Chapter 1

Introduction

1.1. Background

Mesenchymal stem cells (MSCs) are a non-hematopoietic multipotent stromal cells that have the ability to renew themselves and differentiate into multilineages such as endothelial cells, adipocytes, chondrocytes, osteocytes, smooth muscle cells, and keratinocytes (Ding, Shyu & Lin, 2011). MSCs have several unique features in which they express low levels of major histocompatibility complex (MHC) I and do not express the MHC II and co-stimulatory molecules such as CD40 and CD80 (Oh, Kim, Yang & Lee, 2008). This makes MSCs become immune-privileged cells, which are types of cells that can tolerate immune response since MHC I, II and co-stimulatory molecules are antigens presenting molecules on the cell surface in order to be recognized by T cells. Another unique features of MSCs is that it has immunomodulatory properties, which is the ability to alter the phenotype and functional properties of T cells, B cells, NK cells, and macrophages, to help the body from various inflammatory disorders (Müller et al., 2021). These unique features imply that MSCs may revolutionize cell treatments for the repair of injured tissue across a variety of systems, especially the acceleration of wound closure which becomes a potential therapeutic effect of these cells (Lee, Ayoub & Agrawal, 2016).

MSCs release a wide variety of bioactive factors in response to their environment, including proteasomes, proteins, exosomes, nucleic acids, microRNA, and membrane vesicles, which are together referred to as the secretome (Ferreira et al., 2018). These secretomes are crucial for cellular communication and participate in a number of physiological processes, such as signal transduction that results in a biological reaction or also known as paracrine signaling, which contributes to its regenerative and reparative mechanisms to damaged tissue (Wei et al., 2020). In terms of the wound healing process, the secretomes work by accelerating the re-epithelization of tissue to make the

epithelial cells migrate upwards faster to repair the wounded area, improving extracellular matrix (ECM) production and modeling, its immunomodulatory properties, and promoting angiogenesis or formation of new blood vessels during the proliferation phase in wound healing process (Harrell et al., 2019). The incorporation of MSCs into regenerating tissues will also cause the cells to exhibit regenerative, reparative, and immunomodulatory effects through paracrine signaling (Wang, Yuan, & Xie, 2018). Treatment with MSCs for wounds improves granulation tissue development, stimulates angiogenesis, speeds up re-epithelization, and reduces inflammation (Hocking & Gibran, 2010). The production of MSC-secretome (MSC-S) includes the isolation from many types adult tissues such as skin tissues, adipose tissues, umbilical cord, spinal cord, placenta and many others, as well as cell culture to obtain large number of secretome (Damayanti, Rusdiana & Wathoni, 2021). The produced secretome will then formulated and packaged into biopharmaceuticals product, which has high specificity and efficacy compared to small molecules drugs (Lim et al., 2016).

However when it comes to biopharmaceuticals products, especially protein, there are several problems that frequently arise related to its chemical and physical instability, as well as bioavailability. When administered orally, protein-based drugs will encounter an acidic environment and a lot of protease enzymes that will break down the complex protein into amino acids which results in loss of its efficacy and low bioavailability (Singh, Singh, & Lillard, 2008). Moreover, due to its high molecular weight of these drugs, the epithelial barriers tend to be a challenge for the absorption into the periphery, which makes the bioavailability even lower. These problems make the injection route such as intravenous route (IV) become the route of choice for this type of drugs, including the secretome. Pre-filled syringes are often the most suitable primary packaging for biopharmaceutical products either in liquid form or solid forms, however protein-based drugs in solution tend to have physicochemical problems, such as degradation, denaturation of the protein content, and aggregation (Falk, 2019). Since the initial form of the secretome itself is in liquid form, this problem can be overcomed by using the appropriate drying approach to make it into solid form, in which one of them

is freeze-dry method or lyophilization.

Lyophilization or also known as freeze-drying is a type of drying process by freezing the water molecule to become ice, and under a low pressure the ice is removed by sublimation process which is a direct transition from solid phase to gas phase (Adams, Cook & Ward, 2014). There are several critical process parameters (CPPs) in each step of freeze-drying, which are the parameters in the process that can be controlled and will affect the critical quality attributes (CQAs) of the product, in this case the secretome (Koganti et al., 2011). Freezing time is one of the CPPs that will determine whether the water molecules have been completely frozen or not during the freezing process. The presence of unfrozen water molecules can cause evaporation, leading to an imbalance in the resulting freeze-dried product (Barbosa-Cánovas et al., 2005). This occurs as certain water molecules undergo sublimation while others undergo evaporation, which will result in a build up of tensile stress in the freeze-dried cake and might affect its physicochemical properties such as cake appearance and protein profile, which are the CQAs of the freeze-dried secretome (Falk, 2019). There have been several studies that examine the effect of freezing step such as ice nucleation and annealing during freezing process on the cake appearance (Barresi, Capozzi, Arsiccio, Sparavigna, & Pisano, 2018; Esfandiary, Gattu, Stewart, & Patel, 2016; Lu & Pikal, 2004). However, lack of studies have been conducted to examine the effect of freezing time variations on the physicochemical characteristics such as cake appearance, particle size and its protein thermal profile of the freeze-dried secretome, especially UC-MSC. Thus, this study highlights the significance of several freezing time variations on the CQAs of freeze-dried UC-MSC secretome, as well as to determine the optimum freezing time used in order to produce good quality cake in freeze drying.

1.2. Objective

The objectives of this project are:

1.2.1. To perform freeze-drying process on MSC-S with variety of freezing time, and

evaluate freezing time effect on the physicochemical properties of freeze-dried MSC-S (cake appearance, moisture content, reconstitution time, particle size, zeta potential, DSC profile) and compare it with the physicochemical properties of fresh MSC-S.

1.3. Hypothesis

The H0 of this research are:

1.3.1 The secretome cannot be freeze-dried into a stable solid cake with acceptable physicochemical properties criteria;

1.3.2 The variety of freezing time is not the critical process parameter (CPP) in freeze-dry process and will not affect the physicochemical properties of freeze-dried MSC-S.

Whereas the H1 of this research are:

1.3.3 The secretome can be freeze-dried into a stable solid cake with acceptable physicochemical properties criteria;

1.3.4 The variety of freezing time is the critical process parameter (CPP) in freeze-dry processes and will affect the physicochemical properties of freeze-dried MSC-S