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Woodpile structural designs to increase the stiffness of mycelium-bound composites

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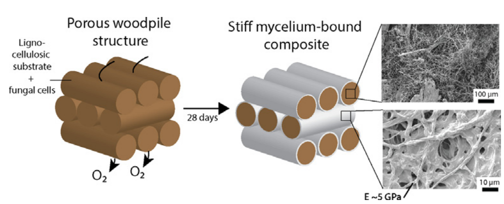
HIGHLIGHTS

- Single fungal hyphae increase in stiffness with time, up to 5 GPa.
- Increase in mycelium density increases the mechanical properties of the composite.
- Woodpiles porous structures lead to increased dense mycelium skin growth.
- Woodpiles porous structures increase the stiffness by 6 times after 28 days mycelium growth.
- Structural design in porous structure is an efficient method to improve the properties of growing bio-based materials.

GRAPHICAL ABSTRACT



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ABSTRACT

Mycelium-bound composites are biodegradable, eco-friendly materials grown by fungi onto solid ligno-cellulosic substrates. Mycelium is an interconnected network made of fungal cells that bind the substrates' particulates together. Uncompressed mycelium-bound composites have typically weak mechanical properties, similar to that of expanded polystyrene. In this paper, mycelium is grown onto porous woodpile struts structures to increase the final mechanical properties. The hypothesis is that increase in porosity can increase oxygen diffusion throughout the material and increase the development of dense mycelium network. Mycelium-bound composites grown from *P. ostreatus* onto bamboo microfibers substrates were studied to test this hypothesis. Constructing porous woodpile structures and monitoring the growth and the mechanical properties under compression, it was found that the porosity obtained through the design was able to increase dense fungal mycelium skin formation. As a result, the stiffness of the porous structures was multiplied by 6 after 28 days of growth. The specific modulus was in turned multiplied by 4 with the addition of 30 % macroscopic porosity. Despite the modest mechanical properties (stiffness about 0.5 MPa), the approach proposed illustrates how appropriate structural design can efficiently increase the properties of grown bio-based materials.

1. Introduction

Mycelium-bound composites are biodegradable materials obtained by growing a white-rot fungus onto a solid substrate

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made from ligno-cellulosic particulates such as wood chips, sawdust, plant leaves, etc [1]. As the fungus degrades the substrate to digest its nutrients, it develops its mycelium, which is a highly interconnected network of filaments called hyphae, composed of elongated cells which bind the substrate's particulates together to yield a mycelium-bound composite. The diversity and research output on mycelium-bound composites have recently increased due to their promising properties: lightweight, sound absorbing, resistance to flames, and most importantly their eco-friendliness [2–6]. With such properties, mycelium-bound composites find applications as sustainable packaging, textiles, furniture, biomedical scaffolds and even architecture [6–9]. Yet, the poor mechanical properties of the material restrict its use. Indeed, naturally grown mycelium composites typically have the properties around that of expanded polystyrene, with a compression modulus of about 0.1 to 0.4 MPa after drying [10]. Strategies to increase the mechanical properties of mycelium-bound composites are therefore needed to expand their range of application.

Various approaches have been explored to increase the mechanical properties of mycelium-bound composites, among which are the choice of the fungus, the choice of the substrate, the use of reinforcing particles, and the use of post-treatment such as cold- and hot-pressing [2]. For example, addition of nanoclay doubled the stiffness of mycelium-bound hemp composites grown using *Trametes versicolor* [11]. Addition of 37.5% natural reinforcement particles to mycelium-bound wheat composites grown using *Pleurotus ostreatus* increased the elastic modulus to 38.5 MPa [12]. Addition of carbohydrates and calcium to the substrate composition doubled the flexural modulus of mycelium-bound corn composites [13]. Furthermore, cold pressing of dried mycelium-bound composites was found to increase the Young's modulus by one order of magnitude, and hot-pressing by two orders of magnitude [14]. Although these methods are efficient in increasing the mechanical properties, pressing might not be applicable for composites with complex shapes and the addition of particles may not be desired. Since the mechanical properties of the composites rely greatly on the packing density and branching of the mycelium interconnected network, it would be interesting to explore ways to increase the properties of that network first; the composites could subsequently be further strengthened following the approaches reported above.

When fungi grow onto a substrate, they form a dense mycelium network at the interface between the substrate and the air, called fungal mycelium skin, which gives the composite its white color and soft leather texture. Mycelium-bound composites have therefore typically a core-shell type of structure with the shell being fungal skin and the core being the substrate's particulates bound together with a less dense mycelium network [15]. The formation of this skin at the air-substrate interface results from a mechanism by the fungus to maintain a certain level of moisture in the core of the composite to avoid drying out, which would result in the death of the fungus [16]. Indeed, the mycelium skin exhibits hydrophobic properties with contact angles with water above 110 ° [17]. The thickness, branching and packing densities, and mechanical properties of the mycelium skin depend on the substrate, the colonization level, the fungal species and other processing methods [14]. At the core of the solid substrate, the mycelium growth is essentially limited by the diffusion of oxygen [18,19], assuming the substrate is homogeneous enough to provide nutrients throughout its volume. Due to this limitation, the growth and penetration of mycelium into substrates has been reported to decrease exponentially from the surface [20]. Increasing the oxygen diffusion in the substrate could therefore be a way to increase the mycelium growth and packing density at the core of the substrate, which would likely result in increased mechanical properties of the overall mycelium-bound composites. One way to increase oxygen diffusion is to increase the porosity of a material. To retain good mechanical properties, a variety of porous 3D structures have been explored in metal or polymeric materials [21–23]. Furthermore, such 3D porous structures are known to provide sites for cell attachment and to promote cell proliferation and growth [24,25].

Here, the approach proposed to increase the mycelium growth and the resulting properties is to intentionally increase the porosity of the particulate-based substrate through design. To increase this design-induced porosity, without decreasing the packing density of the ligno-cellulosic particulates within the substrate, we explore the growth of mycelium onto substrates having a macroscopic woodpile porous struts structure. Thanks to the woodpile arrangement of the struts, where each strut is composed of the ligno-cellulosic substrate, air channels are formed which facilitate oxygen diffusion throughout the structure and increase the surface area of the substrate-air interface. To demonstrate the approach, a system composed of the fungus *Pleurotus ostreatus*, also called Oyster mushroom, was grown onto solid substrates composed of bamboo microfibers supplemented with oats and with the appropriate amount of water. Similar composition has been used and optimized in a previous study [26]. First, the morphology and mechanical properties of the mycelium skin and individual hyphae obtained are characterized on non-porous blocks, to support the claim that more mycelium increases the mechanical properties of the final composite. Then, how the mycelium grows onto porous woodpile structures was studied and the mechanical response of the final composites was tested under compression as a function of mycelium growth time and compared with that of non-porous composite blocks. A closer look at the mechanical properties of single struts from the woodpile structures provides some explanation about the observed compression response of the woodpile composite and highlights the role played by the mycelium skin. Finally, woodpile structures with various struts dimensions are investigated to determine which of the design-induced porosity and the surface to volume ratio is the most important parameter to increase mycelium growth and enhance the mechanical response of the final composites. Our simple woodpile structures are proof-of-concept examples that intentional structural design can be used in bio-based composites to boost their performance. Given the recent efforts in the 3D printing of mycelium composites and the increasing research around mycelium-based materials, the work presented here opens the door for larger exploration of structural designs to increase properties and widen the range of applications of these materials.

2. Materials and method

2.1. Materials

Pleurotus ostreatus spawn bags were sourced from Bewilder, Singapore. *Dendrocalamus asper* bamboo microfibers were graciously provided by Widuz, Singapore, before being grinded and sieved into microfibers of 200 μm length. Food supplements, instant oatmeal, were purchased from the local supermarket (Quaker, 100% Australian wholegrain oats, product of Malaysia). The oats were grounded with a blender mixer (PowerPac) for approximately 3 min. Distilled water was used during the growth process of the mycelium composites.

