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Bacterial Filtration Abilities of Mycelium as a New Gauze Substitute: A Research Protocol

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Abstract

Introduction: Climate change is escalating the demand for wound dressings which are produced from non-sustainable materials such as cotton and synthetic fibres. Mycelium presents a promising biodegradable and renewable substitute for gauze material, which could help reduce the negative impact of the medical textile industry on the environment. Mycelium extracted from *Ganoderma tsugae* exhibits healing properties for the skin, making it an appropriate gauze, but its ability to prevent bacterial infections is not well understood. This study seeks to experimentally investigate the ability of mycelium to filter bacteria from infecting third-degree burn wounds in comparison to a woven cotton gauze and a non-woven Acticoat gauze. No gauze, loosely woven gauze and impermeable gauze will be used as controls. It is hypothesized that a mycelium dressing would significantly affect the bacterial filtration efficiency because previous studies have shown mycelia to be effective at air filtration.

Methods: The mycelium gauze will be made from the mycelium root network of the fungus *Ganoderma tsugae*. Third-degree burns will be introduced on mice followed by an assessment of the bacterial filtration efficiency of each dressing type. During a 28-day period, bacterial samples from the wound will be obtained and plated for cell counting analysis. Wound healing abilities and gauze porosities will also be measured.

Results: It is anticipated that the mycelium gauze will result in the highest bacterial filtration efficiency due to the material's filtration ability and tissue repair mechanisms. It is also expected that the mycelium gauze will result in improved wound healing as it promotes cell growth.

Discussion: ANOVA will be used for a comparative assessment of bacterial filtration efficiencies among the different gauze types to indicate significant differences.

Conclusion: The findings from this research could serve as the foundation for potential mycelium gauzes with enhanced functionality in hopes of contributing to the advancement of environmentally conscious medical textiles.

Keywords: mycelium; *Ganoderma tsugae*; bacterial filtration; gauze; medical textile; murine model; biomedical technology; biodegradable; sustainability; burn wounds

Introduction

With the increasing severity of climate change, there is an unprecedented risk of natural disasters, including wildfires, which drives an increase in the demand for burn dressings such as medical gauzes [1]. Unfortunately, gauze production is heavily dependent on non-sustainable materials such as cotton and synthetic fibers for woven gauzes and non-woven gauzes respectively [2]. Worldwide cotton production consumes 256 Gm [3] of water annually, depleting global water sources. This is best seen in the Aral Sea, which has lost 80% of its volume in 40 years in order to grow cotton in the desert [3]. Furthermore, increased gauze usage will lead to surges in disposable medical waste which already accounts for 5.9 million tons annually [4]. In terms of efficacy, woven gauzes are also known to shed fibres and release lint into the wound, causing foreign body reactions such as granulomas or adhesions [2]. According to a clinical study on peritoneal cavity granulomas, gauze was deemed the causative agent in approximately 71.9% of cases [5]. Consequently, there is an urgent need to explore and develop sustainable, yet effective alternative materials in response to the growing demand for medical textiles while mitigating environmental impacts.

Mycelium, often found in different species of fungi, is a renewable and biodegradable biomaterial that could potentially substitute current gauze materials given its structure [6]. Mycelium is a vegetative root network of branching elongated cells called hyphae present in some fungal species such as *Ganoderma tsugae*. The mycelium

complex is a porous structure consisting of proteins and cross-linkages between chitin and glucans, which allow this material to be strong and rigid [7, 8].

Mycelium composites can be produced from any raw material that can maintain fungal growth, such as agricultural or forestry waste, thus lowering the engineering cost and energy input while promoting waste upcycling [6]. The ability to obtain mycelium composites through low-cost engineering highlights the sustainability of using mycelium and mycelium composites as an alternative to traditional gauze materials. Furthermore, previous research has found that mycelium-made packaging is entirely compostable and can biodegrade within 40 days, hence making it an ideal biomaterial [9].

Additionally, previous studies suggest that the mycelia of *Ganoderma lucidum* and *Pleurotus ostreatus* could act as a skin scaffold to support the regeneration of tissues. The mycelia from these species have been found to exhibit similarities to the extracellular matrix of human tissues, yielding favourable results for biocompatibility. In terms of epidermal growth and wound healing, chitin and other polysaccharides found in fungal mycelium may provide biochemical guidance for cell migration and improve the growth of fibroblasts and keratinocytes. Moreover, mycelium-based materials have the advantage of being customizable, with alterable chemical composition and mechanical properties based on varying fungal strains and growth substrates. This study thus suggests that mycelium's abilities as a self-growing, customizable, bioactive composite makes it a promising scaffold material [10]. With the shared goal of promoting wound healing, these outlined characteristics would also be beneficial in a gauze material.

