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Fungal Engineered Living Materials: The Viability of Pure Mycelium Materials with Self-Healing Functionalities

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Engineered living materials (ELMs) composed entirely of fungal cells offer significant potential due to their functional properties such as self-assembly, sensing, and self-healing. Alongside rapid developments in the ELM field, there is significant and growing interest in mycelium materials, which are made from the vegetative part of filamentous fungi, as a potential source of advanced functional materials. In order to advance the development of fungal ELMs that utilize the organism's ability to regenerate as self-repair, new methods for controlling and optimizing mycelium materials are needed, as well as a better understanding of the biological mechanisms behind regeneration. In this study, pure mycelium materials are fabricated for use as leather substitutes, and it is found that chlamydospores, thick-walled vegetative cells formed at the hyphal tip, may be the key to the material's self-healing properties. The results suggest that mycelium materials can survive in dry and oligotrophic environments, and self-healing is possible with minimal intervention after a two-day recovery period. Finally, the study characterizes the mechanical recovery and physical properties of damaged and healed samples, allowing for the first characterization of fungal ELMs.

1. Introduction

1.1. Engineered Living Materials: A New Class of Advanced Materials with Self-Healing Capacities

The motivation to improve the sustainability of the production processes and life cycles of materials and the need to achieve complex material function is leading engineers and designers to turn to biological systems as an inspiration and as a potential source of next-generation advanced materials.[1] Biological systems are capable of self-assembling structurally complex materials from building blocks of proteins, polymers, and biominerals.[2] Furthermore biological system maintain complex structures throughout the lifetime of the organisms, exhibiting adaptability to their environment and properties such as self-healing.[2]

Utilizing biological systems as both inspiration and substance for new materials has given rise to the field of Engineered Living Materials (ELMs).[2,3] According to Nguyen et al., ELMs are materials that are made up living cells that form the material itself.[2] In this context, "engineered" refers to the cultivation and fabrication of materials through intentional manipulation of the biological growth in a controlled environment, rather than harvesting and processing natural materials (e.g., engineered wood). Because ELMs are "living", they can respond to specific stimuli during their lifetime.[2] This means that these materials can change their properties based on the environment they are exposed to. If needed, the living cells can be killed, leaving the material without the need for ongoing maintenance or any potential biohazard concerns,[2] and thus converting the ELM into a biohybrid material.

Recently, researchers have developed a bacterial cellulose (BC) ELM, where cellulose producing *Komagataeibacter rhaeticus* was co-cultured with engineered yeast *Saccharomyces cerevisiae* that can produce recombinant enzymes and provide the cellulose pellicle with the catalytic properties.[4] The cellulose pellicle could be dehydrated at room temperature and then rehydrated, with enzymatic activity restored, indicating that reactivation occurred after the bacterial cells finished generating the material. Another study by the same research group looked at methods to repair and regenerate BC-based materials that had been damaged, but found that either regrowth failed or mechanical characteristics declined.[5] The punctured holes were seeded with BC spheroids because the initial BC pellicle was unable to regrow new material. Patching large holes is a common strategy employed in the study of self-healing materials.[6]

ELMs can include hybrid living materials (HLMs), combining synthetically manufactured materials with cells that are

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the functional "smart" components.[7,8] An example of HLM reactivation is microbial-mediated self-healing of concrete fissures.[9,10] The method relies on the precipitation of $CaCO_3$ through microbial cells metabolic activities in a concrete mix.[11] The spores are injected while they are metabolically dormant and hence resistant to the harsh conditions of the concrete environment, where they may survive for lengthy periods of time.[10] When the concrete cracks, the encapsulated nutrients and spores are exposed to water and oxygen, causing spore germination. The development of a hybrid living system that incorporates both living and nonliving components increases the complexity and functionality of normally inorganic materials. It enables such composites to be built with self-maintenance capabilities, providing autonomous self-healing.

1.2. Mycelium Materials

The kingdom of the fungi represents a fascinating and evolutionary ancient biological group of eukaryotic microorganisms. More specifically, Basidiomycetes fungi are of particular interest in this study. They are common in wood habitats and grow as long filaments (hyphae) with diameters ranging from 2 to 10 µm, forming a complex network known as mycelium by degrading organic plant-based material. The so-called "white-rot fungi" have even developed the ability to degrade harsh lignin polymers in wood structures.[12]

The intriguing properties of filamentous fungi have not gone unnoticed in the context of bio-fabrication of biodegradable materials as they are seen as a low-cost and ecologically sustainable option when compared to the manufacturing process and life-cycle of petroleum-based materials.[13] Multiple sectors, ranging from construction to the chemical and textile industries, are transitioning toward bio-based and closed-loop circular economy strategies and are showing interest in these mycelium materials. A novel industrial sector focused on this new type of biomaterials is emerging, with the US-based companies Ecovative and Mycoworks being leading players.[14] The initial focus was on the manufacturing of solid composite materials by growing the fungus into lignocellulosic fibers, therefore valorizing organic waste streams and producing dense bulk materials.[15,16] Recently, approaches have been developed in which the fungus is grown as a flexible membrane on top of a liquid or solid substrate.[17–19] These materials consist for the main part of fungal tissue and have textile-, leather- or foam-like properties after post-production treatments that can be used in consumer good applications (e.g., clothes, bags, furniture, etc.).

In currently described production processes and applications, whether a solid or flexible composite, the mycelium material is heat-treated and dried after a growth period of typically a few weeks, killing the fungal organism. Cell death occurs if the cytoplasmic content of hyphae is drained during the drying process.[20] In the case of flexible mycelium materials, post-processing steps (e.g., glycerol treatment, crosslinking and coating) are performed to yield materials with desired mechanical characteristics.[21] Although this strategy can be justified by the need to generate a stable and inert product, avoiding unpredictable further organismal development (e.g., growth or fruiting body development), it misses out on potentially beneficial properties of a living material.

1.3. Fungal Engineered Living Materials

Despite the great promise and rapid progress in this field, there are only a few examples of ELMs that are entirely biological ("bio-ELMs"[3]) and in which the organism—typically a microorganism—provides not only functional capabilities, but also structural properties of the material at the macroscale. Because many ELMs are developed using single celled microorganisms restricted to the nano- and microscales, such as *Escherichia coli*,[3,8] they are typically not structured at the macroscale. These ELMs often require an external scaffold to achieve the macroscale,[7] such as BC bio-ELM[4,5] where the structural component is the cellulose network, a by-product of microbial activity rather than the microorganism itself.

Living fungal materials are potentially a promising novel class of biological macroscale ELMs and its exploration has just started.[7,22] Filamentous fungi have significant promise as ELMs because of their i) ability to assemble macroscale material structures, ii) their cell wall characteristics can provide outstanding tensile strength because of the presence of chitin, and water repellence due to the expression of hydrophobins,[23] iii) their versatility in obtaining nutrients that allows them to colonize any habitat by expressing extracellular carbohydrate-active enzymes, iv) their natural self-assembly and self-healing properties, including the formation of network-like hyphal structures naturally filling void spaces within an existing matrix, v) their capability to survive harsh environments (starvation, drought, high salinity, and extreme temperatures) in combination with superior sophisticated environmental sensing and adaptation mechanisms.

A major opportunity for the development and use of fungal ELMs is to utilize the organisms capacity for regeneration and self-repair.[24] A few pioneering studies have lately investigate the use fungal spores for the microbial-mediated self-healing concrete.[25] Unlike bacteria, filamentous fungi can fill and deposit $CaCO_3$ in larger cracks because of their long mycelium networks with branched filamentous hyphal structures.[26] These materials are generally inert and will only germinate when exposed to specific environmental cues.

Regeneration phenomena of damaged cells have been widely studied in plants and animals with implications in medicine,[27] but not significantly in fungi. Despite the fact that the three eukaryotic kingdoms adopted diverse tactics to adapt to mechanical damage and survive, they share molecular mechanisms and certain signaling components.[20] In general, vegetative filamentous fungi respond to damage by shutting their septal pores or caps, preventing cytoplasm leakage.[20,28] In some cases, an injury will also cause the formation of reproductive structures in the affected regions.[29] In fungi, we may divide the reaction to mechanical damage into at least three stages: wound healing, regeneration of damaged hyphae, and a third phase involving cellular differentiation that leads to the creation of reproductive structures.[20] Although the precise mechanisms are still unknown, researchers believe that damaged-self recognition exists in fungi, for example, by releasing

chemicals upon mycelial injury that are recognized by a transmembrane receptor to initiate the injury response.[30] In recent research, it has been proposed that sensing and signaling capabilities of vegetative mycelium can be leveraged to create intelligent sensing patches for reactive fungal wearables.[31] Researchers found that large mycelium composites respond to weight pressure via changes in their electrical activity.[32] However, this approach necessitates the mycelium to remain vegetative and thus, the material needs to be hydrated, which can pose limitations for many material applications. Moreover, vegetative hyphae will continue to develop and degrade the lignocellulosic scaffold in mycelium composites. While dried fruiting bodies of fungi can survive for many years, it is currently unknown if the same holds true for vegetative hyphae.

An alternative approach to creating fungal ELMs is to introduce an engineered bacterial component in co-cultivation with the mycelium material.[33,34] In this case, it is the bacteria that hold the living sensing-signaling capabilities, while the fungal organism is serving as a passive scaffold.

