Chapter 1

Introduction

1.1 Background

Fungal infections or mycosis are illnesses resulting from fungal organisms including yeast and mold, that might cause a wide range of diseases including pneumonia or peritonitis. Fungal infection can be vary from subcutaneous, cutaneous, superficial which affect the outer layer of the skins, to disseminated or systemic infection, that can spread to vital organs and cause more serious diseases (Kurien et al., 2025; Martins & Miteva, 2019; Walsh et al., 1996). Furthermore, the range of people at risk of getting infected by fungus can vary, from normal to people who are immunocompromised patients (Garnacho-Montero et al., 2024). According to studies done by Fang et al. (2023), the most common invasive infection usually comes from *Candida*, *Cryptococcus*, *Aspergillus*, *Pneumocystis*, and *Coccidioides* species. Additionally, the Centers for Disease Control and Prevention (2024) also stated that the overgrowth of *Candida* is called candidiasis, where it can spread to the bloodstream, organs, or bones and cause more serious infection. As reported by Turner and Butler (2014), the top two *Candida* species that are responsible for fungal infection are *Candida albicans* and *Candida glabrata*. Under certain predisposing factors, including treatment, both species can cause infection that range from superficial or systemic infection which often leads to high morbidity and mortality (Brunke & Hube, 2013).

One of the reasons why fungal infection cases increased is due to the lack of diagnosis, vaccine, and novel antifungal drugs (Vitiello et al., 2023). According to Drummond et al. (2014), major fungal infections are caused by the opportunistic pathogens that do not induce disease unless the host immune system is compromised. Additionally, Houšť et al. (2020) also shows that the overuse of antifungal agents has led to increased resistance in opportunistic pathogens. As a result, many fungal

species now exhibit resistance towards all four classes of antifungal drugs including polyene, triazoles, echinocandins, and 5-fluorocytosine (5-FC) (Fisher et al., 2022).

Fortunately, the human body has its own immune system that is known to fight invading germs and fungal infections, where it is divided into two categories: innate and adaptive immunity. However, individuals with immunodeficiency may be unable to effectively prevent the invading germs and fungal infections due to a weakened or dysfunctional immune system, which may increase the risk of more severe inflammation (Firacative, 2020). Despite of that problem, a new therapeutic tools called mesenchymal stem cells (MSCs) are able to treat various diseases due to their ability to replicate, adherence, and differentiate into three types of cells including adipocyte, osteoblast, and chondrocyte (Zhuang et al., 2021). Additionally, MSCs are used in cell therapy due to their response toward injured tissues, ease of isolation and expansion, and immunomodulatory ability. Additionally, a study by Cho et al. (2019) and Gao et al. (2025), mentioned that MSCs can stimulate pro-inflammatory cytokines such as Interleukin (IL)-2, IL-12, IL-15, and tumor necrosis factor alpha (TNF-α), which activate natural killer (NK) cell to enhance the fungal killing effect and cytotoxicity. At the same time, MSCs are also able to secrete anti-inflammatory cytokines that modulate the activity of NK cells, B cells, T cells, neutrophils, leading to a reduction in the release of pro-inflammatory cytokine and ultimately decreasing inflammation. In addition to modulating cytokine production and immune cell activation, Toll-like receptors (TLR) play a key role in regulating the immunomodulatory function of MSCs (Liu et al., 2023; Zhou & Bai, 2022).

Toll-like receptors play a crucial role in mediating inflammatory pathways and are extensively expressed on both MSCs and immune cells. Where, it can identify pathogen and danger-associated molecular patterns (PAMPs, DAMPs) that originate from either external pathogens or internal cellular products (Liu et al., 2023). Additionally, the activation of TLRs can initiate signaling pathways that directly influence MSCs differentiation, migration, and survival (Li et al., 2024; Shirjang et al., 2017). A study done by Patin and colleagues in 2019, shows that TLR2 plays a critical role in identifying fungal

pathogens by recognizing specific components of their cell walls such as Zymosan, a β-glucan that can be found in fungal cell walls. Additionally Ko et al. (2024) mentioned that TLR2, which is found on MSCs serves as a detector for danger signals released during tissue damage or inflammation. When TLR2 is activated, it will enhance the immunoregulatory properties of MSCs, thus secreting anti-inflammatory cytokine, and facilitating the differentiation of monocytes and macrophages into immunosuppressive phenotypes (Han et al., 2022). This mechanism is essential to lower the inflammation and supporting tissue repair. Therefore, this study utilizes both methods *in silico* and *in vitro* approaches, where the *in silico* analysis using bioinformatics was first conducted to narrow down the gene expressed by the MSCs in responses to fungal zymosan stimulation. This was followed by *in vitro* analysis using qPCR to evaluate and compare the expression of key immunomodulatory genes such as TLR2, *ICAM1*, *PGES2* and *PTGS2* that expressed by the bone marrow, umbilical cord, and adipose tissue MSCs in responses to stimulation with *Candida albicans* and *Candida glabrata*.

1.2 Objective

1. To assess the genes secreted by MSCs during fungal infection using bioinformatics tools
2. To assess the effect of co-culturing *Candida albicans* and *Candida glabrata* on three types of MSCs on the immunomodulatory genes such TLR2, *ICAM1*, *PGES2* and *PTGS2* using qPCR

1.3 Hypothesis

1. H0: Based on bioinformatics analysis, MSCs stimulation with fungi will not increase proinflammatory or anti-inflammatory genes in bioinformatics experiment

   H1: Based on bioinformatics analysis, MSCs stimulation with fungi will increase both proinflammatory and anti-inflammatory genes in bioinformatics experiment
2. H0: MSCs co-cultured with fungi will not increase immunomodulation gene including TLR2, *ICAM1*, *PTGES2*, and *PTGS2*

   H1: MSCs co-cultured with fungi will increase immunomodulation gene including TLR2, *ICAM1*, *PTGES2*, and *PTGS2*