2.2. Growth of the mycelium

For all different mycelium composites made, the same recipe was followed. Bamboo fibres were used as the lignocellulosic substrate and oats were added as a supplement to boost the growth of the mycelium. Similar ingredients have been used in previous work [26]. Water was added to the mixture to increase the moisture content of the composition. The composition was: 25 wt% bamboo, 12.5 wt% oats, 37.5 wt% water, and 25 wt% inoculum containing the fungus. The weights mentioned are the dry weight of the ingredients.

Prior to the preparation of the mixture, all tools and materials except the fungus were sterilized in the autoclave (Hiclave HG 80, Hirayama, Japan) at 121 °C for an hour. The inoculum was added to the composition after the mixture had cooled to room temperature after autoclaving and mixed manually. After the desired shapes of the inoculated substrates were formed, they were placed between two plastic cups to allow air circulation and avoid light. The samples had to be kept in the dark during growth to avoid the development of a mushroom (fruiting body). The samples were also moisturized regularly to maintain a minimum relative humidity of 80 % during the entire duration of the experiment, which was measured with a hygrometer. Each growth experiment was conducted up to 28 days at a temperature of 21 ± 0.5 °C. When the sample reached its time point, it was tested directly on the same day without further treatment. The growth experiments were repeated independently to ensure reproducibility. Samples that were found to be contaminated were discarded.

2.3. Block and woodpile structures fabrication

Block samples were grown in silicon ice cube trays and measured 3 cm × 3 cm × 1 cm (breadth × length × height) by placing the inoculated substrate in the ice cube trays following the procedure described in 2.2. (see Supplementary Information (SI) Figure S1 for pictures of the samples showing the growth variance). Woodpile structure samples were assembled using struts extruded by hand from a 3 ml syringe with a cut made at the nozzle or shaped in poly lactic acid molds that were 3D printed using fused deposition modeling (Prusa). The inoculated substrate was packed in the molds to form the strut and subsequently removed from the mold or packed into the syringe before being extruded. The woodpile samples were assembled manually and measured at 3.5 cm × 3.5 cm in breadth and length with differences in height due to the different strut sizes (see Figure S2 for images).

After growth, the mycelium that was growing 'outside the substrate' was cut out by simply lifting off the samples from the flat surface they were lying on (see Figure S3 for images of grown samples after 28 days).

The porosity induced by the macroscopic design in woodpile structures was measured by calculating the volume of struts divided by the volume of the enveloping block (**eq (1)**). As a guide, for the sample with the 2 struts, the porosity was obtained by multiplying the volume of a strut by two and dividing by the total volume of the enveloping block (breadth × length × height). The design-induced porosity values were then used as a factor for the modulus values calculated through the stress strain curves. Since these porosity values do not take into account the porosity inside the struts, which is supposed to be independent on the struts dimension, the specific moduli calculated are therefore underestimated values.

$$porosity(\%) = \frac{V_{strut} \cdot n}{V_{block}} \quad (1)$$

where V_{strut} is the volume of one strut, n is the number of struts, and V_{block} is the volume of the block of same dimensions.

2.4. Characterization

2.4.1. Scanning electron microscope

For the observation of the fungal mycelium skin, small samples of the white mycelium growing at the surface of the substrate were removed with tweezers. For the observation of the mycelium growing in the core of the samples, the struts were simply broken in half. All samples were then dried in an oven (IKA, Malaysia) overnight at 48 °C. Electron micrographs were obtained using a scanning electron microscope (SEM, JSM-5510LV, JEOL, Japan) on samples deposited onto a double-sided carbon tape and coated with 45 s of gold using the Cressington 108 Gold Sputter Coater, United Kingdom. The size distributions of the pores in the mycelium network and of the hyphae were obtained by measuring the diameters of the pores from the micrographs using the software Image J (NIH, USA). The distribution plots were obtained with more than 100 measurements using MATLAB and fitted using a normal distribution.

2.4.2. Nanoindentation

Nanoindentation was performed using a triboindenter (Hysitron, Minneapolis, USA) with a cube corner tip. Mycelium hyphae were grown onto a glass slide by placing a piece of inoculated substrate on one side of the glass slide and letting the mycelium naturally grow over the other side of the glass substrate (see SI Fig. S4a for a picture of the sample). The tests were carried out in a hermetic chamber at 23 °C and it was verified that the samples were not drying during the experiment. The tests were performed in load-controlled mode with a maximum loading force of 60 N, using a 6 s loading time, 1 s hold at maximum load and 6 s unloading time load function. Surface images were scanned before the indentation process to select valid surfaces for indents (see SI Fig. S4b for image of the scan and indent positions). Multiple indents were done on independent samples to improve reliability.

2.4.3. Compression

Compression tests on blocks and woodpile structures were performed with a Universal Testing Machine (Instron 5567, USA). The samples were tested right after their reach the intended growth time. The samples were compressed at a rate of 3 mm/min up till 50% strain. The Young's modulus E were obtained from the stress strain curves by taking the slope at 2% strain. In the case of the woodpile structures, the modulus calculated is referred to as 'apparent modulus' as the area taken for the calculation of the stress is the same area as for the block specimen, which is 3.5 cm × 3.5 cm. Samples were tested at least in triplicates for each time point and woodpile design, to ensure reproducibility. A portable microscope (Dino-lite AM7915MZTL) was used to record the deformation of the samples during the tests. All of the mechanical testing (compression, flexural and tensile) was done on living samples, and carried out at room temperature.

2.4.4. Bending

3-point bending tests were performed with the Universal Testing Machine (Autograph 10 AG-X plus, Shimadzu, Japan). 10 × 30 × 10 mm samples were subjected to flexural stress at a rate of 2 mm/min up to a displacement of 5 mm. The values of the flexural properties were obtained through an average of 5 samples for each time point of 7, 14 and 28 days. Flexural strength was obtained using the following equation:

$$Flexure\ \ strength = \frac{3 \cdot F \cdot s}{2 \cdot w \cdot h^2} \quad (2)$$

where F is the applied load, s the span, w the width and h the height of the sample. The flexural moduli were then obtained from the stress-strain curves.

2.4.5. Tensile

Tensile tests were performed with the Universal Testing Machine (Instron 5567, USA). Struts grown of dimensions

10 × 30 × 8 mm for 28 days were subjected to a tensile stress at a rate of 2 mm/min until the samples. Pure mycelium samples that were used to measure tensile stress were not uniform in size or shape. Larger dimensions were used which may lead to an underestimation of the tensile strength and modulus. Those pure mycelium samples were obtained by placing struts of inoculated substrates with a small distance from one another, allowing the mycelium to bridge the gap (see SI Figure S5 for images). The portable microscope (Dino-lite AM7915MZTL) was also used to record the behaviour of the samples during the tests.

3. Results

3.1. Fungal mycelium skin growth and properties with time

When a fungus grows onto a particulate-based ligno-cellulosic substrate, fungal cells assemble into filaments called hyphae that form a highly interconnected porous network structure that binds the particulates of the substrate together. At the air-surface interface, the fungus grows a denser mycelium network, known as the fungal skin, to maintain the moisture necessary for the fungus survival at the core of the composite. To support the claim that increased mycelium growth and more packed network would increase the mechanical properties of the final mycelium-bound composites, the growth kinetic of the network and its morphology and mechanical properties were studied (Fig. 1).

