basidiomycetes compared to other model filamentous fungi like *A. niger* from the Ascomycota phylum (Elsacker et al., 2021). This has resulted in relatively fewer studies on fungal ELMs utilizing synthetic biology techniques. However, with the continuous advancement of CRISPR-Cas9 tools for the genetic manipulation of filamentous fungi (Nødvig, Nielsen, Kogle, & Mortensen, 2015; Shen et al., 2024)， the future prospects for genetically customized multifunctional mycelium-based active materials are promising.

Application of fungal-bacterial consortia to promote the development of multifunctional mycelium-based ELMs

Although research on the combined use of microorganisms for producing ELMs is currently limited, existing studies demonstrate that certain synergistic microbial consortia have the potential to save external resources, enhance material sustainability, and achieve multifunctionality (Abeysinghe et al., 2020; Das, Bovill, Ayesh, Stoyanov, & Paunov, 2016; McBee et al., 2022).

Existing case studies of fungal-bacterial co-culture production of ELMs

Gilbert et al. (2021) developed a programmable, functional living material by a stable co-culture of the yeast model organism *S. cerevisiae* and the bacterial cellulose-producing bacterium *K. rhaeticus*. The key point is the rational gene editing of *S. cerevisiae* so that it directs and participates in the synthesis of the material influencing its properties, such as secreting enzymes to catalyze the growth of the material as well as sensing and responding to chemical and physical stimuli. Meanwhile, BC produced by *K. rhaeticus* serves primarily as a structural support.

The similar strategy can be applied to produce novel mycelium-based ELMs, where mycelium materials act as scaffolds and an engineered model organism contributes additional functional properties. This approach effectively addresses the limitations of genetic engineering in the functionalization of living mycelium materials, as discussed in the previous section. For instance, McBee et al. (2022) reported the development of multifunctional fungal-bacterial ELMs using hemp shives as the growth substrate and cardboard as a flexible mold. The researchers utilized an origami-inspired growth and assembly method, leveraging the colonization and bio-welding capabilities of mycelium to produce modular and interconnectable mycelium materials of various shapes. This biocomposite material can form human-scale building objects, exhibits self-healing properties, and possesses a strong load-bearing capacity. To further enhance the multifunctionality of this ELM, the researchers isolated a bacterial component, *Pantoea polymorpha*, from the growth substrate, which can be engineered. They introduced complex multi-gene pathways into this bacterium and incorporated it into the growth substrate as a multifunctional component to extend the ELM's capabilities. As a result, the final ELM acquired new biosynthetic abilities, such as the secretion of antimicrobial non-native metabolites to inhibit microbial infection, as well as the ability to sense, transmit, receive, and report signals (McBee et al., 2022).

The above two cases provide feasible solutions for creating multifunctional mycelium-based active materials with macroscopic structures. Although genetic engineering tools for basidiomycetes are still underdeveloped, bacteria capable of stable symbiosis with fungi can be introduced into the growth substrate. By genetically editing these bacteria, it is possible to regulate material synthesis and impart various functional modifications to the resulting mycelium-based materials. The next section will explore the feasibility of bacterial regulation in the production of mycelium materials.

Exploring the potential of bacterial to dynamically regulate mycelium material synthesis base on the bacteria-fungi interactions

A significant portion of the biomass in soil consists of bacteria and fungi. These two kingdoms, in addition to performing their respective functions, interact within ecosystems through mechanisms such as antibiotics, signaling molecules, co-metabolism, and physical interactions to maintain ecological balance (Nutzmann et al, 2011). Understanding the interactions between bacteria and fungi holds substantial research significance for agriculture, medicine, and environmental science. Although there is currently no research specifically addressing the mechanisms of bacterial-fungal interactions in the production of mycelium materials, McBee et al. (2022) demonstrates that coculturing engineered bacteria with fungi can effectively expand the function of mycelium-based ELMs. Building on this foundation of engineered microbial consortia, further investigation into the potential interaction mechanisms between these microorganisms could enable not only the enhancement of material functionalities but also precise regulation of mycelium material growth by engineered bacteria. This could be achieved by promoting or inhibiting growth through bacterial interactions, ultimately leading to the development of more advanced and intelligent multifunctional ELMs.

Bacteria promoting fungal mycelial growth

Filamentous fungi can form extensive mycelial networks in the soil through extension and branching growth. In contrast, while bacterial cells are highly efficient in movement within liquid environments, their mobility in water-unsaturated soil conditions is often facilitated by fungal hyphae, a phenomenon supported by multiple studies (Kohlmeier et al., 2005; Pion et al., 2013). This interaction is considered commensal, as the fungi do not derive any apparent benefit from providing a "highway" for bacterial movement.

