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Construction of Metal Ion Composite Materials and Their Autophagy Activation to Control the Growth of Malassezia on the Skin

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Abstract:

Malassezia can cause various skin diseases, and even lead to more severe problems for specific populations such as immunocompromised children and adults, people with high sebum secretion, and patients with gastrointestinal diseases. In recent years, a plenty of research has indicated that traditional antifungal treatment methods may lead to drug resistance problems. Due to the traditional methods causing this problem, there is an urgent need for new methods to treat *Malassezia* induced diseases. Metal ions have been utilized in biology, chemistry, and medicine. Existing studies have shown that certain metal ions have bactericidal effects, requiring specific metal ions to be selected for different situations. Here, a method to activate autophagy through copper and iron ions has been proposed. This study focuses on the construction of novel metal ion-conforming materials and their role in activating autophagy in *Malassezia*, developing composite materials based on metal ions to control the growth of *Malassezia*.

Keywords: *Malassezia*, Autophagy, Transition metal, Cuproptosis, Ferroptosis

1. Introduction

The yeast of the *Malassezia* genus is a major eukaryotic member of the skin microbiota and can trigger various skin diseases. Considering the entirety of human skin, *Malassezia* is enriched in sebaceous sites which contain abundant lipids and are often lipid-dependent such as face, scalp, and upper back. (Nowicka & Nawrot, 2019; Ianiri, LeibundGut-Landmann, & Dawson, 2022; Yang, Cho, Lee & Kim, 2023). For specific populations, such as individuals with weakened immune systems, *Malassezia* infections can result in more severe health issues (Ianiri et al., 2022). However, traditional anti-fungal therapies for *Malassezia* may cause limitations and problems like drug resistance, thus posing potential danger to human (Robbins, Caplan & Cowen, 2017; Gamaletsou, Walsh, & Sipsas, 2018; Fisher et al., 2022; Puumala, Fallah, Robbins, & Cowen, 2024). Therefore, finding new treatment methods is crucial for controlling *Malassezia* infections.

Cell death is important in various aspects of mammalian development, homeostasis, and diseases, tightly intertwined with other biological processes. There is a mutual regulatory and synergistic relationship between cell death and autophagy, and they may also affect each other's activity. This complex interaction relationship is of great significance for maintaining cellular homeostasis, resisting external pressure, and preventing the occurrence of diseases. The autophagy process involves a series of evolving autophagic structures. After induction, isolation membranes form within the cell and aggregate with engulfed components needing degradation. The isolation membrane extends, enveloping and enclosing cytoplasmic components to form a double-membrane structure called an autophagosome. The autophagosome fuses directly with a lysosome, forming an autolysosome, or firstly fuses with an endosome to form an autophagic endosome before fusing with a lysosome, with the enclosed cytoplasmic components ultimately degraded and utilized by the lysosome (Glick, Barth & Macleod, 2010; He & Klionsky, 2009; Rodolfo, Di Bartolomeo & Cecconi, 2016). The four functional protein groups involved in autophagosome formation have both conservative and non-conservative components, indicating the plasticity of autophagosome formation in fungi (Wang et al., 2019). By activating the autophagy mechanism within cells, the ability to eliminate fungi like *Malassezia* can be enhanced, reducing the risk of infection.

Research has shown that certain metal ions, such as silver, copper, and iron ions, possess antibacterial properties, capable of inhibiting the growth of bacteria and fungi (Godoy-Gallardo et al., 2021). In recent years, various metal ions and metal nanoparticles have been incorporated into biomaterials for altering their physicochemical properties and providing important antibacterial capabilities to the material. Metal ion composite materials are widely used in biomedical applications, including tissue engineering, disease treatment, and drug delivery. Therefore, incorporating metal ions into composite materials

to achieve antimicrobial effects is a promising research direction. Many transition metal-based compounds have been proven to be capable for modulating autophagy (Luo, Fu, Huang & Li, 2021; Feng et al., 2020). Ferroptosis is an iron-dependent cell death program triggered by dysregulation in the redox mechanism (ROS), ultimately leading to extensive peroxidation of polyunsaturated phospholipids (Dixon et al., 2012; Jiang, Stockwell & Conrad, 2021; Stockwell et al., 2017). Copper cell apoptosis is caused by the excessive accumulation of copper ions, which will cause abnormal aggregation of sulfur-containing proteins, the disruption of mitochondrial respiratory iron-sulfur cluster proteins and the toxic stress responses of protein, ultimately resulting in cell death. Cell death caused by copper ion carriers is a form of cell death induced by excessive accumulation (Tsvetkov et al., 2022; Xue et al., 2023; Chen et al., 2022; Shende, Bhagat, Raut, Rai, & Gade, 2021; Kawakami, Inagawa, Hosokawa, Saito, & Kurasaki, 2008). The relationship between copper and autophagy can be of great significance in the improvement of cardiovascular disease, acute renal injury, and other diseases (Bravo-San Pedro et al., 2017; Karginova et al., 2019). The use of iron induced cell death plays an important role in cardiovascular disease (Wang et al. 2022).