To characterize the formation and the properties of the fungal mycelium skin, bulk mycelium-bound composites were grown onto a solid substrate made of bamboo microfibers, food supplement, and water, according to an established protocol [26]. The substrate was simply deposited into a beaker and the fungal skin formed at the top surface where the substrate is in contact with air. As the fungus grows with time, the fungal skin becomes visible as a white fluffy surface from day 14 onwards (Fig. 1**A**). The mycelium growth in our experiments was conducted till a maximum of 28 days. At longer growing times, the fungus starts to develop fruiting bodies, which are the sporocarps or mushrooms, instead of its mycelium. From day 0 to day 28, the fungal skin was found to increase in packing density, with the diameter of the pores in the interconnected network decreasing with time from about 12 to 6 μm (Fig. 1**B**). At the same time as the network's density increases, the individual fungal filaments, the hyphae, were found to double their diameter from 2 to 4 μm. This increase in diameter might be a result of the composition change in the mycelium. Indeed, it has been reported that after 14 days growth, the content in protein, lipids, polysaccharide and chitin increases, which might also suggest that the mycelium cell walls become stronger [26].

To characterize the properties of the individual hyphae, mycelium was allowed to grow on solid flat glass substrates. Although fungi cannot digest glass, the growth was conducted by placing a small amount of ligno-cellulosic substrate containing the mycelium inoculum on one side of a glass slide. With time, the fungus developed mycelium towards the bare glass side and hyphae bundles as well as single hyphae could be singled out, without the ligno-cellulosic substrate (Fig. 1**C**). Using the mycelium grown onto the glass, nanoindentation was used to probe the elastic modulus of the hyphae with growing time (Fig. 1**D**). It was found that the modulus of single hyphae increased with the growing time, confirming the previous hypothesis (see SI Figure S6 for the load-displacement curves). The modulus obtained after 28 days of growth was of about 5 GPa. This modulus is much higher than the data reported in the literature for the same fungus but measured using atomic force microscopy (AFM), varying between 4 and 28 MPa [27], as well as the moduli reported for other fungi, about 64 to 110 MPa [28]. The larger value measured could be due to the longer time growth as compared to the other studies where the mycelium was tested after 2 days only. It could also be that the cell walls in the hyphae are not homogeneous in their thickness and that stronger components of the cell wall, such as cellulose, have a higher concentration in the inner part of the cell wall. In nanoindentation, the hyphae are probed until a thickness from 200 to 700 μm, whereas AFM probes the first 20 to 50 μm only and might miss the stronger components. Since the hyphae thickness was higher than 2 μm, our high modulus value cannot be an effect from the glass substrate. Also, it was ensured that the hyphae remained hydrated before the experiment. Interestingly, a modulus of 5 GPa for single hyphae is close to the modulus of bacterial cellulose, of about 9–10 GPa, and is lower than the modulus of crystalline cellulose [27], of about 18–50 GPa along the transverse direction [29]. Since mycelium cell walls are composed of proteins, cellulose, and chitin, the value measured of 5 GPa is therefore reasonable. This result supports the assumption that single hyphae are a strong material. Increasing the hyphae concentration in the ligno-cellulosic substrate would therefore increase the mechanical properties of the mycelium-bound composite. The increase in mechanical properties of the mycelium with time across the 28 days also suggests that the mechanical properties of the composites will increase as the mycelium grows within that period.

The fungal mycelium skin formation occurs at the air-substrate interface and shows increase in hyphae density, diameter and modulus, whereas the mycelium formation in the inner of the substrate remains small in comparison, due to the lack of oxygen diffusion. This is visible in the optical image of the cross-section of the sample in Fig. 1**A**, right insert, where the white mycelium is not visible below the skin. Based on this observation, we can suppose that increasing the formation of the dense fungal skin at the core of the substrate would increase the mechanical properties of the global mycelium-bound composites. For this purpose, air channels where intentionally induced by constructing porous woodpile structures and the mycelium growth and the final properties of the composites were measured with time.

3.2. Woodpile mycelium struts structure

To increase the air-substrate interface area and promote mycelium skin formation at the core of the substrate, woodpile structures were produced using a manual method (Fig. 2). Although there exist other structural designs that have high surface to volume area such as triply periodic minimal surfaces (TPMS), including gyroids [30], woodpile structures made from assembled struts were chosen due to the convenient fabrication method. Indeed, simple extrusion of struts of controlled diameter could be carried out in a reproducible manner. Also, woodpile structures are found in many applications such as tissue engineering, lightweight structures, and metamaterials [31].

With growing time, the white fungal skin developed as expected at the surface of the substrate (Fig. 2**A**). The pore size within the fungal skin grown on the struts was found to decrease similarly to the skin grown on the blocks (see SI Figure S7(a)). Interestingly, although the mycelium grown inside the core of the struts exhibited larger pore diameter, this diameter was found to decrease faster than in the skin (-0.39 μm diameter/day as compared to −0.27 μm/ day) suggesting sufficient transport of oxygen and nutrients to the core of the struts (see SI Figure S7(b)). Mycelium was also found to develop at the core of the struts to connect and bind together the bamboo microfibers which are visible in the micrographs in Fig. 2**A** (highlighted with the orange arrows. See SI Figure S8 for closer view of the micrographs after 28 days of growth.). Although this is expected in mycelium-bound composites, more mycelium formed in the case of the woodpile structures