Finally, another approach to developing fungal ELMs involves genetically modifying the fungal organism itself.[35] In a recent study, the genetically tractable *Aspergillus niger* species was used as an engineerable chassis for the creation of fungal membranes.[36] While this method is highly promising, it is noteworthy that most filamentous fungal species used in the manufacturing of mycelium materials belong to the Basidiomycota division, whereas *A. niger* belongs to the Ascomycota division. This divergence is significant since genome engineering tools, such as high throughput transformation systems, are less well-developed in mushroom-forming basidiomycetes than in other model filamentous fungi.[37,38]

There are several approaches for developing fungal ELMs, each with their own unique advantages and disadvantages. The main objective of this work is to develop a biological macroscale fungal ELM in which the functional and viable agent, as well as the structural element are produced by the fungal organism itself. The goal is to employ a species commonly used for mycelium material manufacturing, and without the necessity for bacterial co-cultivation or genetic modification to preserve "alive" capabilities. We, therefore, have to define success criteria to develop a novel class of fungal ELM for leather-like application with self-healing capabilities. First, specific signals mediated by chemical and physical stimuli should activate cell survival responses, such as sporulation or by releasing chemicals upon mycelial injury. However, fruiting body development (including basidiospores) during the life span of the material needs to be prevented. This is due to the fact that primordial formation can cause the material to become heterogenous and basidiospores can form a health hazard.[39] Second, the fungal organism should not be vegetative because the material must remain dry and flexible during its application. Finally, the organism must be metabolically inactive during its functional use, but viable without permanently accessible feedstock. In other words, the mycelium material cannot grow while in use for its intended purpose and may only be revived if the user chooses to heal a specific damage.

To grow mycelium materials, we use the Basidiomycete fungal species *Ganoderma lucidum*, which is a medicinal mushroom that has been extensively studied in a medicinal context.[40,41] Spherical thick-walled asexual chlamydospores (up to 20 µm in diameter) formed at the hyphal tip, are known to be the organism's survival agent, resistant to harsh conditions when the fungus is not actively developing.[42,43] Chlamydospores may therefore play a major role in the material's capacity to revive and self-heal, but have never before been studied in this context.

In this work, we devise a method for sustaining the regenerative capacity of pure mycelium materials when damaged (**Figure 1**D). The regeneration processes of *G. lucidum* that are responsible for reviving the material after particular stimuli are described. The research reveals for the first time the viability of dried and plasticized mycelium materials. We investigate the possible types of minimal interventions needed for the self-healing to occur, with different types of damages, and with a post-treatment that is typically used to give this material leather-like properties. We identify the biological processes that enable this regeneration and analyze the recovery of the mechanical properties after self-healing has taken place. Overall, the ability of this regenerative mycelium material to heal micro and macro defects (Figure 1A–C) opens interesting future prospects for unique product applications in leather-goods replacements such as furniture, automotive seats, and fashionwear.

2. Results and Discussion

Three major objectives are addressed by this work on the development of a biological fungal ELM with self-healing capabilities. The first objective is to understand the biological mechanisms and parameters for regeneration. What element could provide the self-healing capacity? Second, we explore the requirements and functional properties to sustain the fungal viability toward its material application by investigating if post-produced mycelium materials have the capability for regeneration. Can mycelium material survive in dry and oligotrophic environments after having been plasticized? Finally, we determine the self-healing efficiency by characterizing the healing time, mechanical and physical properties. Do the materials recover their mechanical and physical properties after damage and healing, and how quickly?

2.1. Optimal Growth Conditions for *G. lucidum* as Pure Mycelium Leather-Like Material

The factors that impact the growth of mycelium materials were examined as a first step toward developing a self-healing fungal ELM. Using liquid state fermentation, a production pipeline for growing pure, homogeneous mycelium tissues from *G. lucidum* was created. This study builds on the work of Vandelook,[19] Appels,[21] and Gandia,[32] who initiated the first methods for liquid state fermentation of mycelium materials with leather-like characteristics. We chose this method over a solid-state fermentation[44] because the mycelium skins are more homogenous and less prone to primordial formation when grown on a nutritional liquid. Growth physiology was investigated both before and after the fabrication process, with a focus on understanding the influence of some environmental stressors, such

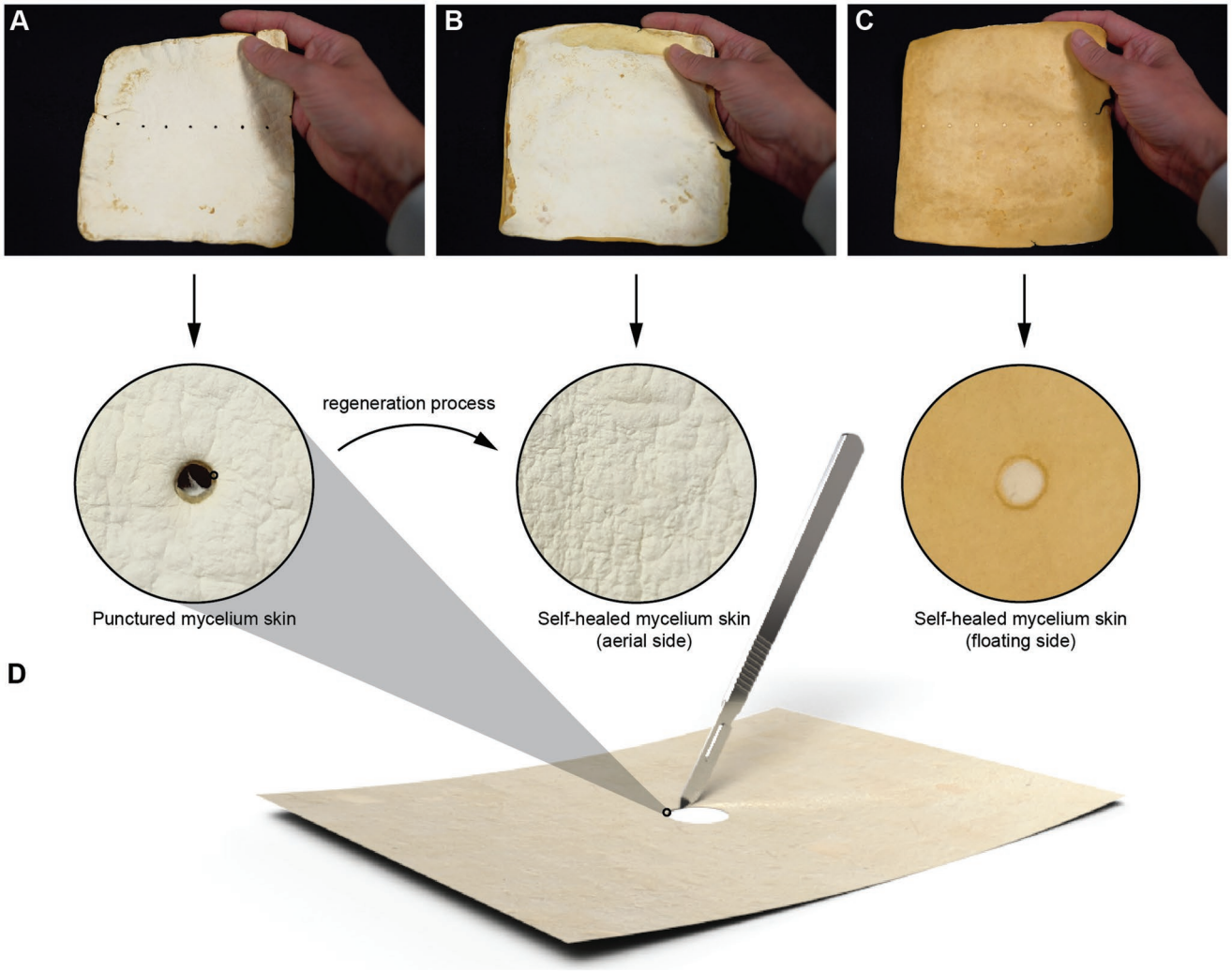


Figure 1. Regeneration process of fungal ELM with self-healing capacities. A) Dry mycelium material damaged with holes but not subjected to a healing treatment. B) Healed mycelium material after treatment, seen from the aerial side where the wounds are not visible. The holes are fully overgrown by aerial white hyphae and therefore unnoticeable. C) Same sample as shown in B but seen from the side that was in contact with the liquid media, which created floating hyphae with a yellowish color. D) Graphical visualization of a process that disentangles the requirements to sustain the self-healing capacity of pure dried mycelium materials upon damage.

as shaking speed and incubation temperature, which underpin the spectrum of fungal developmental processes.

We fabricated the mycelium skins in a two-step process by first inoculating a malt extract broth (MEB) liquid medium with *G. lucidum* plugs in Erlenmeyer flasks and shaking them at high speed to grow fungal pellet biomass (**Figure 2**E, details given in Section 5). The mycelium yield was determined every day over a period of 12 days of growth in a shaking incubator. The submerged mycelium pellets produced the highest cell density after five days (Figure 2F), which was likewise correlated to chlamydospore formation. The six following days resulted in a stationary phase. The chlamydospores were extracted from the mycelium liquid solution daily by homogenizing the pellets first and then filtering the spores (Figure S1, Supporting Information). Spores began to form abundantly on the fourth day. It is likely that as the nutrients were depleted, spore formation was triggered by the organism as a stress response. The cell density of the mycelium liquid solution increased steadily when the shaking speed was increased from 0 to 200 rpm (Figure 2Ga). Despite this, chlamydospore production decreased at 200 rpm while peaking at 150 rpm (Figure 2Gc). Chlamydospores not only formed in submerged agitation, but also appeared in static (0 rpm) submerged conditions that were grown for 5 days (Figure 2Gc). Furthermore, the increased incubation temperature of 40 °C did not result in biomass production (Figure 2Gb).