This study aims to construct skin-adapted metal ion composite materials to enhance their activation of autophagy in *Malassezia* and strengthen their antimicrobial effects. Study the activation of autophagy in *Malassezia* by metal ions, investigate the in vitro control effect of ions on *Malassezia*, and explore the mechanism of protein leakage.

2. Materials and methods

2.1 Reagents and materials

Take 0.1 ml of the sample liquid and add it to 9 ml of sterile water, mix well to make a 10^{-1} solution; then take 1 ml of the 10^{-1} solution and add it to 9 ml of sterile water, mix well to make a 10^{-2} solution, and so on to create a 10^{-x} solution. The 0 mM and 16 mM Fe^{2+}/Cu^{2+} treatment components were diluted to 10^{-6} and 10^{-5}, respectively.

2.2 Strains and growth conditions

Prepare the solid and liquid culture media needed to culture *Malassezia*. The medium used for *Malassezia* is YM medium with the following composition: yeast extract: 0 g/L; malt extract: 3.0 g/L; peptone: 5.0 g/L; glucose: 10.0 g/L; agar: 20.0 g/L. Prepare 4 bottles of medium (each 100 ml), with 2 bottles containing agar as solid culture medium and the remaining two bottles as liquid culture medium.

2.3 Ions treatment

Transfer the *Malassezia* in the logarithmic growth phase to centrifuge tubes and centrifuge (5000 rpm, 5 min) (centrifuge 64 tubes), discard the supernatant, and treat the *Malassezia* with concentrations of 0 mM and 16 mM Fe^{2+}/Cu^{2+}, for 2 and 5 hours, respectively.

2.4 Malassezia protein leakage determination

After centrifuging the bacterial suspension, collect the supernatant in separate large brown centrifuge tubes (8000 rpm, 10 min). Measure the absorbance of the supernatant at 280 nm using a spectrophotometer (UV-1100, MAPADA, Shanghai, China) to indicate the leakage of nucleic acids.

2.5 qRT-PCR detection of autophagy gene changes in Malassezia under 0 and 16mol copper ion treatment conditions

Using the TransStart®° Top Green qPCR SuperMix kit (Beijing TsingKe Biotech), 1 μg of total RNA was reverse transcribed into its complementary DNA (cDNA). Real-time fluorescence quantitative PCR analysis was then performed using a real-time PCR machine (Life Technologies ABI, QuantStudio3). The expression levels of the target genes were evaluated and normalized to the expression level of the internal control gene 26S rRNA. The quantification of target gene expression was based on the cycle threshold for each sample, which was calculated by averaging three replicate measurements.

3. Results and discussion

3.1 The effect of copper ions and iron ions on the number of colonies.

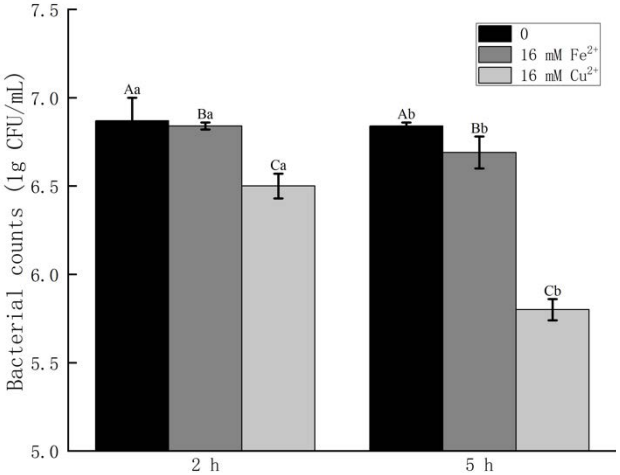

Fig. 1. The effect of copper ions and iron ions on the number of colonies.

After treatment with 16 mM Cu^{2+} and 16 mM Fe^{2+}, the

number of colonies decreased (Fig. 1). Compared to the control group, the addition of 16 mM Cu^{2+} resulted in a significant reduction in colony count. The decrease in colony count was less pronounced after the addition of 16 mM Fe^{2+}. The result indicates that both 16 mM Cu^{2+} and 16 mM Fe^{2+} exhibit bactericidal effects. Under the same conditions and time frame, 16 mM Cu^{2+} shows a better bactericidal effect. The number of colonies decreases over time after treatment with 16 mM Cu^{2+} and 16 mM Fe^{2+}, indicating that within the 2 to 5-hour range, both 16 mM Cu^{2+} and 16 mM Fe^{2+} have bactericidal effects, with the bactericidal effect of 16 mM Cu^{2+} becoming more pronounced as time progresses.

3.2 Analysis of copper ion-induced damage to Malassezia cells

After three hours, the group treated with 16 mM Cu^{2+} showed the greatest decrease in bacterial count. 16 mM Cu^{2+} showed the best bactericidal effect in this experiment, with a sustained bactericidal effect within 2 to 5 hours.