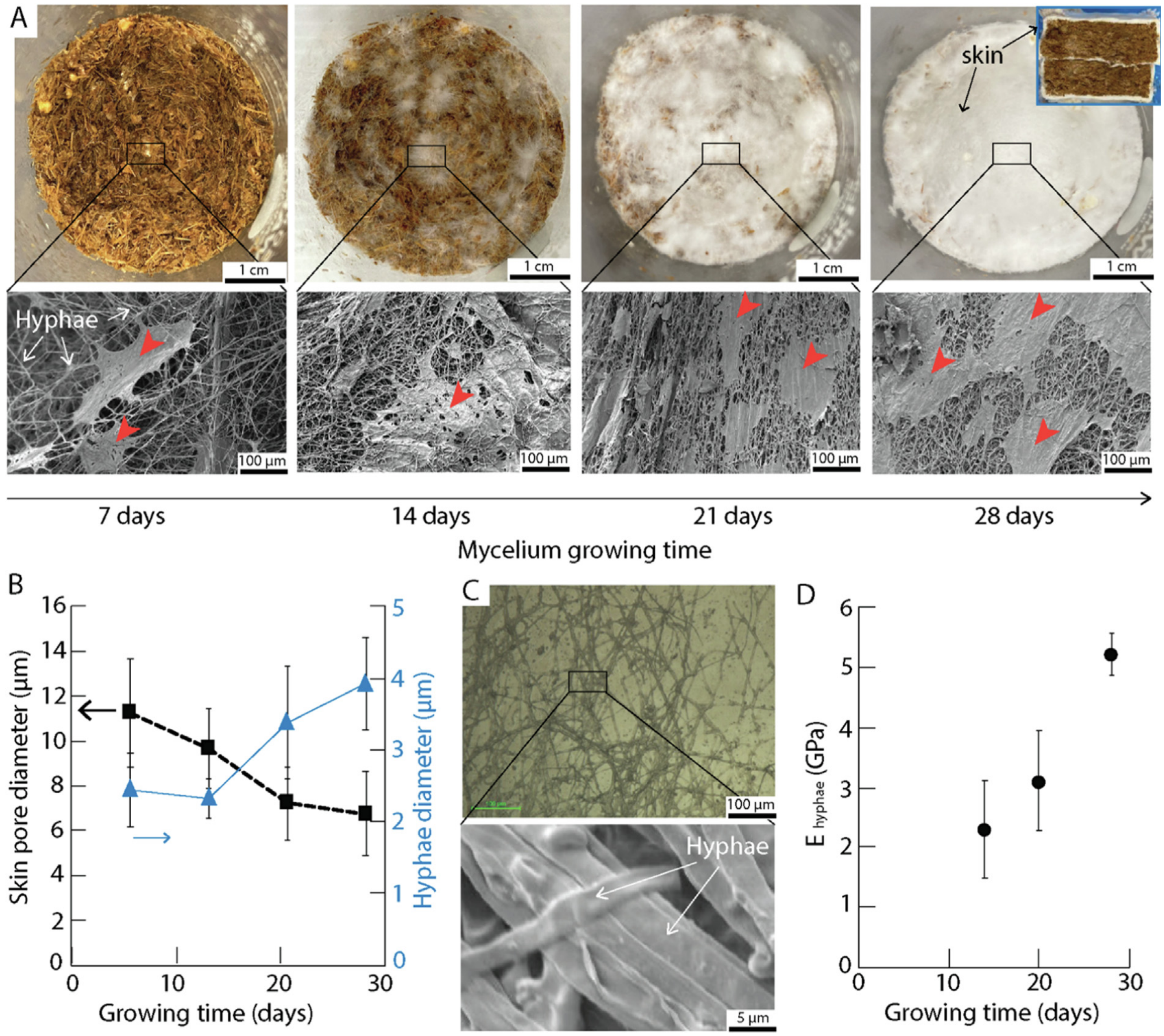

Fig. 1. Skin formation and properties. (A) Optical images and electron micrographs of the skin formation at the surface of mycelium-bound composites grown after 7, 14, 21 and 28 days. The arrows point to densely packed fungal mycelium skin. Insert on the right shows the cross-sections of the 2 halves of a composite after 28 days growth showing that the skin forms on the air-substrate interface. **(B)** Pore diameter and hyphae diameter of the mycelium skin as a function of growing time. **(C)** Optical image and electron micrograph of hyphae bundles. **(D)** Elastic modulus of single hyphae as a function of growing time measured using nanoindentation.

as compared to bulk composites because a larger amount of fungal skin was grown. The exact quantification of mycelium growth inside the substrate is difficult to obtain since the material cannot be de-bounded and the mycelium cannot be easily separated from the bamboo microfibers. Nevertheless, our observations are supported by the literature where increased air flow increases mycelium growth and packing density of hyphae [16,18,19]. The increase in mycelium growth in our woodpile structures is therefore induced by the increased airflow through the gaps between the struts.

Furthermore, mycelium was found to be able to connect the individual struts of the woodpile structure together (Fig. 2**B**). After their extrusion, the individual struts made of the inoculated substrate were assembled by hand, without any 'glue' to hold the woodpile structure together. However, within as little as 2 days, hyphae strands were observed have grown to connect the neighboring struts. Actually, it was observed that mycelium could grow to bridge gaps up to about 5 mm (see SI Figure S9). This growth of mycelium over large gaps is likely a consequence of the 'exploration' mode of the fungus and its ability to grow and translocate across empty areas [32]. In our case, this aerial growth of the mycelium is an extra advantage to increase mycelium concentration in the woodpile structure, not only at the air-substrate interface but also in other gaps that appear between the struts. Interestingly, electron micrographs of the mycelium bridging two struts over a small gap revealed an anisotropic orientation of the hyphae towards to growth direction (Fig. 2**C**). Anisotropic growth and packing of hyphae filament could induce anisotropic properties that might further reinforce the mechanical properties, as observed with bacterial cellulose [33]. Overall, woodpile structures exhibited more dense fungal skin formation. After 28 days of mycelium growth, the woodpile composites had similar dimensions as the composite blocks but contained dense mycelium skin in its interior. How this increased mycelium growth impacts the mechanical properties of the composite as compared to the block specimens is studied in the following section.

3.3. Compression response as a function of growing time

Quasi-static compression tests as a function of the mycelium growing time were carried out to evaluate the effect of increased

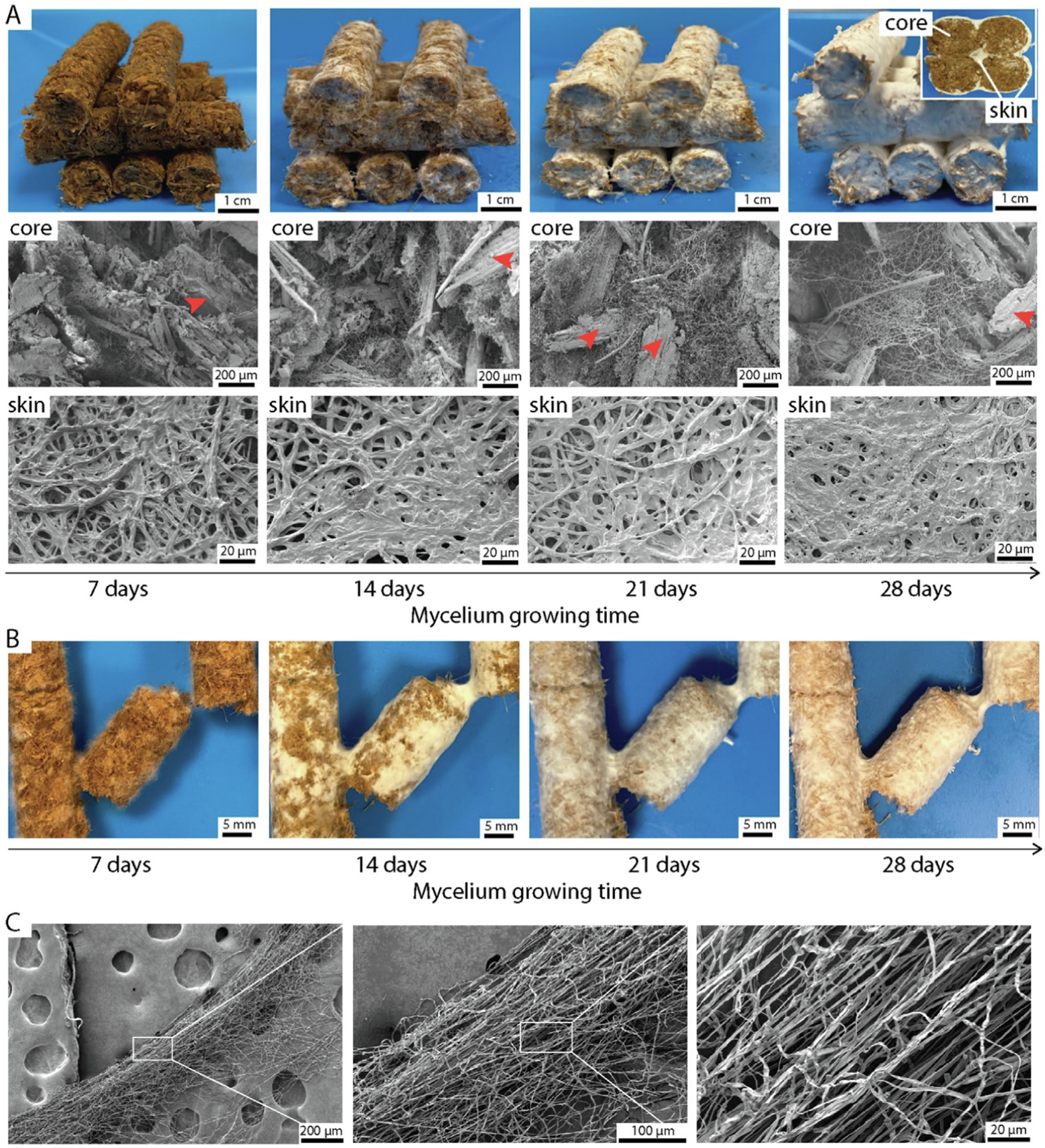

Fig. 2. Mycelium growth on woodpile structures. (A) Optical images of a woodpile structure as a function of the mycelium growing time as well as representative micrographs of the structure at the core of the struts and of the fungal skin on the surface of the struts. Insert shows the cross-section of a similar woodpile structure to indicate the difference between the core of the struts and the skin growing on the struts surface. The orange arrows point to bamboo microfibers from the substrate. **(B)** Optical images showing the mycelium bridging neighboring struts as a function of mycelium growing time. **(C)** Electron micrographs showing the aligned structure of the mycelium when connecting two neighboring struts. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mycelium formation on the mechanical properties of our woodpile structures (Fig. 3).

