Production of Micellar Structures From Medicinal Mushrooms

Nika Kučuk, Mateja Primožič, Željko Knez, Maja Leitgeb

University of Maribor, Faculty of Chemistry and Chemical Engineering, Laboratory of Separation Processes and Product Design, Maribor, Slovenia
nika.kucuk@um.si, mateja.primozic@um.si, zeljko.knez@um.si, maja.leitgeb@um.si

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Fungal micellar membranes, which consist of cellulose, chitin, and proteins, are one of the important and largest groups of microorganisms. Micellar structures are promising biological materials with great advantages, because they can be adapted very well to different cultivation parameters, are biodegradable and their production is relatively inexpensive. Their fibrous structure makes them very promising for biotechnological and cosmetic applications, but also for various industries such as packaging and construction. The therapeutic mushrooms Ganoderma lucidum and Pleurotus ostreatus were used for the production of micellar membranes. In addition, the morphological, chemical, and hydrodynamic properties were also investigated. Micellar membranes were successfully obtained from both therapeutic mushrooms. By optimizing the growth parameters, it was possible to achieve the highest yield and the highest water absorption capacity of the micellar membranes with advantageous characteristics.

Keywords:
micellar structures, medicinal mushrooms, *Ganoderma lucidum*, *Pleurotus ostreatus*, characterization

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1 Introduction

In recent years, the preparation of smart biological materials, sustainable and environmentally friendly products for biomedical applications has become increasingly important (Silva et al. 2021). Various natural, sustainable resources, such as lignin, cellulose, pectin, protein products, etc., are the most important resources for the production of biopolymeric materials. These are environmentally friendly, biodegradable and renewable materials, with many other important characteristics (Baranwal et al. 2022). However, the main problems in obtaining these biopolymer-based materials are the high costs of the development process and purification. Furthermore, production must be accompanied by a high yield of products (Perera et al. 2023).

Therefore, the synthesis of polymeric vital substances (carbohydrates) from biological resources such as fungi (mushrooms) represents an innovative approach, as it is simple, economical, high-yielding and less time-consuming (Martinez-Medina et al. 2021; Sivaprasad et al. 2021).

The micellar structures originating from fungi, which consist mainly of cellulose, chitin and proteins, represent one of the largest groups of microorganisms (Manan et al. 2021). The mycelium is the vegetative part of the fungus and consists of a large group of interwoven hyphae. Micellar structures are important biological materials with many advantages. They can adapt to different growth conditions, are biodegradable and their production is associated with low costs (Majib et al. 2023). Their fibrous structure makes them unique and promising for use in various biological fields. A major advantage of using micellar films over bacterial cellulose membranes is the final purification process, as this step only requires heat treatment of the film (Antinori et al. 2021; Haneef et al. 2017).

Micellar membranes based on medicinal mushrooms are fibrous and self-growing polymeric biocomposites with acceptable properties. They have the ability to strongly mimic the extracellular matrix of human tissue. Micellar structures are potential bioscaffolds, which is why their use in tissue engineering is particularly promising (Alaneme et al. 2023; Khamrai et al. 2018). The desired properties of coatings or wound healing patches include protection of the wound by limiting the loss of body fluid, reduction of water loss from the patches, compatibility with tissue,

and high mechanical strength, which can be achieved by a film of micellar structures (Verma et al. 2023).

The aim of the study was to obtain micellar structures from *Ganoderma lucidum* and *Pleurotus ostreatus*, which are important representatives of medicinal mushrooms. In order to achieve the highest yield of micellar structures and the maximum water absorption capacity, various growth parameters were optimized. A scanning electron microscopy (SEM) analysis was performed to determine the morphological characteristics of the micellar structures and to measure the diameter of the pores and hyphae. Fourier transform infrared spectroscopy (FT-IR) was used to investigate the presence of chemical functional groups on the surface of micellar membranes. In addition, the hydrophilicity/hydrophobicity of the surface of the membranes was evaluated by determining the contact angle. The kinetics of swelling were also investigated. Figure 1 presents the experimental design of the study.