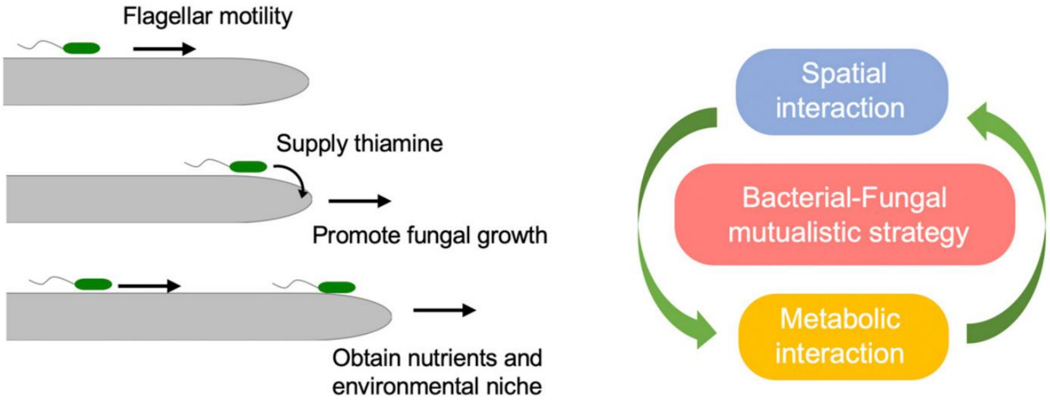

Figure 5. The mutualistic growth strategy between bacteria and fungi involves bacteria rapidly moving and spreading along fungal hyphae. As the bacteria migrate to the tips of the hyphae, they release thiamine, which in turn promotes fungal growth. Image is from (Abeysinghe et al., 2020).

Abeysinghe et al. (2020) reported a mutualistic symbiosis between *B. subtilis* and *A. nidulans* under laboratory agar culture conditions (Figure 5). Through gene deletion studies, molecular mass analysis, and imaging techniques, they demonstrated that motile bacteria utilize fungal hyphae as a "highway" for movement, dispersing as the mycelial network expands. In return, the bacteria at the hyphal tips release thiamine, promoting fungal growth (Abeysinghe et al., 2020).

Thiamine, or vitamin B1, is synthesized by most prokaryotes and by eukaryotes such as fungi and plants. It is an essential cofactor in vital biochemical reactions within all organisms (Jurgenson, Begley, & Ealick, 2009; Kraft & Angert, 2017). *B. subtilis* has been reported to supply thiamine to thiamine-auxotrophic fungi. Based on the research of Abeysinghe et al. (2020), even though *A. nidulans* is not a thiamine-auxotrophic fungus, it uptakes thiamine from *B. subtilis* to save the metabolic cost of synthesizing it. Experiments showed that the fungal biomass in co-culture with *B. subtilis* was 40% higher than in monoculture, whereas the fungal biomass and thiamine content in fungal cell extracts were not increased when co-cultured with *B. subtilis* ΔthiA (a thiamine biosynthesis-deficient mutant). This indicates that thiamine released by *B. subtilis* indeed promotes fungal mycelial growth. Furthermore, the bacterial proliferation in co-culture was higher than in monoculture, suggesting that the mycelium network provides a favorable environment for bacterial migration, dispersion, and proliferation.

Bacteria inhibiting fungal mycelial growth

The mechanisms by which bacteria regulate fungal hyphal growth can be broadly categorized into two types: promotion and inhibition. *B. subtilis* is known to release antifungal cyclic lipopeptides (CLPs) such as fengycin, which inhibit the growth of filamentous fungi (Fan, Ru, Zhang, Wang, & Li, 2017; Hu, Shi, Zhang, & Yang, 2007; Vanittanakom, Loeffler, Koch, & Jung, 1986). The fengycin family is synthesized by five biosynthetic genes: *ppsA*, *ppsB*, *ppsC*, *ppsD*, and *ppsE* (Batool et al., 2011). Fan et al. (2017) identified *ppsB* as a key gene responsible for fengycin synthesis, with fengycin being the primary active compound exerting antagonistic activity of *B. subtilis* 9407 against *Botryosphaeria dothidea* YL1. Fengycin exhibits strong antifungal activity against filamentous fungi, potentially disrupting fungal cell membrane structure and permeability through interactions with sterol and phospholipid molecules, leading to fungal cell inactivation (Deleu, Paquot, & Nylander, 2005; Fan et al., 2017).

Fengycin B, a variant of fengycin, has been shown to inhibit mycelial growth by inducing deformations, oxidative damage, and mitochondrial dysfunction within the hyphae. Results indicated that after a 1.5-hour treatment with fengycin B, zoospore release was fully inhibited. Additionally, the treated hyphae displayed deformations, rough surfaces, and partial separation of the cell membrane from the cell wall. Organelles within the cells also exhibited blurred boundaries and irregular shapes (Y. Wang et al., 2020).

Although current research has yet to explore the use of engineered bacteria to guide mycelium-based material production, this is clearly a feasible and promising area for further investigation. It could potentially lead to innovative ELMs. For example, developing self-healing concrete mediated by a combination of fungi and bacteria could be highly effective. In harsh concrete environments, fungi could not only precipitate calcium carbonate but also act as carriers for bio-mineralizing bacteria. The bacteria could release thiamine to promote further mycelial spread, potentially enhancing the self-healing capacity of the concrete.