First, cubic mycelium-bound composite blocks were produced and tested. Fig. 3**A** shows representative compression curves. Despite some variability due to stochastic variations in the mycelium growth (see SI Figure S10 for the curve of all composites tested), it was found as an overall trend that the mechanical properties of the composites increased with the mycelium growing time, as expected. The increase appeared to be high in the first two weeks of growth which can be explain by the temporal evolution of the fungus that also grows fast in the first two weeks [34]. All mycelium-bound composite blocks showed a typical densification behavior with the absence of the elastic or plastic regions indicating the absence of yield point (or an extremely low one). This type of mechanical response is expected due to the high intrinsic porosity of the substrate made of particulates, and of mycelium-bound composites, with their low density of 0.356 $g.cm^{-3}$. Indeed, the bamboo microfibers in the substrate are not densely packed to allow oxygen diffusion and mycelium growth in its core. In our fabrication protocol, the packing density of the microfibers was not controlled but left 'as it is', similarly to what is done in the literature on mycelium-bound composites. Assuming a density of 1 for

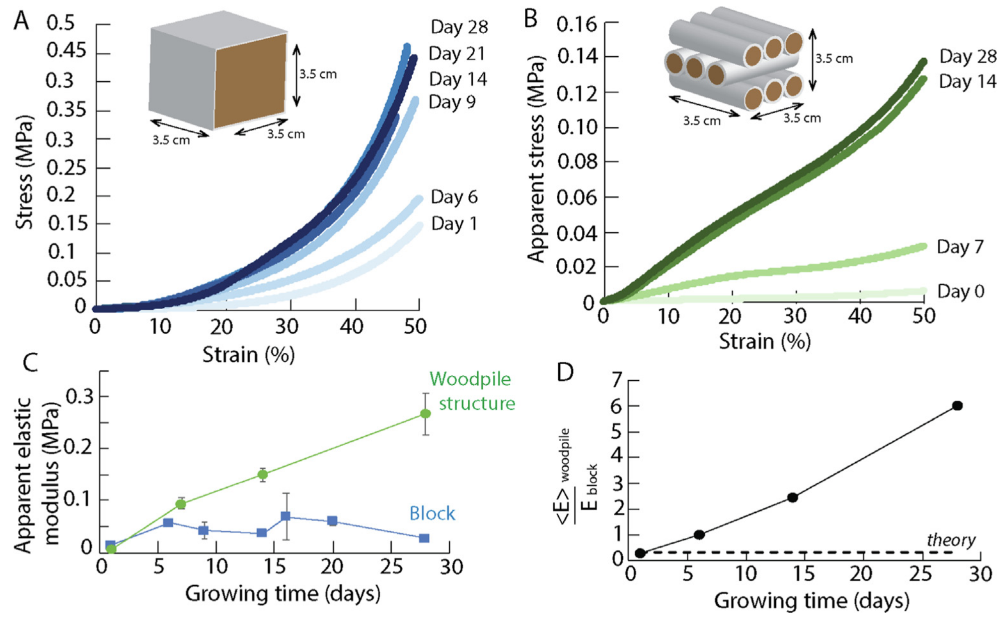

Fig. 3. Compression of blocks and woodpile structures. (A) Stress-strain curves of block composites at increasing various mycelium growth time. The schematics indicates the dimensions of the samples. **(B)** Stress-strain curves of the woodpile samples at increasing various mycelium growth time. The schematics indicates the dimensions of the structure. **(C)** Apparent elastic modulus as a function of mycelium growing time for the woodpile structures (green) and the block composites (blue), measured at 2% strain. **(D)** Ratio of the apparent moduli between woodpile and block composites as a function of growing time. The dotted line represents the theoretical ratio expected from equation (3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the substrate and the mycelium, the intrinsic porosity of the composite blocks can be estimated at 65%. As compared to the block samples, the compression curves of the woodpile structures showed similar increase in mechanical properties as a function of mycelium growth time (Fig. 3**B**). However, the overall apparent stresses are about 4 times lower as compared to the block samples: after 50% strain, the stresses applied to the block samples were of about 0.45 MPa whereas the apparent stresses applied to the woodpile structures were of 0.14 MPa only. Given the added porosity in the woodpile design due to the arrangement of cylindrical struts, calculated to be of about 30%, it is expected to witness a decrease in mechanical properties. Yet, the compression curves show a different shape as compared to the those of the composite blocks, with a sharper increase in the apparent stress at strains below 30% and a curve having a concave shape. Beyond 30% strain, a similar convex curve as for the block samples suggests densification. This transition from concave to convex was reproducible within all the samples tested (see SI Figure S4 for the all the recorded curves) and strongly suggest the appearance of a yield stress at the inflexion point. The initial concave shaped curve demonstrates some elasticity in the material and an increase in stiffness. However, the absence of a horizontal plateau as typically observed during compression of foams indicates the absence of plasticity.

To visualize better the unusual increase in elasticity, we calculated the apparent elastic modulus of the block and woodpile structures as a function of mycelium growth time (Fig. 3**C**). To ensure being in the elastic region, the apparent moduli were calculated at 2% strain for all samples. The results show that although at day 0 the woodpile structures had lower apparent modulus than the blocks, as mycelium develops, the porous woodpile structures exhibited a significant 40-fold increase in their apparent modulus, whereas the block samples only doubled. Such an increase in apparent modulus in the porous woodpile structures can be attributed to the increased content of mycelium skin throughout the structure. Overall, the apparent modulus of the woodpile structures was found to increase with time to higher degree than in the bulk samples, reaching a value about 6 times higher after 28 days of mycelium growth (Fig. 3**D**). In woodpile structures with symmetric staggered arrangement, the ratio between apparent modulus of the structure $< E >_{woodpile}$ and the modulus of the material in the struts E_{strut} should follow:

$$\frac{< E >_{woodpile}}{E_{strut}} = \frac{\pi \cdot r}{4 \cdot \lambda}, \tag{3}$$

where r is the radius of the struts and λ the distance between the middle of the struts [35]. Assuming the modulus of the material in the struts, E_{strut}, is the same as the modulus of the block samples, E_{block}, and $E_{strut} = E_{block}$. Furthermore, our woodpile structures are constructed with $\lambda = 2r$, hence $\frac{<E>_{woodpile}}{E_{block}} = 0.39$. Interestingly, this is the value obtained at day 0 only, with the experimental ratio $\frac{<E>_{woodpile}}{E_{block}}$ being much higher at other time points, confirming the effect of mycelium growth.

Therefore, although the material remains weak, it appears that increasing the porosity and the mycelium skin content through structural design in woodpile arrangement significantly increases the apparent stiffness of the final mycelium-bound material.

To better visualize the effect of mycelium growth on the mechanical properties as a function of growing time, videos were recorded during the compression tests (see SI movies S1 to S4). Images were extracted from these movies at increasing strains from 12.5 to 50% (Fig. 4).