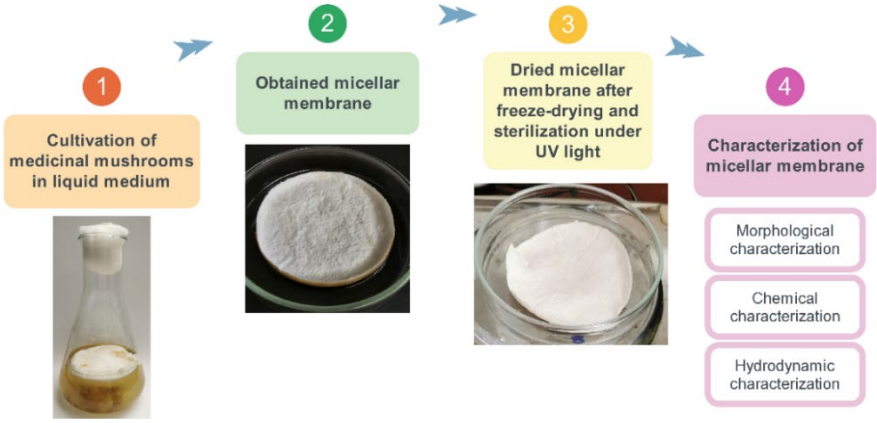

Figure 1: Experimental design
Source: own.

2 Materials and methods

2.1 Preparation of mycelium membranes

The freshly prepared fungal culture of *P. ostreatus* and *G. lucidum* was transferred to the liquid growth medium in the Erlenmeyer flasks and incubated at 27 °C under static conditions. After complete growth, the resulting micellar membranes were

removed from the culture medium and purified by subsequent washing with deionized water. The micellar membranes were subjected to a freeze-drying process. They were then sterilized under UV light.

2.2 Water uptake capacity

To determine the water uptake capacity, the micellar membranes were cut into pieces of 10 mm diameter. The dry membranes were first weighed and then immersed in deionized water at room temperature. The membrane pieces were removed from the water and placed on blotting filter paper to remove the excess, unabsorbed water molecules on the membrane surface. The percentage of swelling was calculated using Equation 1:

$$\%\ of\ swelling = \frac{w_{wet} - w_{dry}}{w_{dry}} \cdot 100 \quad (1)$$

where w_{wet} represents the weight of wet micellar membranes, and w_{dry} represents the weight of dry micellar membranes.

2.3 Scanning Electron Microscopy Analysis

SEM analysis was performed using a scanning electron microscope (FE, SEM SIRION, 400 NC, and FEI) to examine the morphology of the dried mycelial membranes. The diameter of the hyphae and the diameter of the pores in the membranes were determined. Before analysis, the membranes were coated with a layer of gold.

2.4 Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR analysis was performed to determine the presence of chemical functionalities on the surface of the obtained micellar membranes. The spectra were detected over a 4000–500 cm−1 range and recorded by the FT-IR spectrophotometer (Perkin Elmer 1600 Fourier transform infrared spectroscopy).

2.5 Determination of hydrophobicity/hydrophilicity of membranes

The hydrophobicity or hydrophilicity of the surface of the micellar membranes was determined by measuring the contact angle with a digital camera Basler Aca1300-200um connected to a computer with a CCTV lens (Tamron, Japan) using the Open Drop algorithm. The membrane samples were illuminated, then the water absorption in the membrane was recorded with a camera. The contact angle was measured using the ImageJ program.

3 Results and discussion

Micellar membranes were successfully prepared from the two selected therapeutic mushrooms *P. ostreatus* and *G. lucidum*. After harvesting, the mycelium was completely inactivated to stop its growth, one of the most important properties for possible use as a potential bio-scaffold. Freeze-dried mycelial membranes were sterilized under UV light. The mycelial membranes were spread on potato dextrose agar (PDA) plates and did not regrow. Both fungal strains appeared inactive after UV light treatment.

The maximum yield of micellar membranes with favorable properties and the highest water uptake capacity was achieved by the combination of selected cultivation parameters. The best characteristics for micellar membranes of *G. lucidum* were obtained with malt extract medium and of *P. ostreatus* with glucose medium. The composition of the optimal media (malt extract medium and the glucose medium) is shown in Table 1. Complete growth of the mycelial membranes of *G. lucidum* and *P. ostreatus* was achieved after 14 days and 21 days at a constant temperature of 27 °C under static conditions.

Table 1: The composition of the optimal medium.

