Chapter 1

Introduction

1.1 Background

Mesenchymal stem cells (MSCs) are multipotent stromal cells whose ability to undergo differentiation into various cells such as adipocytes, osteoblasts, fibroblasts, chondrocytes, and myoblasts. MSCs primarily perform tissue replacement via multipotent differentiation, anti-inflammatory, immunomodulatory actions, and molecular secretion, which aids in tissue repair. Therefore, stem cell-based products have gained a great popularity in the world of regenerative medicine (Putri et al., 2019), by restoring any damaged or dead cells for maintaining tissue homeostasis (Rahimi et al., 2021). MSCs, in particular, can release trophic substances that promote tissue regeneration and repair tissue damage (Damayanti et al., 2021). The stem cell therapy is performed by injecting the stem cells medication toward the damaged tissues for continued development and the regeneration of novel tissue, or by secreting signaling molecules to stimulate a series of biological processes at the damaged sites to undergo reparation and regeneration. However, the therapy using MSCs may lead to unfavorable immune reaction, along with poor engraftment rates, low transplanted cell survival rates, impaired self-renewal and differentiation abilities after implantation, and cellular senescence as a result of population growth to obtain sufficient numbers of cells for therapy. Despite being supported by clinical data and the necessary regulatory permission, the mainstream clinical acceptance of MSCs therapy is still being constrained, such as due to the inadequate deeper studies to ensure the long-term efficacy of the MSCs therapy. Delivery of the stem cells locally or systemically may also occasionally have unfavorable effects, such as the development of tumors (Tan et al., 2022).

Secretome is a bioactive substance secreted by MSCs in a conditioned media, with many growth factors and other substances that are able to provide a biological response by undergoing signal transduction. A secretome-based therapy can address issues with the utilization of living cells, as well as for treating various skin diseases, such as to regenerate and repair injured tissues (Damayanti et

al., 2021). In Japan, South Korea, and the US, secretome is a novel class of biopharmaceuticals with the prospective to be used in cosmetic products including anti-aging, wound healing, and anti-pigmentation (Tan et al., 2022). However, due to the protein instability limitation, preservation of the protein integrity is beneficial to preserve the efficacy (Izutsu, 2018). According to Tan et al. (2022), in order to concentrate and produce a stable secretome in a convenient, ready-made preparation for subsequent formulation, storage, and transport, freeze-drying is frequently used. Therefore, freeze-dried (FD) secretome can be produced, packaged, and distributed more easily (Putri et al., 2019). According to Assegehegn et al. (2018), freeze-drying consists of freezing, primary drying, and secondary drying. Where freezing is the process of freezing most of the solvent onto a frozen solid, that has been studied to be a crucial step, as it affects the primary and secondary drying processes, thus it must be monitored and managed well. In which, the freeze-drying process yielded a free-soluble secretome powder (Bari et al., 2018).

Freezing process is the foremost critical part in the process of freezing drying that might affect the morphology of ice crystals (Severo et al., 2017). As stated by Kasper and Friess (2011), regarding the biological activity and stability of pharmaceutical proteins, frozen sample protein is required to be achieved during the freezing phase, thus when stressors occur during the drying process, protein destabilization can be prevented. This research evaluated the effect of the freezing time variations in freeze-drying toward the secretome, in terms of potency on 3T3 fibroblast cells. In evaluating FD secretome cytoprotective potency, the cellular metabolic activity of the treated 3T3 cell can be evaluated through oxidative stress assay, where the oxidative stress using peroxide insult were introduced into the cells and the capability of secretome treatment protect the cells from oxidative stress would be evaluated (Bari et al., 2018). Additionally, wound healing property of the FD secretome was evaluated through *in-vitro* scratch assay, where wound repairing enhancement in the cell due to the accelerated cell migration and formation of extracellular matrix (ECM) by the secretome was observed (Walter et al., 2010).

1.2 Objectives

The objective of this research is to evaluate the effect of varying freeze-drying parameters, that is freezing time towards the potency of secretome through cytoprotective evaluation against oxidative damage and capability to improve cell proliferation and migration through *in-vitro* scratch assay of FD secretome treatment in 3T3 cells.

1.2 Hypothesis

The hypotheses of this research are:

1. The freezing time parameter on the freeze-drying process performed on the secretome may significantly affect the secretome potency measured through cytoprotective capability as well as cell proliferation and migration improvement.
2. The longer freezing time during the freeze-drying process conducted on the secretome does not negatively affect the secretome potency.