At day 1, when the mycelium is not grown yet, the struts were found to break transversally at very low compressive strains and the structure densified through the breakage and packing of the struts. Similar mechanism was observed after 7 days growth, although the fracture of the struts was delayed to higher compressive strains as compared to the samples after 1-day growth. Also, it is noticeable that the struts at the bottom of the pile started to compress, with their height diminishing from 1.3 to 0.6 cm from strain 0% to 50%. In comparison, the struts diameters did not appear to vary for the samples after 1-day growth, as the struts broke through their transversal cross-section instead. This suggest that the mycelium grown in the structure after 7 days was preventing the fracture of the struts and maintaining their length, there-

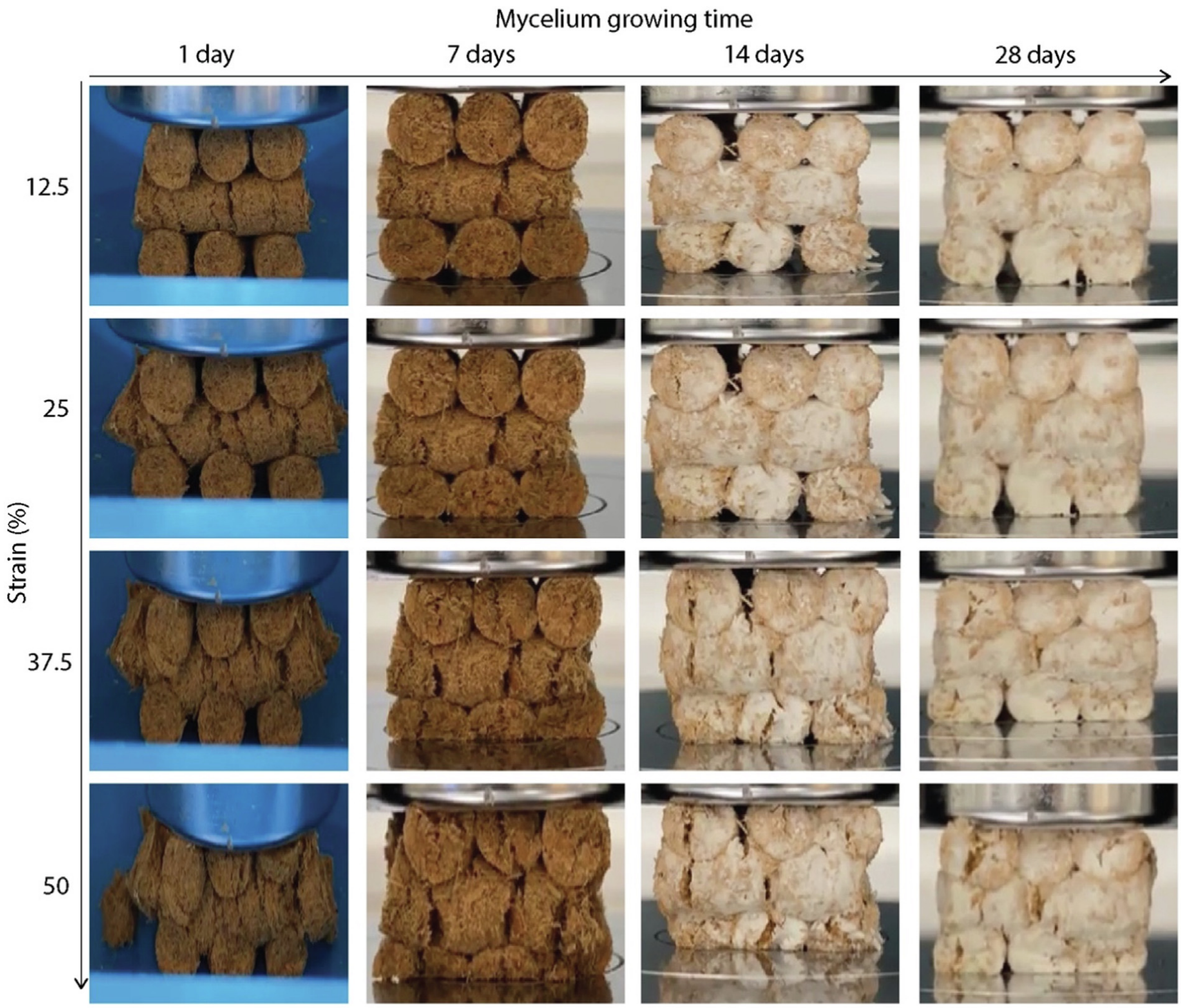

Fig. 4. Pictures of the woodpile structures after 1, 7, 14 and 28 days of mycelium growth taken during the compression test, at strains of 12.5, 25, 37.5 and 50%. The initial height and width of the samples were 3 and 3.5 cm, respectively.

fore applying more load onto the bottom layer of struts. Similar compression of the bottom struts was observed for the samples after 14 and 28 days of growth. In these samples, the fungal skin had extensively developed. During compression, almost no cracks or struts fracture were visible until 35% strain was applied and the structure could resist the compressive load. This behavior differs greatly to the samples at day 1 and 7. Beyond 35% strain, fracture of the struts and compression of the bottom layers were observed too, leading to the densification of the structure. The evolution of the deformation and fracture response of the composites to compressive loads strongly support the previous hypothesis that the fungal skin holds the shape of the struts together and increases the stiffness of the overall composite.

To tentatively explain these compression results, the properties of the individual struts were measured under bending and tension (Fig. 5). Indeed, the transversal fracture of the struts is a result from tensile stresses developing along the length of the struts. Also, pressure from the struts from one layer onto the struts in the layer underneath should induce some bending.

Flexural tests carried out on individual struts at increasing mycelium growth time show increase in flexural modulus and flexural strength as the mycelium grows (Fig. 5**A,B**). At day 0, the struts did not exhibit any flexural strength and were found to crumble and densify, reflecting what was observed in the compression tests. From 7 to 28 days of mycelium growth, the flexural modulus was found to be multiplied by 2.5 whereas the flexural strength was multiplied by about 2.8. The increased resistance to bending explains the lesser deformation and fracture of the struts during the early stages of the compression tests, below 30% compressive strain. The fungal skin was presumably able to maintain the shape of the struts together and to resist the deformation thanks to the high stiffness of individual hyphae. Furthermore, tensile tests were carried out onto struts after 28 days of mycelium growth to better observe their fracture behavior (Fig. 5**C**). Under tension, the struts exhibited a typical mechanical response with an elastic regime until yielding point. Interestingly there is a little plasticity before the struts fracture. By recording videos during the tensile tests (see Movie S5 in SI), it could observe that the large drops in the stress-strain curves corresponded to the mycelium skin tearing apart. At the same time as the mycelium skin covering the struts broke, bamboo microfibers were found to bridge the gap. This is likely the reason why there is a remaining apparent stress after the drop. To confirm the sharp tearing of the mycelium skin, pure mycelium was also grown for 28 days and tested under tension while recording the deformation (see Movie S6 in SI, Fig. 5**D**). Obtaining pure mycelium skin of defined area was challenging, hence the area of the cross-section was estimated which may lead to an underestimation of the calculated stresses and of the moduli and yield strengths. Nevertheless, it was observed that the mycelium skin teared off catastrophically under tensile loading and