Ganoderma tsugae and *Ganoderma lucidum* are phylogenetically related species of fungi, both falling under the same genus, *Ganoderma*. Various botanical sources, including *Ganoderma*, are comprised of polysaccharides such as β-1, 3-D-glucan [11]. As a specific enantiomer of β-1, 3-glucans, this polysaccharide is recognized by Langerin receptors expressed on the surfaces of Langerhans cells — resident macrophages of the skin that recognizes and internalizes foreign antigens [11, 12]. Upon activation, Langerhans cells secrete factors including the epidermal growth factor, promoting cell growth. Additionally, the presence of chitin found in the cell wall of fungi contributes to its high affinity for skin while ensuring regular skin proliferation and function — possibly having significant effects in the application of mycelium as a gauze substitute [11]. Moreover, glucans derived from oats and barley have applications as a skin emollient, and glucans found in mycelium may have similar effects which could be an additional benefit as a gauze material [11].

Although previous studies have shown mycelia to be effective at air filtration of NaCl particles in the context of a mask application [13], current research lacks insight on its bacterial filtration abilities, its reaction to severe burns, or its function on live models. Hence, this proposal attempts to investigate these gaps in research and further explore the feasibility of a mycelium-based gauze alternative and its effectiveness in preventing bacteria from infecting burn wounds in live mice models. Additional factors such as wound healing, gauze longevity, and gauze porosity will also be investigated throughout this proposal.

Methods

Mycelium Gauze Preparation

The fungi, *Ganoderma tsugae*, will be used to produce the mycelium gauze, as adapted from Su's procedure which has successfully produced mycelium-adjacent membranes using the fruiting bodies of *Ganoderma tsugae* [11]. The mycelium root network of *Ganoderma tsugae* will be neutralized and deacetylated using a solution of 45% NaOH at a temperature of 85°C for 24 hours. The washed solution will then be freeze-dried to preserve the original structure of the mycelia. The freeze-dried solution will then be sieved, resulting in fibres between 10 to 50 µm in length and 2 µm in diameter that are suspended in deionized water. This will further be autoclaved, filtered, and freeze-dried under aseptic conditions to form the final porous membrane, which will act as the mycelium gauze in this study.

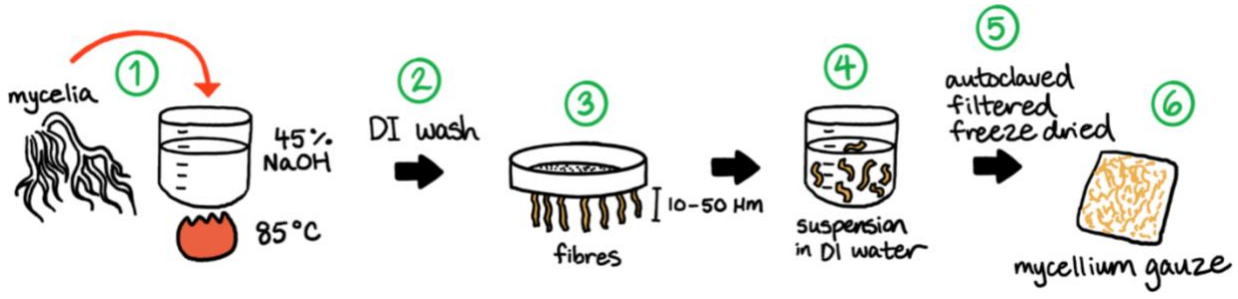

Figure 1. Illustrates the preparation of a mycelium gauze following Su's procedure, forming a porous membrane structure that will be cut into smaller pieces before gauze application. Figure made using Procreate.

Mice Care

Female and male BALB/cArc mice of 6-8 weeks, weighing 25 ± 5 g, sourced from the Ozgene mouse line will be used as an animal model to assess the effectiveness of mycelium gauze following a third-degree burn wound. Regulation of these parameters would limit data variance due to physical differences among individual mice. Mice serve as a representative animal model as they possess the major skin layers of a human (endoderm, mesoderm and ectoderm), allowing third-degree burns to be mimicked in vivo. Furthermore, third-degree burns require an extended healing period, providing an ample time frame to observe and collect data for further analysis. Recommended living conditions, following guidelines set by the Canadian Council on Animal Care, will be kept constant for all mice to minimize the effect of environmental differences on data and abide by animal ethic regulations [14].

