

Project title: Biogenic Mycelium Feedstocks for 3D-printed Architecture

Industry partner(s): Advanced BioCarbon 3D Ltd.

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**Introduction & Background:**

Mycelium is the root-like network of fungi. Biofabrication with mycelium in the last 20 years has gained considerable interest for the sustainable production of materials that have the potential to replace fossil-fuel plastics [1]. Mycelium biocomposites can be prepared by combining fungi with lignocellulosic substrates in a template mould and mycelium biocomposites prepared in this way have found early commercial success as interior furnishings and thermal and acoustic insulation panels [2]. Advancing this technology as a 3D printing feedstock to produce mycelium-based materials additively can yield new properties and sophisticated products. A diverse range of nutrients, thickeners, and gelling agents can be combined to produce hydrogels suitable for 3D printing. However, few studies have demonstrated the success of printing living mycelium. In this study, we outline a reproducible workflow to infuse a 3D printing feedstock consisting entirely of easily sourced food grade reagents with living *Pleurotus ostreatus* (oyster mushroom) mycelium.

**Relevance to Circular Economy:**

Lignocellulosic substrates used in mycelium biofabrication are typically byproducts of forestry and agricultural sectors. These biocomposites sequester and utilize the carbon of these substrates for the remainder of the lifetime of the biocomposites. At their end of life, mycelium biocomposites, which are entirely bio-based and organic, can be composted or broken down to be reused as substrate for future mycelium biofabrication processes. Furthermore, architectural 3D printing to date has largely focused on cement as the printing feedstock, even if the printed component does not require the strength and resilience of cement. However, cement is responsible for 7% of global anthropogenic CO<sub>2</sub> [3]. Development of a biogenic printing feedstock to replace cement in targeted architectural applications can greatly reduce the emissions associated with the construction industry.

**Methodology:**

*P. ostreatus* mycelium was chosen for this study as it is among the strains most commonly used in mycelium biofabrication. In this work, we first developed and characterized a new bioprinting hydrogel feedstock which consisted of 18 g of carboxymethylcellulose, 9 g of cornstarch, 7 g of agar powder and 300 g of deionized water mixed in a high shear mixer and autoclaved to achieve homogeneity. Hydrogel rheology, print resolution and ability for the gel to bridge suspended regions were characterized with a rheometer and image analysis software. *P. ostreatus* liquid culture incubated at 30 °C and between 7 to 14 days old was used to inoculate the hydrogel at a 1:100 w/w ratio in preparation for 3D printing. In most experiments, we printed a hydrogel block (50 mm in width × 50 mm in length × 20 mm in height) with waffle-like infill and incubated the block at 30 °C for 14 days. Prints were performed on a non-sterile indoor benchtop environment or a sterile laminar flow hood. Prints were characterized for their change in height and mass as well as their gel hardness between Day 0 and Day 14. Finally, hydrated hardwood sawdust was blended into the gel formulation at a 1:1 w/w ratio and inoculated with *P. ostreatus* to print mycelium biocomposites.

**Results and Discussion:**

Mycelium-infused hydrogels printed on a non-sterile indoor benchtop (Fig 1A) and in a laminar flow hood (Fig 1B) both yielded prints which were propagated by *P. ostreatus* mycelium after 14 days, as indicated by visible pinning – early indicators of mushroom growth.

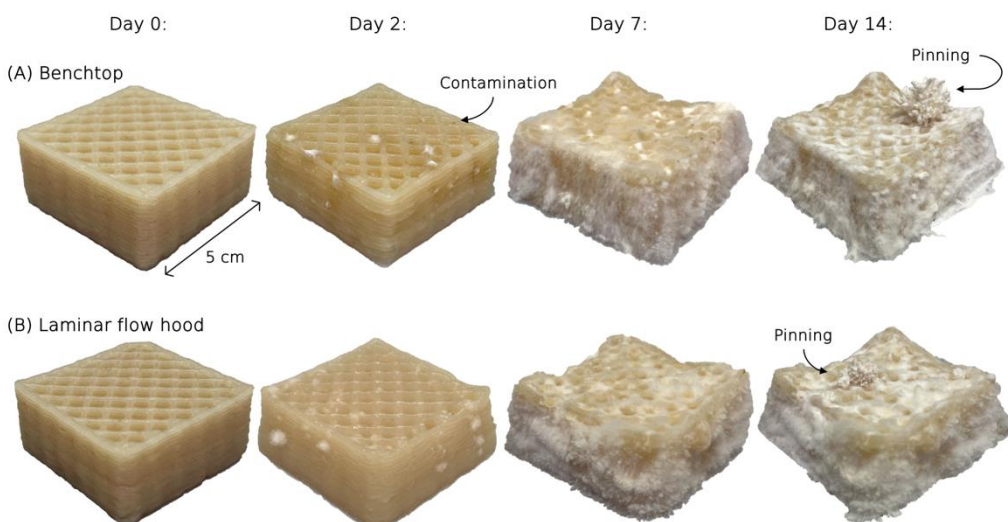


Figure 1: Mycelium-infused hydrogel blocks 3D printed on (A) non-sterile benchtop and (B) sterile laminar flow hood.

