Chapter 1

Introduction

1.1. Background

The development of stem cells as a regenerative medicine have long gained the interest of researchers. Stem cells are undifferentiated cells that are able to develop into various cell types and have the ability to repair damaged tissues (Xia et al., 2019). The mesenchymal stem cells (MSCs) are a specific stem cell type that can secrete bioactive molecules called secretomes or MSC-secretome (MSC-S) in a conditioned medium, in which the molecules include soluble factors and extracellular vesicles (EVs) (Damayanti, Rusdiana, & Wathoni, 2021; Xia et al. 2019). The secretome can be obtained by the isolation of MSCs from many types of adult tissues and perinatal tissues, then the isolated cells are cultured to obtain greater amounts of secretome (Damayanti, Rusdiana, & Wathoni, 2021; Meiliana, Dewi, & Wijaya, 2019; Sandonà et al., 2021).

The secretome itself is utilized by the stem cells for their inter-cell communications. However, it holds a promising role for regenerative medicine since it is a cell-free therapy that would not have a rejection reaction and give an ease for future treatment development. Some mechanisms possessed by secretome are tissue repair and wound healing, promoting angiogenesis, anti-apoptosis, and immunoregulation effect (Meiliana, Dewi, & Wijaya, 2019). Secretome is also known to have potential therapeutic effects on various diseases such as cardiac, oncology, diabetes, neurodegenerative disease, anti-aging, hair loss, joint osteoarthritis, and psoriasis (Damayanti, Rusdiana, & Wathoni, 2021; Xia et al., 2019). Compared to the conventional stem cell therapies, secretome therapy has lower risk of immune compatibility, tumorigenicity, transmission of infections, and emboli formation. The preparation and evaluation of the secretome can follow the conventional pharmaceutical agents, and the secretome can be kept for a long time without losing its potency or using potentially hazardous cryopreservatives. Moreover, the ability of secretome to be produced in advance and

rapidly available when needed for urgent treatments can significantly minimize the cost and time related to growing and maintaining cell lines (Meiliana, Dewi, & Wijaya, 2019; Xia et al., 2019).

In order to be able to deliver the secretome as a therapeutic agent, a suitable and effective formulation process must be carried out to make it into an active pharmaceutical ingredient (API). The conditioned media containing secretome is originally produced as a liquid aqueous preparation and can be administered by injection through the intravenous, intramuscular, intrathecal, intraperitoneal, and subcutaneous routes. Topical routes using cream or gel, also administration through inhalation using liquid suspension or dry powder are other options of secretome preparation (Ahangar, Mills, & Cowin, 2020; An et al., 2021; Bari et al., 2019; Ibrahim et al., 2022). Among all preparations, intravenous injection is the most chosen route of administration due to its fast onset of action and has high bioavailability (Bari et al., 2019). However, several problems regarding protein stability can arise due to the liquid aqueous formulation including chemical degradations such as deamidation, hydrolysis, and disulfide bond cleavage, and physical degradations like perturbation of higher-order structures and aggregation; which all can lead to the loss of its effect as a therapeutic agent (Izutsu, 2018). Moreover, the liquid formulation should be able to survive from shaking and temperature changes during handling and transportation. Therefore, many approaches have been suggested to improve the protein stability of secretome, to increase the shelf life, and also to ease the storage and distribution, one of them is through the freeze-drying process or lyophilization which turns the liquid formulation into a solid formulation (Bye, Platts, & Falconer, 2014; Falk, 2019; Mocchi et al., 2021)

Freeze-drying is a type of drying method that removes water from the product in a frozen state under a high vacuum environment to preserve the perishable or thermolabile materials, increase the shelf life, and ease the transportation of the product. The freeze-drying process consists of three interdependent steps which are freezing, primary drying or sublimation, and secondary drying. Each

step of the freeze-drying process has several critical process parameters (CPPs), the parameters that can be modified, which contribute to the critical quality attributes (CQAa) of the lyophilized product (Gaidhani et al., 2015; Kawasaki, Shimanouchi, & Kimura, 2019). In this study, the freezing time is chosen as one of the CPPs to be controlled during the freeze-drying process. The freezing time or duration in the freezing step can determine whether the water molecules are completely frozen or some still unfrozen in a liquid sample. Completely frozen water molecules can easily undergo sublimation, while unfrozen water molecules will evaporate at higher temperatures (Barbosa-Cánovas et al., 2005; Ratti, 2013). The imbalance between the frozen and unfrozen water molecules upon drying would affect the final lyophilized product, especially the cake appearance and thus the protein stability of the freeze-dried secretome or lyosecretome.

Although numerous studies are available on the importance of freezing step of freeze-drying, especially the freezing rate and freezing temperature, there is little or no information available that discusses the effect of freezing time or duration on the protein stability of lyosecretome. Hence, this study discussed the effect of freezing time variations towards the lyosecretome protein stability which was evaluated by several protein characterization methods and also to determine the most optimal freezing time that would retain the highest protein content in the lyosecretome.

1.2. Objective

The objectives of this study were to perform freeze-drying of the secretome under various freezing times and to compare the effect of various freezing times towards the protein characteristics of the freeze-dried secretome and compare with the fresh secretome.

1.3. Hypothesis

1. Freezing time variations in the freeze-drying process affect the protein characteristics of the freeze-dried secretome differently.

2. Freeze-dried secretome with the longest freezing time would have better protein characteristic results as evaluated from protein characterization.