When the mycelium pellets reached maturity on day 5 (Figure 2A), the liquid solution was homogenized with a blender and transferred to 6-well plates. In static conditions, a mycelium skin formed at the liquid–air interface and grew for several more days (Figure 2B). In contact with the liquid, the skins created floating and more compact hyphae with a yellowish color, whilst in contact with the air, aerial white hyphae developed. As the hyphae continue to grow into the air, the

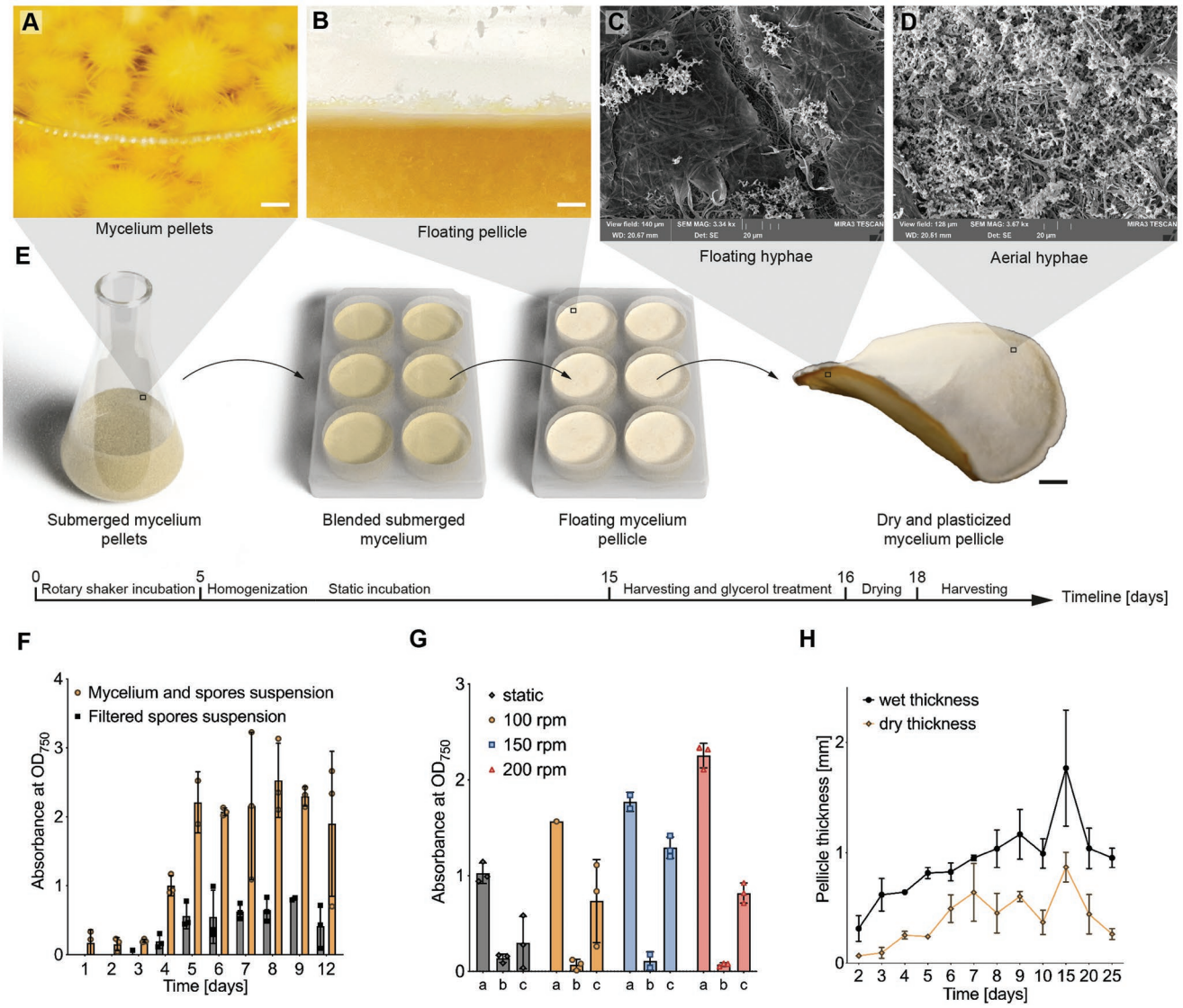


Figure 2. Optimal growth conditions for *G. lucidum* to produce mycelium skins. A) Submerged *G. lucidum* mycelium pellets after orbital shaking incubation of 5 days. Scale bar represents 10 mm. B) Floating mycelium skin forms on the liquid–air interface after 5–10 days of static incubation. Scale bar represents 10 mm. C) Scanning electron microscopy of the flat and thickly intertwined hyphae on the liquid interface. Scale bar represents 20 µm. D) Scanning electron microscopy of coral-shaped hyphae on the aerial interface. Scale bar represents 20 µm. E) Schematic illustrating the production pipeline for growing pure, homogeneous mycelium tissues from a liquid culture. Submerged mycelium pellets are formed through agitation on a shaker. Then, these are blended and transferred to a container in which it can grow in static conditions. Skins are harvested, plasticized, and dried, resulting in the final mycelium skin. Scale bar represents 10 mm. F) Absorbance at 750 nm of mycelium liquid solution and filtered spores grown on shaker (200 rpm at 30 °C) over 12 days. Chlamydospores were filtered from these mycelium suspensions. All samples were prepared in triplicate. G) Absorbance at 750 nm of mycelium liquid solution and filtered spores grown for 5 days at different shaking speeds (0, 100, 150, 200 rpm) and temperatures (30 and 40 °C) a) mycelium liquid culture incubated at 30 °C, b) mycelium liquid culture incubated at 40 °C, c) spores filtered from mycelium liquid culture incubated at 30 °C. All samples were prepared in triplicate. H) Determination of thickness at wet and dried skins over time. All samples were prepared in triplicate.

density of the packed mycelium layer underneath increases to an extend that the hyphae in contact with the liquid become anaerobic.[45,46] These floating hyphae were distinguished by their flat and thickly intertwined structure (Figure 2C). The thin biofilm that covers the hyphae in Figure 2C might be a polysaccharide coating produced by the fungi during fermentation, which is only visible on the liquid-interface. The aerial side was covered by a highly branching network of coral-shaped hyphae (0.5–1 µm diameter) (Figure 2D).

Mycelium skins were harvested each day to define the maximal thickness. On the 10th day of incubation, the wet skins were ≈1 mm thick and continued to grow until they reach 1.77 mm on day 15 (Figure 2H). The thickness of wet skins decreased on day 20 and 25 to ≈ 1 mm, which could be related to an autophagy process initiated by the organism due to nutritional starvation. After drying, the thickness of day 10 and day 15 samples reduced by 49%–62%. The standard deviations of the thickness measurements are rather large, which is most

likely due to the division of one liquid culture (400 mL) over various wells (9 mL). Even though the liquid culture was mixed to promote inoculum homogeneity, the distribution of mycelium in the liquid was not fully uniform. We also observed that the harvested mycelium skins were fragile and often not thick enough to serve as a leather substitute. Further optimization of the growth conditions, fungal strains and procedures is needed, but out of the scope of this work. This could be achieved by combining different mycelium skins or adding reinforcement layers such as natural woven textiles. Another option could be to supplement the mycelium skins with more nutrients during their growth (i.e., batch fed fermentation) and by increasing CO_2 levels.

2.2. Self-Healing Mechanism of the Living Mycelium Material to Regenerate

This section's goal is to identify the potential revival agent responsible for the material's capacity for self-healing. Is the reviving process caused by hyphae (which might have preserved cell viability even after drying), chlamydospores, or both? To do this, different species and strains were selected that are commonly used in the production of mycelium materials.[19] Our model-organism *G. lucidum* (GL-M9726) has been reported in the literature to contain chlamydospores.[42,43,47,48] We chose to produce mycelium skins from two other GL strains (GL-M9720 and GL-OS), as well as another genus of *Ganoderma*, *G. resinaceum* (GR), because *Ganoderma* strains are known to show a significant genetic diversity, due to the often incorrect naming of newly cultivated strains.[49,50] In addition, two other genera of filamentous fungi frequently utilized to produce mycelium materials were tested, including *Trametes versicolor* (TV) and *Pleurotus ostreatus* (PO). Only a few articles have described the morphology and colony formation of *G. lucidum*. Notably, not all *Ganoderma* species form chlamydospores: *G. lucidum* (a hardwood isolate) does, but *G. tsugae* (a conifer isolate) doesn't.[51] *T. versicolor* (TV) is reported to generate chlamydospores less frequently[52] or not at all.[48] Finally, *P. ostreatus* (PO) has, to our knowledge, not been reported to produce chlamydospores so it served as the control species in this investigation.

PO had considerably lower cell density in mycelium liquid solutions after 5 days of shaking than all other species (**Figure 3**A). The growth rate of the PO mycelium skins was likewise slower since complete development occurred only on the eighth day of incubation (Figure 3C). Both the strain GL-M9720 and the species GR showed much lower cell density than our model-strain GL-M9726 (Figure 3A). Under the microscope, all of the GL strains displayed an abundance of chlamydospores with an oval shape (20 µm) (Figures 3B, and 5B,C). GR chlamydospores were tapered at the ends. TV chlamydospores were 5–10 µm long and hence more difficult to discern. Finally, PO had no chlamydospores. A viability test was carried out by incubating dry and plasticized mycelium skins (5 mm diameter) of all species and strains on malt extract agar (MEA) (Figure 3D). The reviving rate was assessed during a 10-day period (Figure 3E). PO was the only species that could not be revived, supporting the hypothesis that chlamydospores are the healing agent. Only five of the six replicates of the TV samples revived, perhaps due to a lack of or insufficient chlamydospores on that replicate. However, because only a small number of species were evaluated in this study, we underline the need for additional morphological characterization and viability tests of other fungal species.