Another promising research direction involves engineering *B. subtilis*, such as by incorporating light-sensitive pathways to regulate gene expression related to thiamine or fengycin synthesis. By modulating light exposure, it may be possible to precisely control the production of these compounds, thereby dynamically regulating fungal mycelium growth. This approach could potentially address the challenge identified by Elsacker et al. (2023) regarding that living pure mycelium materials are unable to directionally self-repair damage sites. Alternatively, engineered mycelium-based materials with customized properties could be developed, leading to more advanced, responsive, and multifunctional engineered living materials (ELMs).

Conclusions and Outlook

As the development and commercialization of mycelium-based materials continue to advance, the potential of filamentous fungi in producing ELMs is gaining increasing attention. This thesis reviews the progress in fungal ELMs research and highlights several inspiring case studies, demonstrating the significant research value and application potential of fungal ELMs across multiple domains, as well as the opportunities and challenges that lie ahead.

The dynamic responsiveness of living fungal materials to external physical and chemical stimuli positions them as promising candidates for the production of novel biosensors (Gilbert et al., 2021; K. Li et al., 2023), human-scale wearable smart devices (Phillips et al., 2023), and robotic skins (Gantenbein et al., 2023). Their inherent abilities for self-assembly, self-regeneration, and self-repair, enable the development of antibiotic-releasing (Gerber et al., 2012b) and self-cleaning (Gerber et al., 2012a) surfaces and self-repairing construction materials (Adamatzky et al., 2019; Elsacker et al., 2021; Menon et al., 2019). Additionally, the combine of biological and structural functionalities in mycelium makes it feasible to produce ELMs with macrostructures in situ.

This thesis highlights two additive manufacturing techniques for producing fungal ELMs. One approach is 3D printing, which shows promise for creating complex, specific structures. However, challenges remain in scaling up to large, integrated structures, such as substrate collapse under excessive load, nozzle clogging, and high contamination risks (Elsacker et al., 2021). The other, more ingenious method involves a modular production strategy that leverages mycelium's biological welding ability to join and shape modular blocks using a robotic wire-cutting machine. This method enables the construction of complex, architectural-scale shapes entirely from mycelium, without the need for additional materials like mortar or adhesives, demonstrating its potential for large-scale biobased structures.

This thesis highlights two additive manufacturing techniques for producing fungal ELMs. One promising approach is 3D printing, which offers the potential to create ELMs with specific and complex structures. However, challenges remain in scaling up to large, integrated structures, such as substrate collapse under excessive load, nozzle clogging, and high contamination risks. The second technique involves a modular production strategy that combines biological welding and robotic wire-cutting (Elsacker et al., 2021). This approach demonstrates the potential for constructing complex architectural-scale shapes using only mycelium, without the need for mortar, adhesives, or metal fasteners. It also underscores the applicability of modular mycelium production in building large-scale biobased structures.

A review of the last decade's research on fungal ELMs reveals that most studies, particularly those focused on basidiomycetes, rely heavily on the intrinsic biological processes triggered by physical interventions and environmental responses (Elsacker et al., 2023). There has been minimal exploration into using synthetic biology techniques to engineer fungi for guiding the synthesis or functional modification of fungal materials. The primary limitation stems from the relatively restricted availability of genetic information and manipulation tools for fungi (Nguyen et al., 2018). However, this thesis emphasizes pioneering work in introducing engineered bacteria during mycelium material growth (McBee et al., 2022), offering an innovative solution for creating multifunctional, macro-structured, active materials based on mycelium.

This review then further explores the interactions between fungi and bacteria, where *B. subtilis* can migrate along the mycelium, releasing thiamine to promote mycelial growth (Abeysinghe et al., 2020) or fengycin (Hu et al., 2007; Vanittanakom et al., 1986) to inhibit the growth of filamentous fungi. This opens a viable and promising research direction: utilizing engineered bacteria to guide the production of mycelium-based materials. Given the spontaneous nature of fungal growth, precise artificial intervention in promoting or inhibiting mycelial development is challenging. However, by engineering *B. subtilis* to modulate the expression of genes related to thiamine or fengycin synthesis, it may become possible to achieve precise, dynamic control over fungal mycelium growth.

In conclusion, while the field of fungal ELMs is still in its early stages, the potential for innovation is immense. Future research that integrates synthetic biology with traditional mycology could lead to groundbreaking advancements in the production of sophisticated, multifunctional materials. The continued exploration of fungal-bacterial co-culturing, coupled with advances in genetic engineering, promises to unlock new possibilities for the design and application of mycelium-based ELMs across various industries. However, as fungal ELMs transition from research to potential commercialization in the future, it is crucial to consider public acceptance of fungal materials, as well as the biosafety concerns associated with the use of living fungal materials, particularly their pathogenicity to humans.

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