Anaesthesia and Burning

Analgesia (buprenorphine, 0.05 mg/kg) and anaesthesia (Ketamine, 75 mg/kg and Xylazine, 0.5 mg/kg) will be administered intraperitoneally [15]. Additionally, 1 mL of saline is injected along the dorsum to protect the spinal cord from possible injuries. Unconsciousness will be confirmed by occasional firm mouse foot pinches. Following confirmed unconsciousness, a 2 cm x 2 cm square mark on the dorsum of each mouse, representing approximately 25% of the total body surface, will be razor-shaved to allow for even burning. The dorsum serves as an ideal area to introduce wounds as it is a difficult area for mice to reach, reducing the chances of scratching or injury [15]. The shaved area will be cleaned with an alcohol wipe prior to burning to ensure a sterile wound site. The shaved area will then be submerged in 100℃ boiling water for 10 seconds to produce the third-degree burn. Constant temperature and period of submersion will allow for control over the depth of the wound for a consistent severity of burning. The mice will be transferred into separate cages with post-surgery monitoring for 14 days following animal care guidelines set by the CCAC.

Figure 2. Illustrates anaesthesia and burning protocol introducing a third-degree burn in the dorsum of mice. Figure made using Procreate.

Gauze Application

Three experimental groups (cotton gauze, Acticoat gauze, and the mycelium gauze) and three control groups (impermeable gauze, loosely woven gauze, and no gauze) will be studied, with 6 mice randomly assigned to each group. Cotton and Acticoat gauzes will be used as comparisons to the mycelium gauze, representing woven and non-woven gauzes respectively. In the cotton gauze condition, the widely-used commercial 2" x 2" Medline Sterile 100% Cotton Woven Gauze Sponges will be used. In the Acticoat gauze condition, the commercial 2" x 2" ACTICOAT Silver Antimicrobial Barrier Dressing manufactured by Smith & Nephew and supplied by Medline will be applied. In the mycelium gauze condition, the synthesized mycelium gauze following the Mycelium Gauze Preparation protocol outlined above, will be utilized. The positive control condition will utilize the Transeal Transparent Dressing, manufactured by DEROYAL and supplied by Medline, to serve as an impermeable gauze to bacteria. The incorporation of this positive control group standardizes the experimental procedure, as the bacterial filtration efficiency is known to be high. Additionally, Kerlix Gauze Bandage Rolls, manufactured by Cardinal Health and supplied by Medline, will serve as the loosely woven gauze for the negative control. The negative control will standardize the experimental procedure as it is known to exhibit a low bacterial filtration ability. An additional negative control group of no gauze will represent the bacterial filtration efficiency in the absence of a physical barrier. All experimental and control gauze conditions outlined above will involve the adherence of appropriately-cut sterile gauzes of size 2 cm by 2 cm onto the shaved dorsum of the mice following third-degree burns.

Porosity Tests

To further examine the potential effects of gauze porosity on bacterial filtration efficiency, prior to gauze application, tests of porosity will be conducted for the different gauzes applied across all treatments. Measurement of porosity will be calculated using the porosity formula for polyurethane (PU) dressings:

$$p = \left(1 - \frac{m}{\rho_{polymer} \times v}\right) \times 100\%$$

where p, m, $\rho_{polymer}$, and V indicates porosity, foam mass, true density of the polymer, and volume [16]. Polyurethane

is used as a synthetic dressing and scaffold material similar to gauze, making it an appropriate method in determining the porosity of traditional and non-traditional gauzes.

Infection Protocol

Since gram-positive bacteria such as *Staphylococcus aureus* commonly colonize burn wounds and cause complications in terms of wound closure [17], this bacterial species will be used in the wound infection protocol. A bacterial solution of *S. aureus* strain 8325-4 will be grown in brain-heart infusion broth at 37°C until it reaches a concentration of 10^8 CFU/mL (OD=0.6-0.8 at 600 nm) during the mid-log growth phase. Using phosphate-buffered saline solution (PBS), the bacteria will be serially diluted 10-fold.

After the initial gauze application, the exterior of the gauze will be inoculated with 50 µL of the diluted bacterial sample. In the case of the no gauze control, the bare wound would be inoculated with 50 µL of the same bacterial sample. The gauze or wound site will be re-inoculated with the same bacterial solution every day.