2.3. Requirements of the Material to Sustain Viability

Having developed the first protocol for growth of these materials under mild conditions, we investigated the requirements for viability. Post-processing conditions, namely the drying temperature and plasticizing treatment, had to be established. First, we wanted to understand which drying temperature would alter the organism's survivability. Generally, 10% of most ascomycetes species' conidia do not survive heat treatments > 60 °C.[53] The ability of *G. lucidum* chlamydospores to withstand high temperatures has to our knowledge not yet been investigated. In order to confirm that it is the chlamydospores and not the hyphae that allow the mycelium material to revive, three sets of experiments were carried out with *G. lucidum* (GL-M9726): heating dry and plasticized mycelium skins containing chlamydospores and inert hyphae (**Figure 4**A,B), heating a filtered chlamydospore solution (Figure 4C,D), both at 40 and 60 °C, and heating a liquid solution containing chlamydospores and vegetative hyphae (Figure S2, Supporting Information). Filter paper was soaked in those liquid solutions and incubated at 28 °C for a day.

As such, mycelium skins dried at 40 °C developed new hyphae after 1.5 days (Figure 4A), and the spore solutions on filter paper dried at 40 °C required ≈ 3 days to revive on MEA at 28 °C (Figure 4C). Nonetheless, heating the mycelium skins and spores' solutions at 60 °C had a substantial negative influence on revivability, since growth was only observed after 5 days with the mycelium skins. We were unable to observe growth on the filtered spore samples heated at 60 °C. The experiments were repeated with filter paper soaked in a mycelium and spore liquid solution heated at 22 °C, 40 °C and 60 °C (Figure S2, Supporting Information), and the reviving rates were comparable to those of mycelium skins. Mycelial growth may be seen in the samples starting on day 3 and 4, heated at 22 °C and 40 °C respectively. Samples heated at 60 °C did not revive. According to these survival data, the double-walled chlamydospores of *G. lucidum* can easily survive at 40 °C but not at higher temperatures. Because fewer spores are likely to have attached to the filter paper than are expected to be present in the mycelium skins, mycelium skins dried at 60 °C can revive at a low rate after 5 days.

Second, the effect of glycerol post-treatment on the revivability of mycelium skins was investigated. Glycerol is among the most commonly used plasticizers to decrease the brittleness of biofilms.[21] Previous research has found that increasing the glycerol solution percentage (from 1% to 32%) alters the mechanical characteristics of mycelium materials from natural material to elastomeric.[21] Since high percentages of glycerol increase the elasticity of the material,[21] we sought to investigate if the percentage of the glycerol solution (20% and 50%) also affects re-growth, and if there is a difference in revivability between glycerol injected during or after the growth of

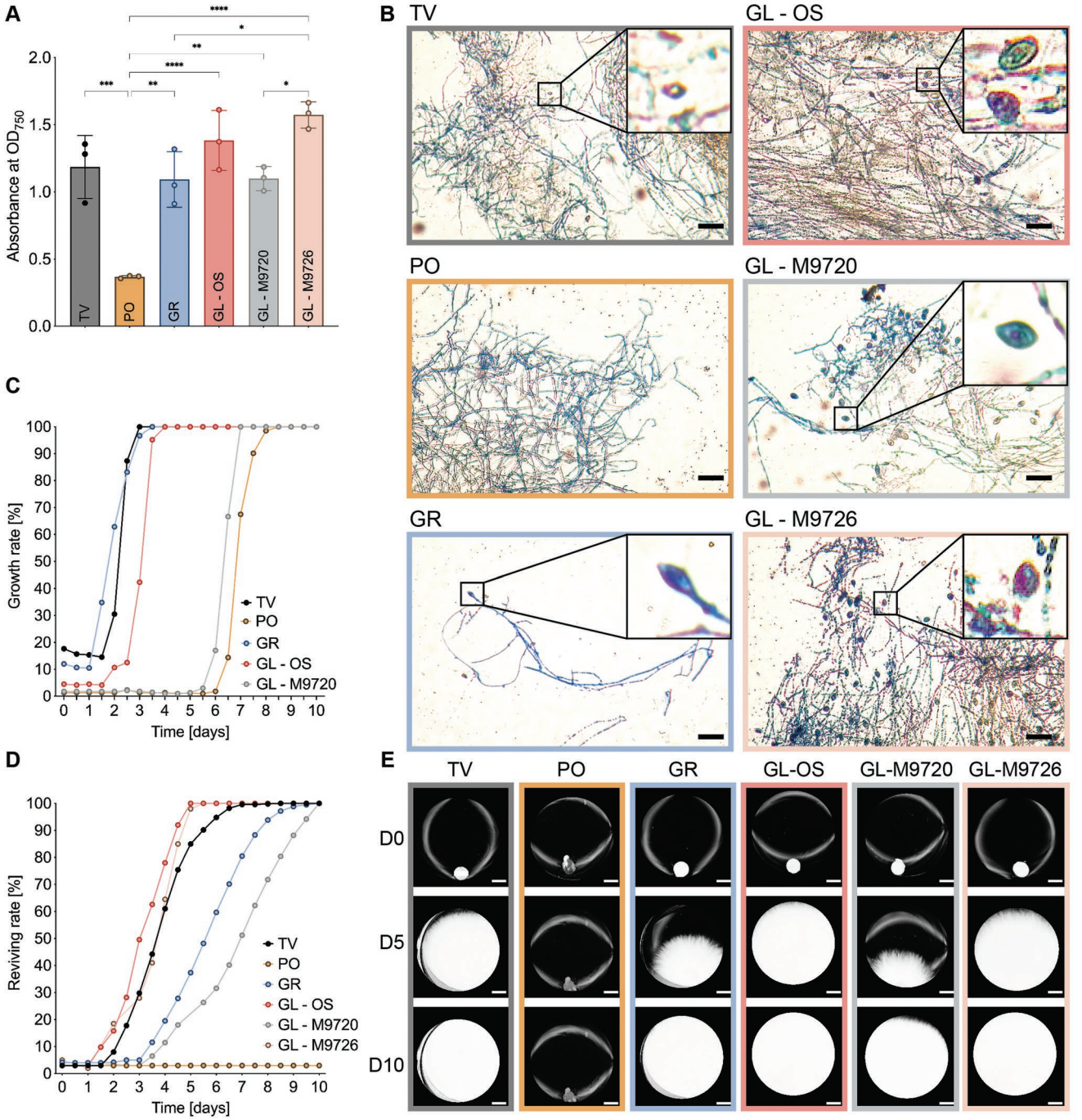


Figure 3. Revival agents in mycelium materials. A) Absorbance at 750 nm of mycelium liquid solution (TV: *Trametes versicolor*, PO: *Pleurotus ostreatus*, GR: *Ganoderma resinaceum*, GL-OS, -M9720, M9726: different strains of *Ganoderma lucidum*) grown for 5 days. All samples were prepared in triplicates. B) Microscopic images of submerged hyphae of different species and strains, zoom showing chlamydospore formation (zoom x5). C) Growth rate percentage of mycelium skins (B), taken every 12 h over a 10 day-period. All samples were prepared in six replicated, mean values are shown on graph. Scale bar represents 50 µm. D) Reviving rate percentage of mycelium skins (C), grown on MEA, taken every 12 h over a 10 day-period. All samples were prepared in six replicated, mean values are shown on graph. Scale bar represents 5 mm. E) Scans of mycelium skins reviving on MEA, shown on day 0, 5, and 10 of the incubation.

samples. The revivability is slowed down by 64% when the glycerol solution is increased from 20% to 50% (Figure S3, Supporting Information). Skins treated in 20% glycerol solution revived faster and had filled the MEA area on day 3, but samples soaked in 50% glycerol solution required 5 days to cover the same area. Spores can be microencapsulated using glycerol.[54] The high concentration of glycerol used in this study (50%) may therefore have prevented the chlamydospores from