Growth medium	Chemical	Concentration (g/L)
Malt extract medium	Malt extract	10.00
	Yeast extract	4.00
Glucose medium	Glucose	49.20
	Yeast extract	4.90
	KH_2PO_4	0.88
	$MgSO_4 \cdot 7\ H_2O$	0.50

The average diameter of the mycelial membranes obtained was measured with a beak scale. In general, the membranes of *G. lucidum* were slightly heavier and thicker than those of *P. ostreatus*.

SEM examinations determined the average diameters of hyphae and pores. The micellar structures of *P. ostreatus* resulted in a larger average diameter of the hyphae. However, the membranes of *G. lucidum* were found to have a larger average pore diameter.

Using FT-IR analysis, no significant differences in the chemical functionalities of the micellar structures were detected when different liquid growth media were used.

Table 2 presents characteristics of the obtained micellar membranes grown in the optimal medium for both medicinal mushrooms used.

Table 2: The characteristics of the obtained micellar membranes grown in the optimal medium for the individual medicinal mushroom.

Characteristics	*G. lucidum*	*P. ostreatus*
Average diameter of the membrane (mm)	0.94	0.77
Average diameter of the hyphae (μm)	0.43	1.31
Average diameter of the pores in the membrane (μm)	40.30	25.10
Hydrophilicity/ hydrophobicity	Hydrophobic surface	Hydrophilic surface
Present functional groups	O-H stretching, CH_2 asymmetric stretching, CH_2 symmetric stretching, amide I (β-sheet), amide II, C-H bending, PO_2^- asymmetric stretching, C-C stretching + C-O stretching + C-H deformation, C-OH stretching, C-O stretching, C-C stretching, glucan β-anomer C-H bending	

The water absorption capacity of the membranes of the two medicinal mushrooms was also investigated. The results of the time-dependent swelling profile are shown in Figure 2.

The time-dependent swelling profile for the micellar membranes from both selected therapeutic mushrooms was comparable. However, the micellar membranes of P. ostreatus were found to have a higher water uptake capacity (> 600 %) than the membranes of G. lucidum (500 %) after 24 hours.

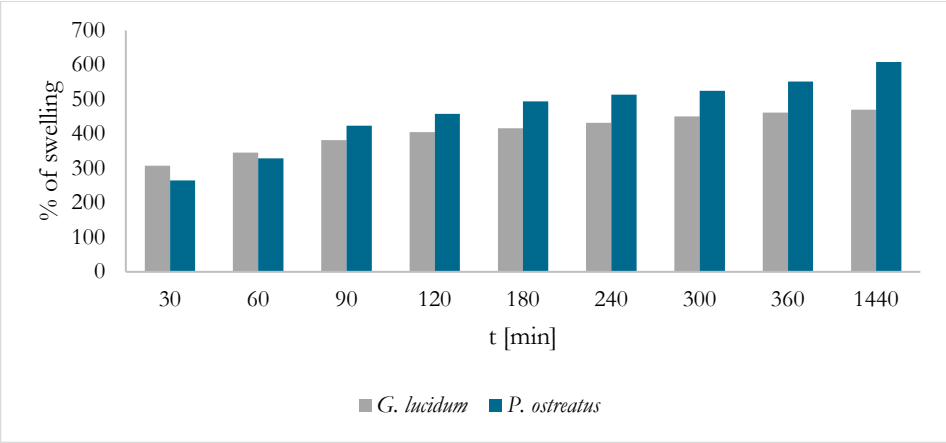

Figure 2: Percentage of swelling of micellar membranes.
Source: own.

4 Conclusion

Micellar membranes from therapeutic fungi are self-growing biocomposites with suitable characteristics. The micellar structures obtained from therapeutic mushrooms are promising fibrous, self-growing polymeric biocomposites. They have favorable properties for potential use in various industries such as packaging and construction. In addition, micellar structures can also be functionalized with different bioactive compounds, which can be successfully used for various cosmetic and biomedical applications, as they mimic the extracellular matrix of human body tissue.

They are a particularly promising platform for tissue engineering applications, especially for wound healing and innovative skin materials. To the best of our knowledge, only mycelium extracts and their derivatives have been used and tested for various applications, e.g. in cosmetics and biomedicine, but not the membranes themselves as functional materials.

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