Bacterial Sampling

To determine the number of colony forming units (CFU), bacterial samples will be collected every day for a 28-day period. The initial bacterial count will be derived from swabbing the wound 15 minutes after the burn procedure. Otherwise, once a day following a 24-hour period after inoculating the gauze, the gauze will be removed, a sterile swab will be rotated using the Levine swab technique across the centre of the shaved burn area, and the same gauze will be reapplied [18]. This gauze will be replaced with a new gauze every 7 days. Prior to re-inoculation but after swabbing the wound to count colonies, the wound will be gently cleaned with soap and water. At this point, another swab of the wound will be taken to establish a baseline number of CFU before bacterial inoculation.

The Levine swab technique will be used for all mice to reduce differences in bacteria count due to swabbing. This method has been observed to be more effective than other swabbing techniques for acute wounds [19]. The swab will be placed in a 10.0 mL neutralizing buffer and a serial dilution to 10^{-3} will be performed. 1.0 mL of the solution will then be plated on an agar plate and incubated at 37°C for 24 hours, assuming all the bacteria will grow at this temperature [20, 21]. Plating provides a quantitative indicator of the amount of bacteria in the wound.

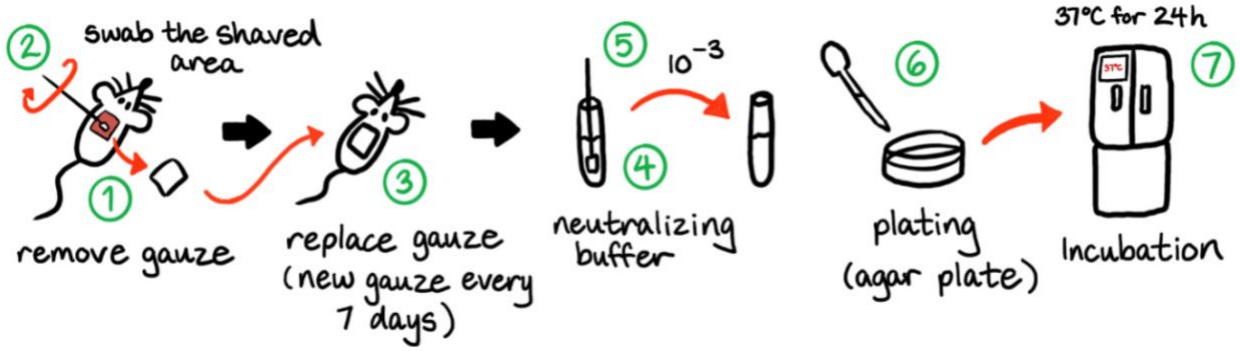

Figure 3. Illustrates colony counting procedure involving serial dilutions to 10^{-3}, agar plating and 37°C incubation. Figure made using Procreate.

Wound Healing

For each day, wound healing for each individual mouse will be documented using macrophotography. Wound size will be measured using a metric ruler, and wound area calculations and colour analysis will be performed using ImageJ software [22]. Wound morphology (colour, signs of inflammation, scabbing, signs of infection, etc) will be observed.

Analysis

Daily bacterial colony counts (CFU counts) of individual mice from experimental (cotton, mycelium, Acticoat) and control groups will be conducted and averaged to determine the mean initial and final CFU counts per day for each treatment group. Initial and final CFU counts per day will be paired to determine the bacterial filtration efficiency of each day for all treatments by applying the formula:

$$\%efficiency = \frac{final - initial}{initial} \times 100\%$$

The average of the bacterial filtration efficiencies will be taken across 7 days for each treatment group to serve as a comparison with other treatment and control groups. Furthermore, to conduct a wear test for each gauze type, the bacterial filtration efficiency for each day within the 7-day period (across all 4 weeks of the study) will be averaged. Day-to-day differences will be examined to determine the effect of time on the gauze's bacterial filtration efficiency. For instance, the bacteria filtration efficiency of the first day of each of the four weeks will be averaged and will be compared with the second day average (across the 4 weeks)

for each treatment group.

Potential correlations between the level of porosity and bacterial filtration efficiencies will be considered by comparing the porosity of each gauze type with the mean bacterial filtration efficiency of each gauze type determined over the 4-week period (28 days). Wound healing efficiency will be quantified by the number of days it takes for the wound to completely heal, as seen by a wound area of 0. If the wound is not entirely healed by the end of the 28 days, the difference between the wound area on day 28 and the wound area on day 1 will be calculated to measure healing.

Evaluation of significant differences between datasets will be conducted using a one-way Analysis of Variance (ANOVA) test. ANOVA will determine if the differences are due to random error by comparing it to an appropriate critical value (α-value), and comparing the mean bacterial filtration efficiencies between the groups. ANOVA may be followed with confidence intervals or a post-hoc test to determine the group that is statistically different for further analysis.