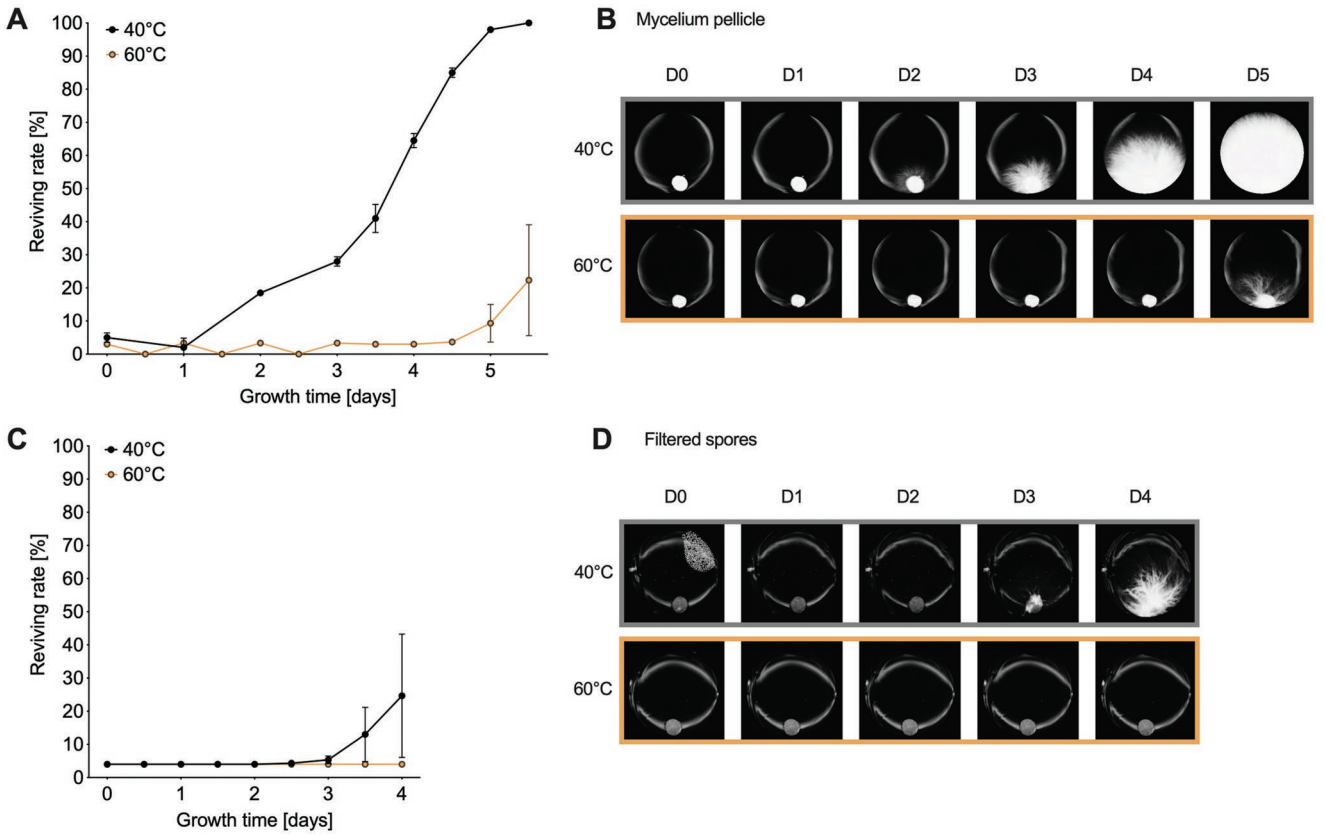


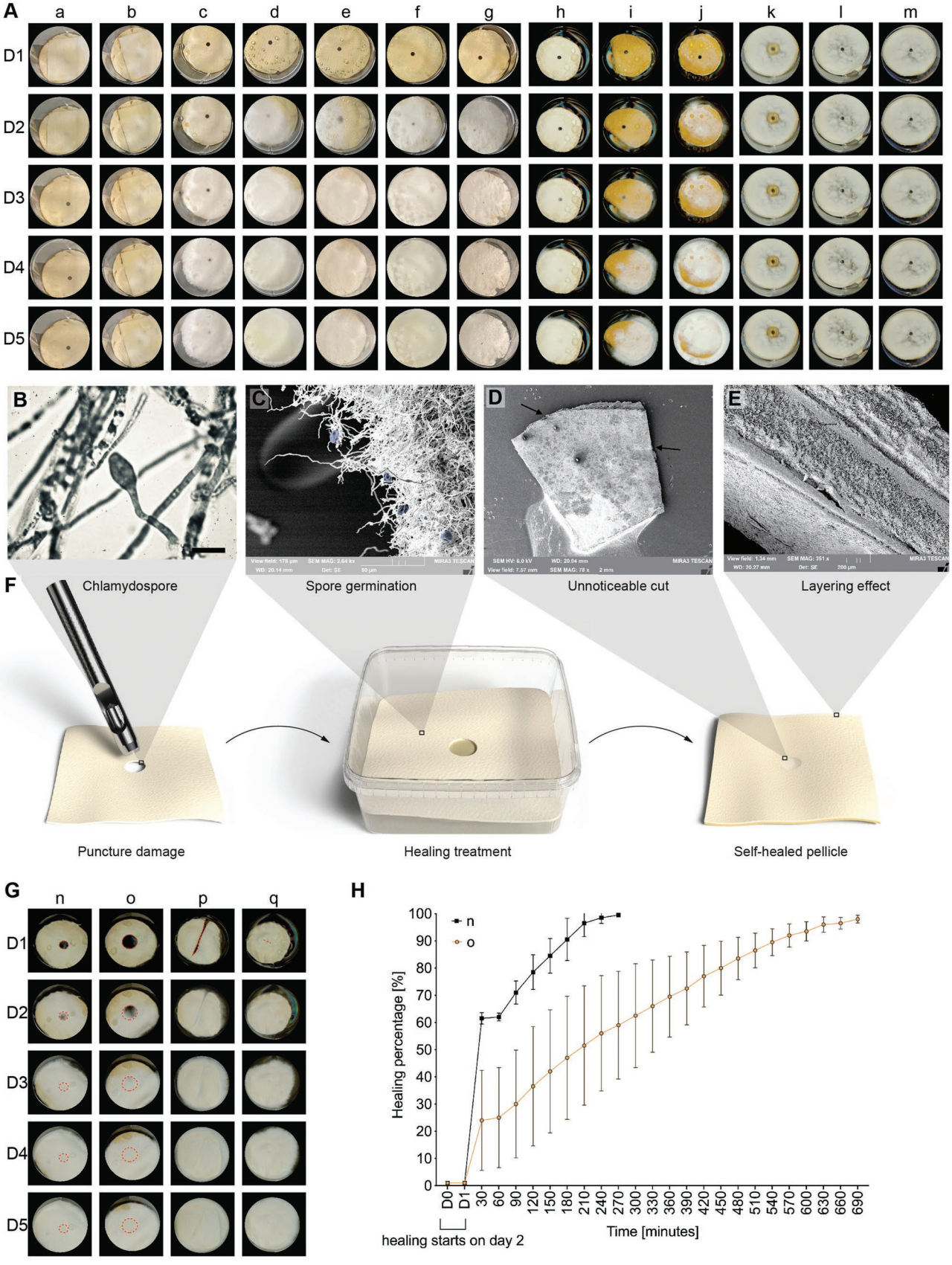
Figure 4. Impact of drying temperature on viability. A) Surface areas of reviving samples shown in (B) taken every 12 h over a period of 5 days. All samples were prepared in triplicate. B) Scans of mycelium skin heated at 40 and 60 °C, observed over 5 days on MEA. C) Surface areas of reviving samples shown in (D) taken every 12 h over a period of 5 days. All samples were prepared in triplicate. D) Scans of filter paper soaked in chlamydospores solution heated at 40 and 60 °C, observed over 4 days on MEA.

germinating. On the other hand, injecting glycerol either during or after the formation of the mycelium skins had no effect on the revivability or the ability of the submerged hyphae to form the skins. This discovery permits skipping a post-treatment step during the production of mycelium materials.

Mycelium materials retain viability after drying at 40 °C and plasticizing with a 20% glycerol solution, suggesting that our method may be used for its application as a leather substitute. Additional coating and tanning agent testing are required to ensure that additional postproduction treatments to obtain leather-like materials are fulfilled.

2.4. Interventions Activating the Self-Healing Capacity of the Living Mycelium Material

We performed an initial screening of the treatments and nutrients that can revive the organism after being dried. How should the nutrients be added? What types of damage, such as holes and cuts, can be healed? When migrating in the direction of nutrients, hyphae can bridge air gaps[24] or non-nutritional surfaces (such as plastic).[55] However, because we had perforated the mycelium material with a 2 mm hole (**Figure 5**A), we reasoned that nutrients should be provided as a temporary scaffold, such as the surface of liquid or solid nutritional medium. First, a wound-dressing patch was applied over the holes (Figure 5Aa,b). The patch was soaked in MEB until it reached maximum absorbance capacity, then it was applied on the mycelium skin for 2 or 5 days. None of the replicates re-grew, probably because the nutritional supply was insufficiently accessible for the organism. Dispensing 1 mL MEB with a syringe was more effective (Figure 5Ac), although only two out of three replicates fully closed the hole. We then soaked the mycelium skins in 5 mL of MEB for 2 and 5 days (Figure 5Ad,e) to speed up the regeneration process. The perforations were completely sealed on the third day of incubation. The healing percentage leaps from nearly 0% on day 2 to 100% on day 3 (Figure S4, Supporting Information) since it takes the mycelium ≈ 48 h to germinate, after which it covers a small hole in < 4.5 h, as shown in Figure 5H. Placement of the skin on a MEA surface for 2 or 5 days (Figure 5Af,g) resulted in steady wound healing on day 3 and was equally effective as the soaking technique in MEB. Unexpectedly, the skins also revived on H_2O (Figure 5Ah,i) after 4 days, regardless of whether the aerial or liquid surface was in touch with the water. Samples were similarly capable of regrowth when placed with aerial-side in touch with MEA (Figure 5Aj). Although we noticed excellent regeneration, the material's consistency and appearance were not restored. Finally, different sizes of MEA plugs were put on top or under the mycelium skin to localize



the healing region near the hole (Figure 5Ak–m) but did not result in regrowth.

Localizing the damage-repair would be an interesting future step, because the chlamydospores germinate everywhere and in all directions (Figure S5, Supporting Information) on the initial mycelium skin due to the bulk-feeding technique used in this study. Chlamydospores do not perceive damage, nor do newly germinated hyphae have a sense of orientation to grow only in the direction of the damage. Further research is needed to trigger damaged-self recognition in the material, for example, by releasing chemicals upon mycelial injury that can initiate healing. When the container size was increased from 6-well plates to boxes of 185×185 mm, it became evident that a new layer of mycelium had formed on top of the old one (Figure S6B, Supporting Information). On the SEM images of a cross-section of a mycelium skin, the layering effect is obvious, as two distinctive compressed floating hyphal structures (Figure 5E) are stacked between two aerial hyphal structures. Moreover, regrowth did not occur uniformly when the mycelium skin was not in direct contact with the MEA (Figure S6A,B, Supporting Information). However, when the mycelium skin floated on the surface of MEB liquid it became more homogenous (Figure S6C,D, Supporting Information).