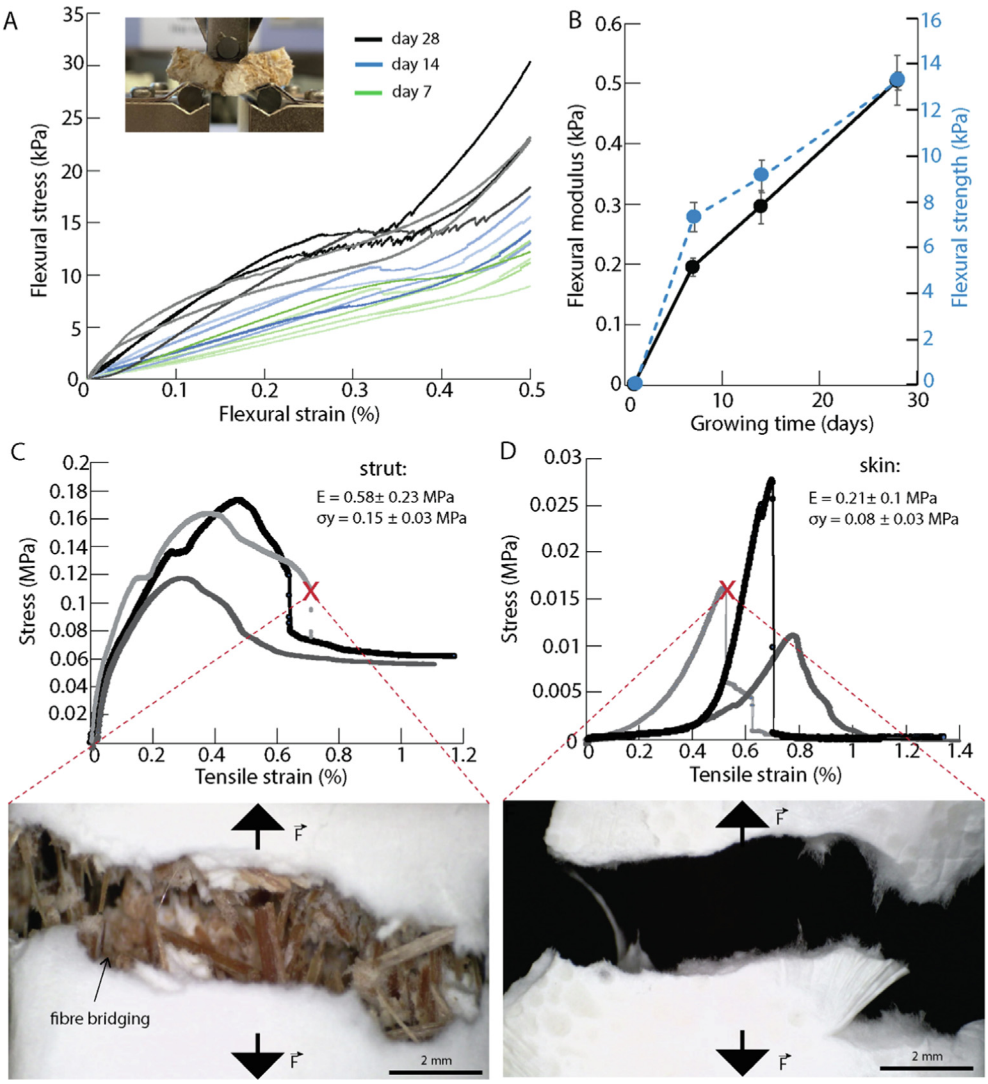

Fig. 5. Flexural and tensile properties of the struts. **(A)** Stress-strain curves of the struts recorded under 3 points bending (see image in the insert) after 7, 14, and 28 days of mycelium growth. **(B)** Flexural modulus (black) and flexural strength (blue) of the struts as a function of the growing time. **(C)** Stress-strain curves of the struts grown after 28 days under tensile test. The optical image shows the fracture of the strut and bamboo microfiber bridging the gap. **(D)** Stress-strain curves (underestimated area) of pure mycelium skin grown for 28 days and optical image showing the sharp tearing of the mycelium under tension. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

exhibited almost no plasticity. This suggests that the plasticity observed in the struts results from the substrate, while the stiffness and elasticity are likely provided by the mycelium skin. As the mycelium grew in the woodpile structure and connected the struts together with a highly packed mycelium skin, the structure gained in stiffness. After the mycelium teared off and the struts became disconnected or fractured, the remaining apparent stress was low as compared to the composites blocks. Increase in porosity in the woodpile structure as compared to the blocks therefore increased fungal skin formation on the inside of the composites and increased its stiffness. Since the fungal skin grows at the air-substrate interface, varying the struts diameters while maintaining the same level of porosity would help to determine which of the porosity and the surface to volume ratio plays a major role.

3.4. Effect of struts diameter

To explore if the mycelium growth and the mechanical properties of the woodpile structures could be further enhanced by increasing the air-substrate surface area, four structures with varying strut sizes but constant porosity of about 32% were constructed and tested under compression (Fig. 6).

The four structures were woodpile structures of same volume, constructed with increasing number of struts as the struts' diameter decreased (Fig. 6**A**). Decreasing the struts' diameter increased the surface to volume ratio of the substrate. After 28 days mycelium growth, all structures were found to have grown the expected dense fungal skin at the interfaces between air and the substrate. Dense fungal skin could therefore form inside the woodpile structures as shown by the white areas in Fig. 6**B**. The samples after 28 days growth was then tested under compression (Fig. 6**C**). The block samples exhibited the same compression behavior as previously discussed, with convex-shaped curve showing densification as the load is applied, without a yield point. The structures with the 2 struts exhibited weaker mechanical properties at high compressive strains as mentioned previously for woodpile structures, but a concave-shape curve was observed at compressive strains below 20%. This suggests an increase in stiffness and in elasticity was already induced. Similar observations were made for the woodpile structures with 6 struts, although there was a larger vari-

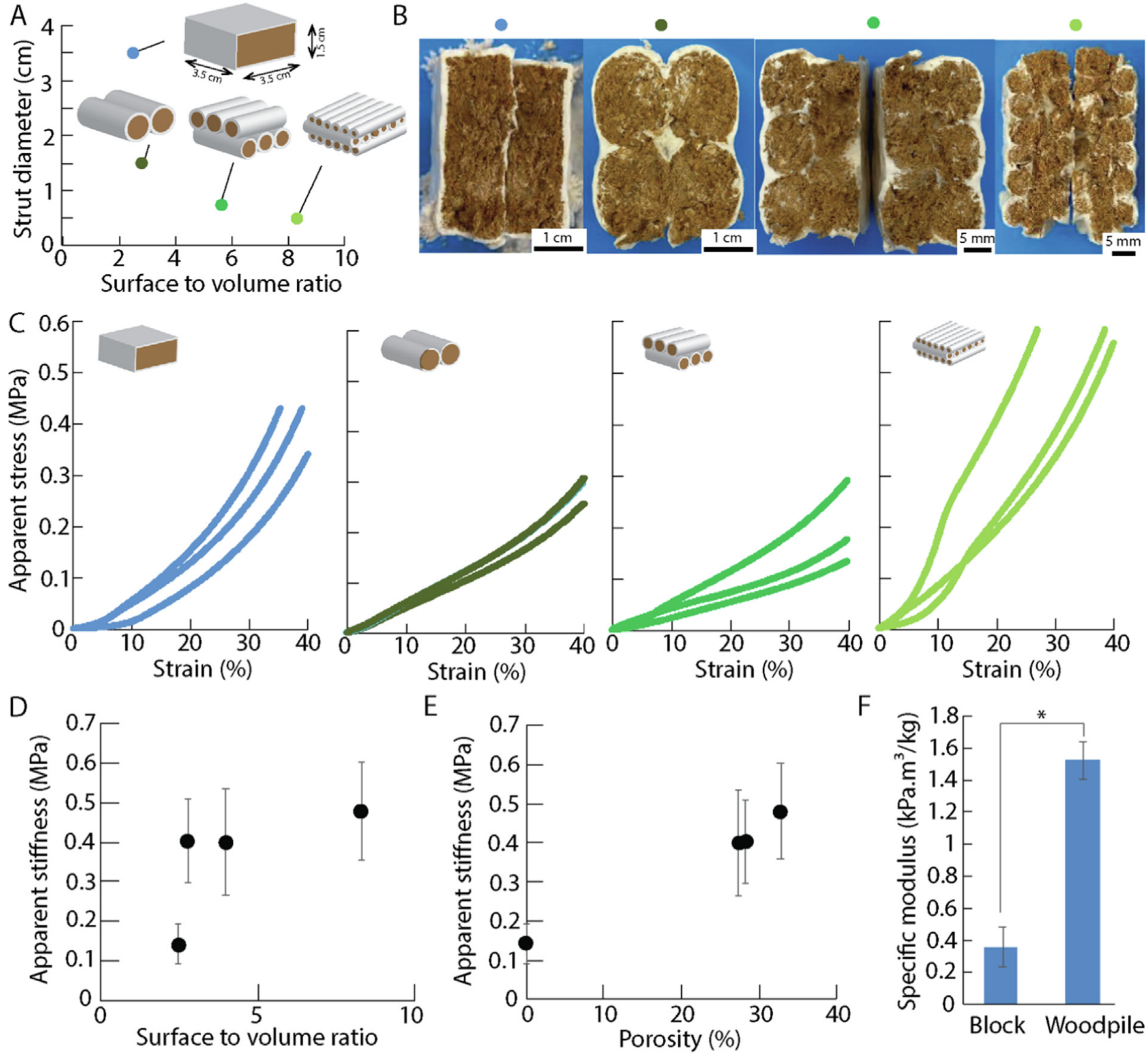

Fig. 6. Woodpile structures with varying struts diameter. (A) Strut diameter as a function of surface to volume ratio for a constant porosity about 32%, and the schematics representing the structures made. **(B)** Images of the cross-sections of the mycelium-bound composites after 28 days growth showing the fungal skin formation (white). **(C)** Compression curves of the four structures after 28 days mycelium growth. **(D)** Apparent stiffness as a function of the surface to volume ratio. **(E)** Apparent stiffness as a function of the porosity induced by the woodpile design. **(F)** Specific modulus of the block and of the woodpile structures. * indicates that the difference is significant ($p < 0.05$).