**Results & Discussion**

Practicality of 3D printing with biogenic feedstock is often limited due to need for sterility. Interestingly, we found the mycelium withstood airborne microbial contaminants, as evidenced by the success of the non-sterile benchtop prints. Furthermore, differences in change in height (Fig 2A), change in mass (Fig 2B), and change in gel hardness (Fig 2C) between mycelium-infused prints performed on the benchtop or in the laminar flow hood were negligible, suggesting that the properties of the prints were comparable. As a proof-of-concept demonstration, we printed a single layer of the hydrogel mixed with sawdust particles. small patches of mycelium growth were visible by Day 2 and the entire surface was covered with mycelium by Day 7.

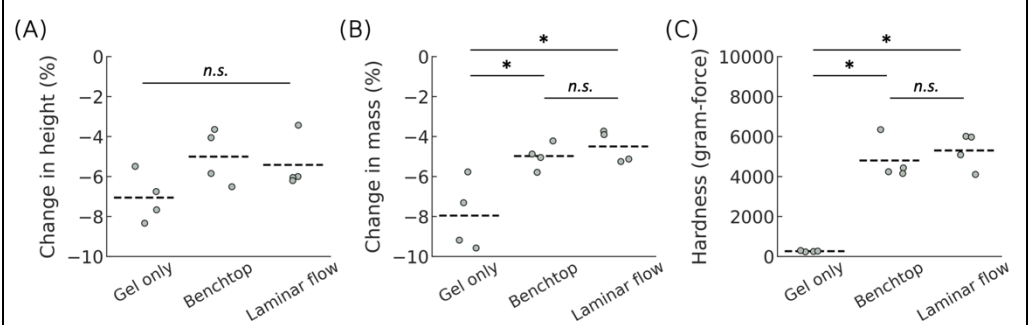


Figure 2: (A) Growth of mycelium-infused hydrogel with sawdust over 14 days of incubation, (B and C) Digital renders of hypothetical large-scale 3D bioprinting of mycelium with sawdust for architectural applications.

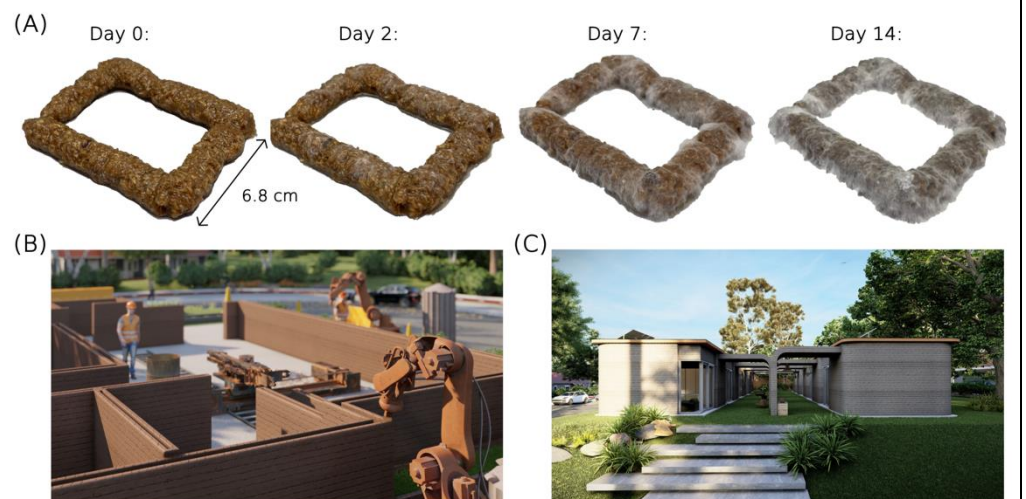


Figure 3: (A) Growth of mycelium-infused hydrogel with sawdust, (B and C) Digital renders large-scale 3D printing of mycelium for architecture.

**Conclusion & Next Steps:**

In this study we designed and evaluated a 3D printing hydrogel optimized for *P. ostreatus* mycelium consisting of four easily sourced food grade reagents: carboxymethylcellulose, cornstarch, malt, and agar. The hydrogel can be supplemented with sawdust to 3D print mycelium biocomposites. Results demonstrate that engineered living materials in the form of mycelium-infused food grade hydrogels and mycelium-infused biocomposites can be successfully 3D printed at scales of ~50 mm in non-sterile environments.

**References:**

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**Further reading:** Lin, Taghizadehmakoei, Polovina, McLean, Sanata-Martinez, Naese, Moraes, Hallam, Dahmen. 3D Bioprinting of Food Grade Hydrogel Infused with Living *Pleurotus ostreatus* Mycelium in Non-sterile Conditions *ACS Applied Bio Materials* 2024 <https://pubs.acs.org/doi/10.1021/acsabm.4c00048>