The kind of damage and intended application, such as scratches, punctures, cuts, and cracks, all impact the self-healing efficacy. To demonstrate the self-healing of large volume damages, several types of mechanical injuries were tested for their regeneration capacity (Figure 5Gn–q). Two puncture damages, with hole defects of 5 and 10 mm, were applied to the mycelium skins. It took two days for the organism to reactivate on MEB nutrients, however from the moment germination began, it took 4.5 h for 5 mm and 10 h for 10 mm holes to be entirely healed (Figure 5H). The growth rate isn't affected by the size of the hole and healing happens at an average rate of 3.98 ± 1.05 $mm^2\ h^{-1}$.We acknowledge that the self-healing period is slow compared with other state-of-the-art polymeric self-healing materials that can heal in a few seconds.[6] However, the main advantage of fungal ELMs over other state-of-the-art synthetic self-healing materials[56,57] or self-healing proteins,[6,58,59] is that they can generate new biomass and therefore fill larger damages. When large volumes of the mycelium material are destroyed or removed, fungal ELMs can recover by completely replacing damage with freshly generated biomass.

Finally, we looked at the healing of a total cut injury, in which the damaged skin was entirely divided into two parts and reconnected for healing (Figure 5Gp). The cut-damaged samples healed at the same pace as the hole-damaged samples. On day 2, the cut was totally covered, and by day 4 it was unnoticeable (Figure 5D). A 5 mm cut-damage also showed complete healing (Figure 5Gq). In summary, self-healing is possible with simple interventions for a variety of large damages, after a healing time of two days.

2.5. Recovery of Mechanical Properties After Self-Healing

To understand the post-healing strength of mycelium materials and kinetics of the healing process, the regeneration of 2 mm holes was analyzed in function of contact time with the healing treatment. The healing procedure increased the tensile strength of the mycelium materials substantially (**Figure 6**A), as samples that were subjected to the self-healing treatment during 3, 5, and 10 days were twice as strong as the samples that were not subjected to the treatment (day 0). This is caused by the regeneration of fresh biomass inside and on top of the existing mycelium sheet. Both punctured and undamaged samples were subjected to the healing treatment. The tensile strength results showed no significant difference between self-healed and undamaged samples. Mycelium materials that had a self-healing treatment during day 3, 5, and 10 days could withstand tensile stresses of the same magnitude (1.11 ± 0.2, 1.00 ± 0.12, and 0.92 ± 0.19 MPa respectively). There is no clear trend for the elongation at break over time. However, self-healed samples seem to have a slightly (but not significant) lower elongation than undamaged samples (Figure 6B). The Young's modulus of self-healed samples on day 3 and 5 is significantly higher (16.04 ± 1.79 and 22.56 ± 1.91 MPa, respectively) than undamaged samples (11.85 ± 2 and 15.87 ± 3.53 MPa, respectively) (Figure 6C), resulting in a stiffer material which could reflect a denser fibrous structure of self-healed samples. Primordial formation occurred on samples that healed for 10 days, resulting in a heterogenous sheet and a large standard deviation in the results.

Mycelium materials in this study are classified as a new category between natural cellular materials and natural elastomers (Figure 6D). Glycerol, the plasticizer used in post-processing to make the mycelium skins flexible, is most likely what gives them their elasticity.[21] However, changes can be made to the manufacturing procedure to increase the tensile strength and make them equivalent to the qualities of synthetic or animal leather.

All samples that self-healed for 5 and 10 days did not break in the healed area but instead broke at random non-healed places after being stretched to the point of failure (Figure 6E), implying that the cut faults were successfully healed and that

Figure 5. Different types of nutritional interventions to heal various damages in mycelium skins. A) Mycelium skins punctures with a 2 mm hole with different types of healing treatments: a) plaster soaked in 5 mL MEB and removed on day 2, b) plaster soaked in MEB and left in place for whole healing period, c) dispensing 1 mL MEB with a syringe on day 0, d) soaked in 5 mL MEB during whole healing period, e) soaked in 5 mL MEB and removed on day 2, f) placed on 5 mL MEA during whole healing period, g) placed on 5 mL MEA and removed on day 2, h) soaked in H_2O with the aerial side up, i) soaked in H_2O with liquid side up, j) placed on MEA with liquid side up, k) 5 mm MEA plug placed on top, l) 5 mm MEA plug placed under skin, m) 10 mm MEA plug placed under skin. All samples were prepared in triplicate. B) Microscopic image of chlamydospores produced by GL-M9726. Scale bar represents 20 µm. C) Scanning electron microscopy of hyphae with deflated chlamydospores (colored in blue). Scale bar represents 50 µm. D) Scanning electron microscopy of self-healed mycelium skin, with arrows indicating where the cut was. Scale bar represents 2 mm. E) Scanning electron microscopy of mycelium skin in cross-section, with arrows indicating the floating hyphae. Scale bar represents 200 µm. F) Schematic displaying the principle of damage, bulk-healing treatment and recovered mycelium skin. G) Mycelium skins punctures with different types of damages: n) 5 mm punctured hole, o) 10 mm punctured hole, p) cut into two pieces, q) 5 mm line cut in the center. All samples were prepared in triplicate. H. Healing percentage of 5 mm (n) and 10 mm (o) holes measured every 30 min, with healing starting on day 2 after the puncture. All samples were prepared in triplicate.

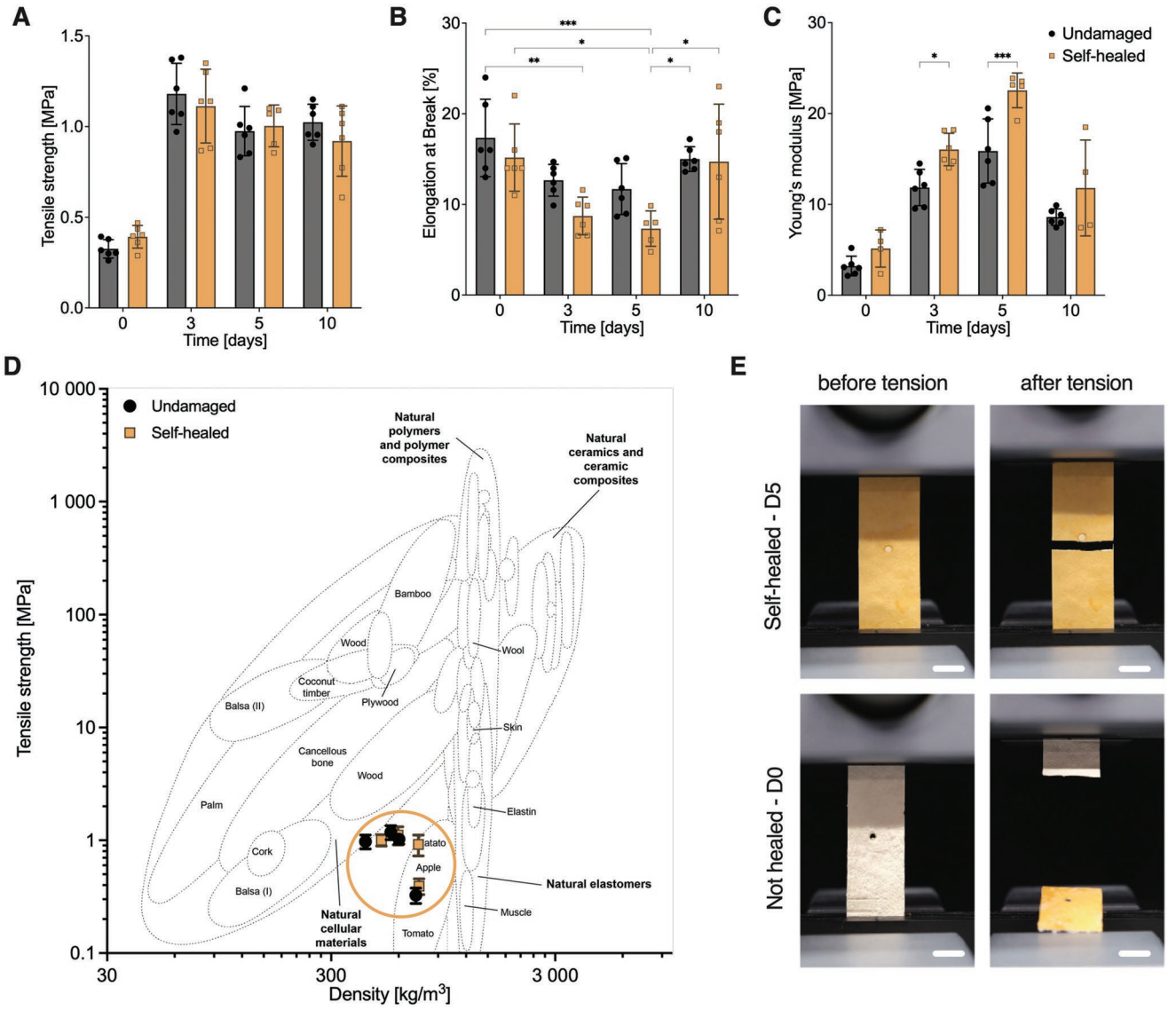


Figure 6. Mechanical characterization of self-healed mycelium skins. A) Tensile strength (MPa) of undamaged and self-healed samples with intervention lasting 0, 3, 5, and 10 days. B) Elongation at break (%). C) Young's modulus (MPa). D) Tensile strength (MPa) versus density (kg m^{-3}) plotted on Ashby chart for engineering materials.[60] E) Photographs of self-healed (shown from the floating hyphae side to see the damaged area) and unhealed sample (shown from the aerial hyphae side) before and after tension, showing that failure happened at a random non-healed or non-damaged region. Scale bar: 10 mm. The standard deviation of all experiments is performed with 6 specimens.

the healed areas were at least as strong as the undamaged material. Five out of six replicates of mycelium sheets that healed for 3 days failed in the healed area.