Results

The use of traditional, impermeable, and mycelium gauze is expected to exhibit an increased mean bacterial filtration efficiency when compared to the negative control groups of loosely woven gauze and no gauze. However, based on the differences in the physical composition between traditional and mycelium gauzes, it would be unlikely that these gauzes will have equal bacterial filtration efficiencies. It is anticipated that if a mycelium dressing is used, then the mean bacterial filtration efficiency will be higher than traditional gauze groups (cotton, Acticoat), with ANOVA indicating it as a statistically significant difference when compared to the critical α-value. Statistically significant differences between treatment groups may be correlated to the porosities of the different gauze types, where it is predicted that lower porosity values suggest a higher bacterial filtration efficiency.

It is expected that the bacterial filtration abilities of the gauzes will decrease as the time using the same gauze increases. By collecting bacterial counts for the same gauze over 7 days, changes in CFU can indicate changes in gauze performance as it ages, acting as a wear test to determine the longevity of each gauze type. In terms of wound healing, it is expected that the mycelium gauze will confer additional therapeutic benefits and would consequently perform better than other gauze types. This is expected to be demonstrated through shorter healing times, decreased wound area, and decreased visual inflammation.

Discussion

This proposal mainly aims to determine the differences in bacterial filtration abilities of mycelium-based gauzes and traditional gauzes through the assessment of bacterial filtration efficiency throughout a 28-day period. A higher mean bacterial filtration efficiency in mycelium-based gauzes when compared to traditional and control groups would suggest its potential in preventing bacterial infections. This anticipated result is supported by previous studies that have explored the enhanced filtration abilities of mycelium-based materials, particularly in terms of their increased abilities to entrap NaCl particles [13]. It was found that mycelium with a small diameter, when layered on top of one another, can create small pores for improved physical filtration. It is anticipated that the enhanced physical filtration given by the small diameter of mycelium fibres used in this study will increase bacterial filtration efficiency. Additionally, a statistically significant difference between the mycelium-based gauze and traditional gauze groups, if suggested by ANOVA, is an indication that the difference in mean colony counts can be attributed to the efficacy of using mycelium as a gauze material.

Conclusions

Wound dressings such as gauzes are essential products in medical industries, which reinforces the importance of discovering sustainable production methods for these products. As a biodegradable material with promising filtration abilities, mycelium might serve as a potential gauze substitute. The primary purpose of this proposal is to determine whether mycelium gauze made from *Ganoderma tsugae* is effective at filtering bacteria to further explore the possibility of mycelia-based gauze substitutes in the medical textile industry by measuring the mean bacterial filtration efficiencies between the control groups, traditional gauzes and mycelium-based gauzes.

A potential limitation to this proposal lies in the different immune and inflammatory responses observed between animal models and humans, potentially leading to inaccurate predictions of the effect of mycelium-based gauzes on bacterial filtration efficiencies in humans. Additionally, the use of in vivo models makes it difficult to control individual variations, such as healing efficiencies. Those with a higher healing efficiency will have an increased reduction of the wound area, hence affecting bacterial colony counts, and potentially complicating the collection and interpretation of results. Future research should consider the effect of *Ganoderma tsugae*-based gauzes on healing efficiencies through histopathological analyses and immunoassays for inflammation markers, provided its suggested role in activating an immune response [11]. Additionally, future research should also consider temperature resistance, lint release, inflammatory pathway response, longevity, and absorption of mycelium gauzes.

With the increasing burden of medical waste, it is more important, now than ever, to invest research into developing sustainable materials. If this study is able to find that mycelium gauzes have similar or superior bacterial filtration when compared to current gauze products, we would be able to further develop a greener material for this crucial medical product and contribute to climate change mitigation efforts.

Conflicts of Interest
The author(s) declare that they have no conflict of interests.

Ethics Approval and/or Participant Consent
This study did not require ethics approval and/or participant consent since it is a hypothetical proposal for a study.

Authors' Contributions
AS: made substantial contributions to the design of the study, drafted the manuscript, illustrated the figures, and gave final approval of the version to be published.
LZ: made substantial contributions to the design of the study, drafted the manuscript, and gave final approval of the version to be published.
YJ: made substantial contributions to the design of the study, drafted the manuscript, and gave final approval of the version to be published.
CC: made substantial contributions to the design of the study, drafted the manuscript, and gave final approval of the version to be published.

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