3.3 Analysis of copper ion-induced damage to Malassezia cells

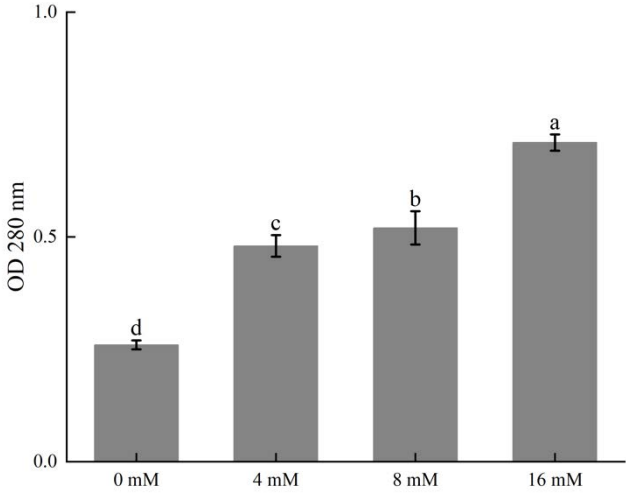

Fig. 2. The effect of copper ion on proteins of *Malassezia* cells.

At 280 nm, the absorbance shows an overall increasing trend from a Cu^{2+} content of 0 mM to 16 mM (Fig. 2), indicating an increase in protein concentration. Proteins are the fundamental structure and important components of cells. Therefore, the leakage of proteins indicates that the cell structure has been disrupted. This suggests that copper ions can damage *Malassezia* cells, leading to the leakage of internal cellular proteins, thereby increasing the protein content in the liquid and consequently raising the absorbance value.

3.4 Analysis of autophagy related genes

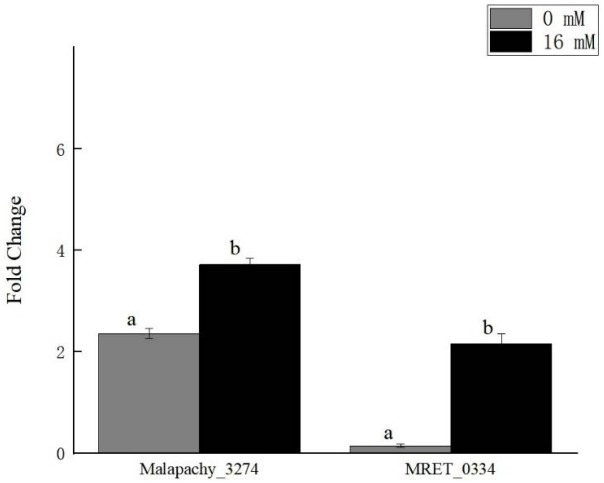

Fig. 3. The effect of copper ions on autophagy related genes.

Verified through RT-PCR experiments. After adding 16 mM copper ions, autophagy related genes Malapachy 3274 and MRET 0334 were found to increase (Fig. 3). The principle of RT-PCR (Reverse Transcription Polymerase Chain Reaction) is a technique that combines RNA reverse transcription (RT) with cDNA polymerase chain amplification (PCR). Firstly, cDNA is synthesized from RNA by reversing the action of the enzyme. Then, using cDNA as a template, PCR amplification is performed under the action of DNA polymerase to obtain the target fragment. Because autophagy leads to genetic changes, this result proves that copper ions can trigger autophagy.

3.5 Synthetic material analysis

Fig. 4. Composite material synthesis.

The addition of copper (II) sulfate pentahydrate facilitates the dissolution of the solution, making the solution clearer (Fig. 4). Besides, materials synthesized by deionized water, sodium alginate, and agar have good ductility and transparency after heat under 80℃. Sodium alginate can serve as a drug carrier, providing assistance in stability, solubility, viscosity, and safety of composite material. It has been widely used in medical and bioengineering fields. Agar exhibits unique gelling properties and gel stability, serving as an excellent thickening and stabilizing agent. Therefore, in the process of constructing metal ion composite materials, sodium alginate and agar can enhance stability and water retention. Some studies have combined alginate with metal organic frameworks to exhibit antibacterial activity.

4. Conclusions

Experimental results have demonstrated that both copper ions and iron ions exhibit bactericidal effects on *Malassezia*. Cu^{2+} showed a better bactericidal effect under the same conditions compared to the Fe^{2+}. The 16 mM Cu^{2+} group performed the best in the experiment for inhibiting the growth of *Malassezia*. The binding of copper with thiol groups deactivates thiol enzymes, leading to copper-induced cell death and apoptosis induction through this mechanism. Ferroptosis is an iron-dependent programmed cell death process triggered by dysregulation of the redox mechanism (ROS), ultimately resulting in extensive peroxidation of polyunsaturated phospholipids. According to the results of qRT-PCR for gene detection and spectrophotometer for protein leakage detection, copper ions can trigger autophagy in *Malassezia* cells, causing protein leakage and damaging cell structure. When introducing copper ions into synthetic materials, the copper ions themselves can enhance the visuality of the membrane structure. The use of agar and sodium alginate to construct composite materials is a good choice, as it can ensure the stability and owns good water retention ability. It is promising to combine copper ions with agar and sodium alginate to construct an aqueous membrane for inhibiting the growth of cutaneous *Malassezia*. Future research can focus on more applications of copper induced cell death, as well as the effects of more metal ions on human skin beyond antibacterial effects, while studying potential risks to human health.

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