In conclusion, mycelium skins that healed for 5 days produced the most favorable materials because they did not break in the damage (unlike day 3 samples) and did not undergo primordial formation (like day 10 samples).

2.6. Water Contact Angle and Water Absorption Before and After Self-Healing

It is common knowledge that assemblies of the surface-active protein hydrophobin are responsible for the filamentous fungi's hydrophobic characteristic.[61–65] We assessed the change in contact angle and water absorption of non-healed and self-healed samples to determine the impact of the healing intervention on wettability. The self-healed samples had gone through two cycles of growth and post-treatment. The first to form the mycelium skin, and the second to self-heal the punctured hole. Non-healed skins, on the other hand, were grown and treated once. Non-healed samples had a much lower initial water contact angle (116 ± 12°), which reduced with time (92 ± 22°) (**Figure 7**A,B). Self-healed mycelium materials were hydrophobic (145 ± 3°) with no variations in contact angle during the measurement period (141 ± 7°). Water absorption of self-healed mycelium skins immersed in water increased significantly (Figure 7C), but water absorption of unhealed

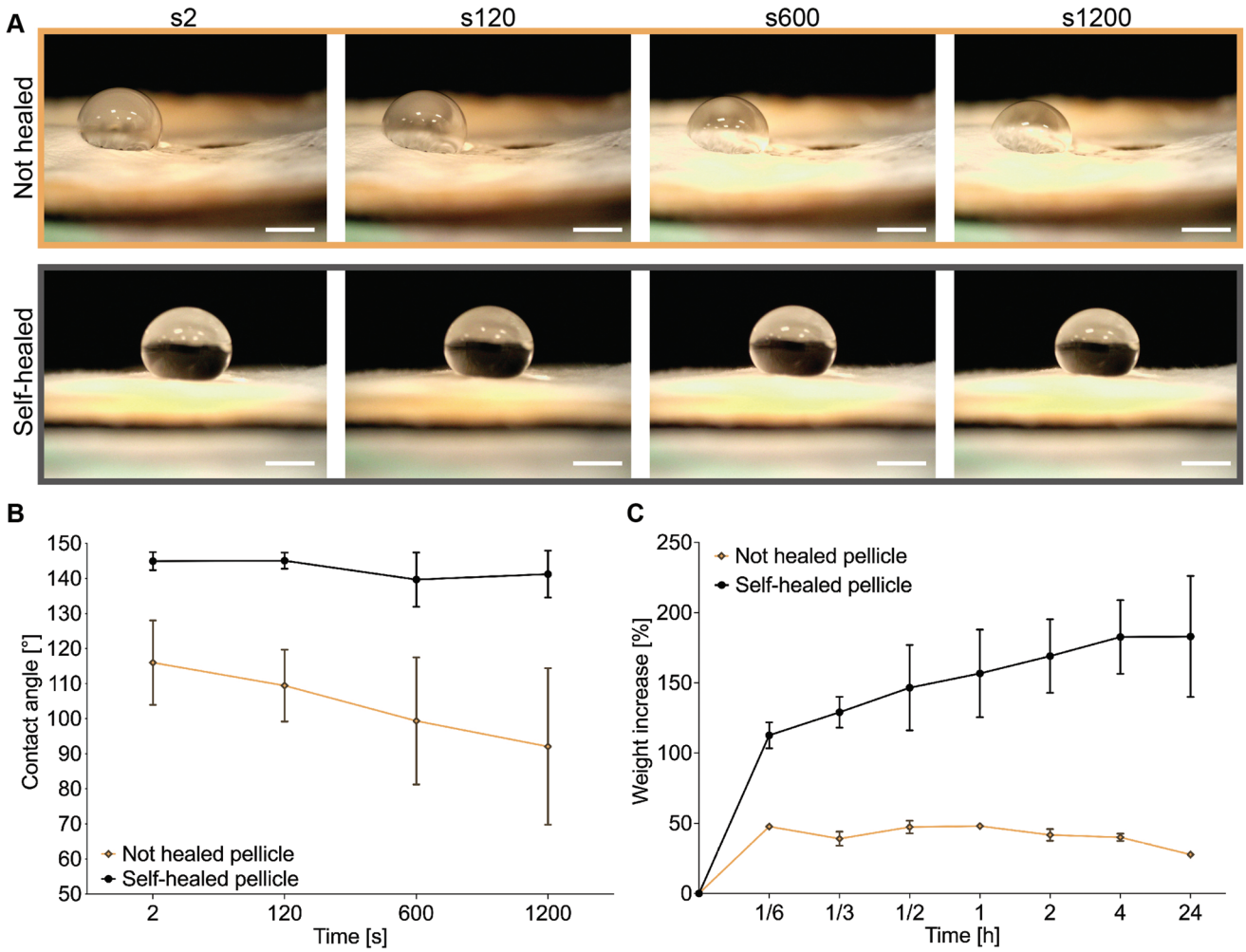


Figure 7. A) Contact angle images of a non-healed and self-healed mycelium skin, captured at 2, 120, 600, and 1200 s after placing a drop of water on a random location of the surface. Scale bar: 2 mm. B) Water contact angle (deg). C) Weight increase (wt%) after water submersion of non-healed and self-healed mycelium material. All samples were prepared in triplicate.

samples was much lower and even reduced. This shift in wettability might be attributed to the presence of more air pockets (and consequently more water molecules trapped in the mycelium structure) in the self-healed skins, which had been exposed to two growth cycles and so created a second mycelium layer on top of the existing skin. It is possible that the surface hydrophobin proteins reassembled during the self-healing intervention, making the surface more hydrophobic. According to Sun et al., densification and amphiphilic hydrophobin rearrangement can contribute to changes in wettability and surface hydrophobicity.[66]

3. Outlook

This first screening study of the revival of fungal mycelium skins has opened new possibilities for the development of self-healing interventions, but it also raises a host of new questions. One of the most critical considerations is whether the fungal organism can survive in harsher conditions that could occur during its use as a leather substitute, such as chemical treatments, coatings, or washing cycles. While water is sufficient to revive mycelium skins, it is not ideal for applications such as leatherwear or furniture. Thus, controlling regeneration at inopportune moments needs to be established.

Another important requirement is to localize the revival more effectively in the crack or hole area. At the moment, chlamydospores are unable to detect damage, and newly germinated hyphae are not able to recognize the direction in which they should grow to heal the damage. More research is needed to induce damaged-self recognition in the material, for example, by releasing molecules that can initiate healing upon mycelial injury. Furthermore, the bulk-feeding approach employed in this work causes the chlamydospores to germinate everywhere and in all directions on the initial mycelium skin. Therefore, encapsulating fungal chlamydospores could be a method to restrict germination to the areas where the encapsulation is diluted. The encapsulation can potentially function as a nutritional supplement. Additionally, it is also important to ascertain the optimal nutritional concentrations required for revival.

It is still unclear whether subsequent healing is possible, as not all chlamydospores germinate during the first healing intervention. More research is needed to determine if new spores form during the healing process. If not, it may be necessary to add more spores to the nutritional patch or trigger their formation in another way.

These experiments were conducted in a lab environment, where sterility of the mycelium skins could be maintained. However, in their intended applications, the mycelium materials might become contaminated by other environmental organisms. Further research is needed to define the life cycle scenarios and whether the chlamydospores embedded in the mycelium matrix can survive for extended periods when other organisms are present in the intended applications.

Improved fabrication systems must be developed to allow for larger-scale production and implementation in existing mycelium material production facilities. Our findings can be implemented in numerous other sectors than the textile industry, such as building materials, environmental applications, and medicine.

Finally, this research project delivers practical proof-of-concepts that enable follow-up research to investigate the broader perspective of using biological ELMs in an ethical and societal perspective. A topic worthy of further investigation is to what extent the public will accept living materials.

4. Conclusion

The development of a novel class of fungal ELMs that makes use of the organism's ability to regenerate itself as self-repair opens the possibility of new biodegradable textile technologies with additional functionalities. We demonstrated that the presence of chlamydospores embedded in a dry and flexible mycelium matrix grown with *G. lucidum* is essential for the self-healing of fungal ELMs. This research deepens our understanding of living fungal materials, where the spores are metabolically dormant until they are stimulated to germinate, and the hyphae are rendered inert. Our method demonstrates specifically how chlamydospores initiate the formation of new hyphae, which can fill up gaps and damages in the material and fuse with other hyphae. In this work, self-assembling mycelium materials are transformed into functionalized fungal ELMs by the development of chlamydospores at the tips of hyphal structures. It is crucial to this discovery that the functional agent (chlamydospore) is produced directly by the mycelium. We evaluated the growth physiology of mycelium skins before and after fabrication, as well as the survival of various species that generate or do not create chlamydospores. In submerged agitated cultures at 150 rpm and 30 °C, chlamydospores emerged after 4 days. After drying at 40 °C and plasticizing with a 20% glycerol solution, the fungal ELM maintained vitality. Furthermore, the *P. ostreatus* species, which does not generate spores, was incapable of reviving, validating our hypothesis. Our approach demonstrates that self-healing is achievable with minimal interventions for a range of large damages following a two-day recovery period. The healing procedure increased the tensile strength of mycelium materials, but it did not produce significant difference compared to undamaged samples. Self-healed samples broke at random non-healed places, showing successful healing and equal strength of healed and undamaged areas. Non-healed samples had a lower initial water contact angle, while self-healed samples were hydrophobic.