ation between the specimens. Finally, the woodpile structures with 18 struts showed significantly increased mechanical properties with the concave-shape curve showing elasticity and stiffness appearing more evidently between 10 and 20% strain. The initial start of the curves has a convex shape which can be attributed in this case to the levelling of the samples at the first stage of the test. Indeed, due to the large number of struts it was more difficult to ensure flat surfaces. These results confirm the previous observations that porosity and increased mycelium growth influence the compression response of the woodpile structures and increases their stiffness. However, there is no obvious conclusion with regards to the effect of the struts' diameter.

To look further into this, the apparent stiffness of the structures was plotted as a function of their surface to volume ratio (Fig. 6**D**) and as a function of the porosity (Fig. 6**E**). As expected, the stiffness increased in the woodpile structures as compared to the block samples, but no significant influence of the struts thickness could be observed. It is therefore probable that the porosity is the critical parameter, rather than the surface to volume ratio. It could also be that the strut diameter and surface to volume ratio had a role to play but at higher struts' diameters. The results suggest that the mycelium growth is greatly reduced in block samples of 3.5 cm length, and the growth is more efficient for any strut below 1.5 cm diameter. Producing bulk samples of higher thickness and woodpile structures with struts diameters larger than 3.5 cm could be done to confirm if there is a maximum thickness above which mycelium does not grow at all in the core of the struts. Taking into account the density of the composites, a 4-times increase in the specific modulus was thus obtained using woodpile structures as compared to block samples. This increase was significative with a Student T-Test p-value of 0.0266 (Fig. 6**F**).

By varying the diameter of the struts in our woodpile structures, it could be confirmed that the increased porosity led to increased mycelium growth which in turn increased the stiffness of the composites.

4. Discussion

Woodpile structures lead to mycelium-bound composites with more fungal skin formation and higher stiffness than block shapes. The mycelium filaments, known as hyphae, bind the individual substrate particles together to strengthen the composites. This interconnected network also increases the stiffness of the overall composite due the stiffness of the individual hyphae. The diameter of the struts, in the range considered, did not had any effect on the stiffness. Therefore, it seems that the porosity is the key factor that increased the mycelium growth and stiffness. As a consequence, we can conclude that the design-induced porosity has led to an increase in the compressive stiffness mainly due to the increase in mycelium density in the core of the composite. The increase in compressive strength observed for smaller diameter could be the result of this increase in mycelium density as well as the increase in the packing of the bamboo microfibers in the struts of smaller diameter. One might thus be tempted to produce similar woodpile structures with increased porosity by increasing the spacing of the struts. This might induce the growth of more mycelium as mycelium has been found to be able to bridge gaps of several mm. However, the increase in spacing between the struts would also largely decrease the initial apparent stiffness in absence of mycelium, following equation (3). In turn, lower porosity than 32% cannot be achieved using cylinders in woodpile structures.

To achieve higher stiffness, it might therefore be preferable to (i) produce structures of higher porosity with a pattern other than woodpile. However, current limitations in mycelium-bound composite fabrication does not enable this option. (ii) increase the mechanical properties of the struts themselves by tuning the substrate. In the present case, the substrate was composed of bamboo microfibers of 200 μm length. Previous results showed that reducing the length of the bamboo microfibers tended to increase the mechanical properties due to higher packing density [36]. Furthermore, despite no influence of the struts' diameter on the stiffness, our results suggest that smaller struts diameter led to higher mycelium growth and overall mechanical properties, especially at high compressive strains. One hypothesis that could explain this result is the higher packing of the bamboo microfibers is the small diameter struts due to the fabrication process, as the substrate was loaded into a syringe of diameter about 5 mm. The substrate had therefore to be compacted during loading and extrusion, which might have led to the higher results. For the other struts' diameters, the preparation of the samples was less tedious and therefore less unusual packing of the bamboo microfibers is expected, which may be reflected again in the lower or similar mechanical stresses measured as compared to the block samples. In addition, despite the little influence of the struts' diameter, it was suggested that a maximum sample thickness might exist, above which the mycelium growth in the interior of the substrate is not efficient. Although the experiments carried out in this work suggest it to be of 1.5 cm, it could be confirmed by producing struts of larger diameters and testing their individual properties after 28 days growth, for example using 3-points bending. (iii) the individual mycelium hyphae could also be strengthened by using another fungal species. Another way to increase the properties of the hyphae could be by adding cellulose nanocrystals which can be internalized by the fungus into the cell walls [37]. Finally, other treatments could be conducted to increase the plasticity of the hyphae and prevent their sharp tearing, for example using glycerol [38]. (iv) drying the composites. As compared to the literature, our mycelium-bound composites exhibited low mechanical properties overall. The reason for this is that our composites were not dried before the testing. Hence, drying would be expected to further increase the properties and allow more comparison with the literature.

Overall, our experiments demonstrated an increase in stiffness thanks to structural design of the substrate. Combining this enhancement of the stiffness with other methods to increase the mechanical properties of mycelium-bound composites would therefore further increase the performance.

5. Conclusions

In this study, the impact of structural design of the substrate onto mycelium growth and the resulting mechanical properties of the mycelium-bound composites in the case of simple woodpile structures was studied. All woodpile structures exhibited increased stiffness by 4 times as compared to the composite blocks as a result of the increase in porosity throughout the structure, which facilitated air flow and the growth of a compact mycelium skin in the interior of the structure. No effect of the struts' diameter was observed for the range of sizes from 0.5 to 1.5 cm, suggesting that below 1.5 cm, air flow through the struts is similar and would depend on the substrate material. Although the mechanical properties remain very modest, the increase in stiffness observed is a demonstration of how design can increase properties. Using a stiffer substrate or a stiffer mycelium strain would likely yield much more applicable properties. Finally, the choice of woodpile structure was chosen as it is typical structure used in 3D printing, which is a growing technology for mycelium-bound composites. More complex structure designs could be studied in the future to optimize the porosity for more optimal airflow, mycelium growth, and mechanical properties. The reported increase in stiffness by design-induced porosity is relevant for structural applications of mycelium-composites, when used as bricks in structures, packaging protecting against deformation, or pieces of furniture, for example. This increase in stiffness can be expected to be larger for composites that are dried or for similar woodpile structures where the struts have high intrinsic properties. This work therefore provides a proof-of-concept demonstration of fabrication and design using growing materials and more complex designs can be expected as fabrication methods are being developed, such as 3D printing.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

H.L.F conceived the study. E.S. carried out the experiments and analyzed the data. H.L.F. and E.S. interpreted the results and edited the manuscript. All authors have read and agreed to the submitted version of the manuscript.

Data availability

All data are available in this manuscript and attached supplementary files. Additional information can be requested to the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matdes.2022.111530.

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