This fungal ELM has a self-healing and structural function that is created by the cells themselves, as opposed to being an inert by-product of the organism's metabolic activity. While most ELM procedures rely on synthetic biology techniques, such as co-cultivation of wild-types and genetically modified organisms, this fungal ELM relies on biological processes initiated by physical manipulation and environmental response. This work thus advances biological ELMs toward unique product applications in sustainable leather-goods replacements such as furniture, automotive seats, and fashionwear. This study broadens our understanding of fungal ELMs and paves the way for the development of a new generation of living materials that could become even more "living" and dynamic than those presented in this research. For example, by programming complex behaviors such as environmental sensing, morphological changes, or cellular reorganization over time.

5. Experimental Section

Strain and Culture Conditions: The *Ganoderma lucidum* (GL-M9726), *Ganoderma lucidum* (GL-M9720), *Pleurotus ostreatus* (PO-M2191) strains were purchased from Mycelia, Belgium. The *Ganoderma resinaceum* (GR), *Ganoderma lucidum* (GL-OS) and *Trametes versicolor* (TV) species were purchased from Osmose Studio, United Kingdom. *Ganoderma lucidum* (GL-M9726) was used in all experiments, unless when mentioned differently. Mother cultures were maintained in 20 mL tubes (called slants) containing 10 mL of malt extract agar (MEA, Merck & Co., USA) that was poured and dried diagonally in the tube to increase the surface area. Mother cultures were transferred to 100 mm petri dishes, containing 25 mL of MEA (Merck & Co., USA), every 60 days. Five mycelium disks (5 mm diameter) cut from the malt extract plates were used to inoculate 1 L Erlenmeyer flasks containing 400 mL liquid medium of malt extract broth (MEB, Merck & Co., USA). The flasks were incubated for 5 days at 30 °C, in the dark, with continuous shaking at 180 rpm. The experiments that analyzed the cell density over time and at different shaking speeds (0, 100, 150, and 200 rpm) (Section 2.1) were performed by inoculating 1 mycelium disk in 50 mL falcon tubes containing 10 mL of MEB. The experiments that compare the different species and strains (Section 2.2) were performed by inoculating 2 mycelium disks in 250 mL flasks containing 100 mL of MEB.

Fabrication of Fungal Material: Depending on the initial amount of MEB, 400 or 100 mL of fresh MEB was added to the pre-cultures described in previous section. Pre-cultures were homogenized for 10 s at 80 000 rpm using a blender. Resulting 800 or 200 mL mycelium slurry was transferred to Cell-Culture Treated Multidish 6-well (Sarstedt, Germany) with a 10 mL Serological Pipette (Sarstedt, Germany) containing 9 mL of mycelium slurry. Only for the experiments testing the impact of glycerol during the growth (Section 2.3), 1 mL of 20% or 50% (v/v) aqueous glycerol solution (Sigma, USA) was added to the wells before incubation. The mycelium slurry was then incubated for 10 days at 28 °C, in the dark in static condition, allowing the hyphae to form a floating mycelium mat on the surface of the liquid. Then, 1 mL of a 20% (v/v) aqueous glycerol solution (or 50% (v/v) for experiments in Section 2.3) was added to the wells with a Serological Pipette and left at room temperature for 24 h. The mycelium skins were harvested, washed in sterile demineralized water, and placed between two layers baking paper that was wiped with 70% (v/v) ethanol, blotting paper, foam and cardboard in a paper press. The press was then placed in a dehumidifier at 40 °C for 72 h.

Self-Healing Treatments and Interventions: Dried skins were damaged with a sterile leather hole punch cutter (2–10 mm diameter) or with a sterile scalpel, and immediately subjected to healing. The different types of healing treatment consist of: a) wound-dressing patch soaked in MEB until it reached maximum absorbance capacity which was applied on a mycelium skin inside a 6-well plate; b) dispension of 1 mL of MEB with a syringe on top of a mycelium skin inside a 6-well plate; c) mycelium skin soaked in 5 mL of MEB; d) placement of a mycelium skin on 5 mL of MEA; e) mycelium skin soaked in 5 mL of H_2O; f) 5 and 10 mm MEA plugs put on top or under the mycelium skin. All samples were incubated at 28 °C for 5 days. Then, the mycelium skins were soaked in a 20% (v/v) glycerol solution, washed in sterile demineralized water, and placed between two layers baking paper that was wiped with 70% (v/v) ethanol, blotting paper, foam and cardboard in a paper press. The press was then placed in a dehumidifier at 40 °C for 72 h. Experiments were performed with three biological replicates and repeated at least two times at different moments.

Determination of Mycelium Yield and Filtration of Chlamydospores: Liquid mycelium cultures were homogenized using a 10 mL glass tissue homogenizer and 1.5 mL was transferred to cuvettes (Sarstedt, Germany). Chlamydospores were filtered from homogenized cultures with a 30 µm cell strainer (pluriStrainer, pluriSelect, Germany). Spectral data (200–800 nm) were obtained with a JenWay 7205 Spectrophotometer. Experiments were performed with three biological replicates.

Determination of Mycelium Skin Thickness: Mycelium skins were harvested daily over a period of 25 days. Skins were dried at 40 °C in a dehumidifier. The thickness of wet and dry samples was measured with a digital caliper. Experiments were performed with three biological replicates.

Growth Rate and Assessment of Regenerative Ability: Growth rate of mycelium skins were assessed by placing 6-well plates containing mycelium slurry on a flatbed scanner (Epson Perfection V600 Photo, Japan) inside an incubator at 28 °C. Reviving rates of dry and plasticized mycelium skins were assessed by cutting a 5 mm diameter plug with a sterile leather hole punch cutter and placing it on 6-well plates containing 5 mL of MEA. Reviving rates of liquid mycelium and chlamydospore solutions were assessed by soaking sterile 5 mm diameter filter paper in the solution, and incubating it for 24 h at 28 °C. These filter papers were then placed on 6-well plates containing 5 mL of MEA. All experiments were performed with three biological replicates and repeated at least two times at different moments. Scans were taken every 30 min using Automator and Epson Scan 2 software. The images were treated in Photoshop (Adobe Systems Incorporated, USA) by adjusting the brightness and contrast, and black and white levels. All images were adjusted in the same way. Image processing package Fiji (ImageJ2) was used to analyze the areas. Stack-images properties were changed to 16-bit. The threshold was adjusted to "Default". The growth area was measured using the Particle Analyzer.

Scanning Electron Microscopy: Scanning electron microscopy (SEM) measurements were performed using a field emission TESCAN MIRA 3 (acceleration voltage, 5 kV). Before imaging, the samples were coated with ca. 10 nm of gold via thermal evaporation to increase surface conductivity and thus image quality.

Light Microscopy: Two slide mounts were made for each species by taking 1 mL from a liquid culture and placing a drop on a slide. Chlamydospores and hyphae were visualized in a drop of Lactophenol Cotton Blue Staining under 40x or 100x magnification using a Bresser Science TRM-301 (Germany) light microscope and photographed with a FujiFilm X-T2 (Japan) camera. The size of chlamydospores was measured using Fiji (ImageJ2).

Mechanical Characterization of Mycelium Materials: Samples were grown by incubating 800 mL of mycelium slurry (as described in Fabrication of Fungal Material Section) in microbox containers of 185×185×120 mm having 4 white filters on the lid (SacO2, Belgium). The mycelium skins were plasticized and harvested as described in Fabrication of Fungal Material Section. Then, 5 mm holes were punctured, and self-healing treatment was performed by soaking the sheets in 400 mL MEB as described in Self-Healing Treatments and Interventions Section. Undamaged samples were subjected to the same treatment, but without a hole being punched in them. Day 0 samples were not subjected to the treatment, while the other samples were harvested after being soaked in MEB for 3, 5, and 10 days. Then, the mycelium skins were plasticized and dried again as described in Fabrication of Fungal Material Section.

Six specimens were cut from one dry and plasticized mycelium sheet into rectangles of 20×110 mm. The thickness, width and height were measured at three points along the axis of the specimens using a digital caliper and the average was taken. Tensile tests that were performed following EN ISO 3376:2020, at room temperature with an Instron (68TM-50 with Bluehill 208 Universal Version 4.21 software) using a preload force of 0.25 N with a 0.2 mm min^{-1} test speed. Specimens were mounted on the machine clamps 30 mm from each end. The fixture separation was set at 50 mm. Specimen were deformed at a rate of 5 mm min^{-1} until failure. The tensile strength (MPa) was obtained from the maximum load (N) per unit area (mm^2) of the specimen. The elongation at break (%) was obtained by calculating the strain (mm) at break. The Young's modulus (MPa) was determined at the linear part of the stress/strain curve. The density (kg mm^{-1}) was calculated by dividing the weight of the specimen by the volume of the specimen.

Water Contact Angle Measurement: Hydrophobic properties of the samples were observed using a portable microscope (Dino-lite Edge 3.0, Taiwan). A 1 µL of water drop was placed onto a random area of the surface with a pipette and left undisturbed. The tests were performed on three biological replicates of non-healed and healed mycelium skins. Pictures of the droplets were recorded for 20 min, and the contact angle of the droplets were measured using Fiji (ImageJ2).

Water Absorption: Water absorption of non-healed and healed mycelium skins was determined by submerging three biological replicates of mycelium skins (35 mm diameter) in demineralized water, each in a separate beaker at room temperature. Water absorption was measured at different time points (1/6–1/3–1/2–1–2–4–24 h) by determining the percentage of weight increase. Excess water was removed with filter paper before weighing.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

chlamydospores, engineered living materials, mycelium materials, regeneration, self-healing

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