Chapter 1

Introduction

1.1 Background

Aging significantly impacts human health by increasing the risk of various age-related disorders. Senescent cells accumulate during aging, promoting cardiovascular and neurodegenerative diseases (Chen et al., 2021). Aging may also result in impaired tissue regeneration, posing additional difficulties in regenerating bone defects. It has been stated that the recovery process is slowed or fails in 5-10% of all bone fractures owing to aging (Maruyama et al., 2020). Any substance that might impede the body's degenerative processes related to aging is called an anti-aging. Anti-aging agents on the market include antioxidants such as vitamin C and E as well as manufactured agents like ascorbic acid and retinol (Liu., 2022). Synthetic anti-aging agents, on the other hand, are frequently associated with several adverse effects, as excessive doses may promote DNA damage and premature senescence (Lourenco et al., 2019). As a result, people are becoming more aware of the need to minimize synthetic anti-aging use and seek other alternatives.

Mesenchymal stem cells (MSCs) have recently grown into a promising tool for treating various diseases because of their tissue-protective and reparative abilities (Chen et al., 2021). MSCs have the potential to significantly slow the aging process (Lv et al., 2022). They control their therapeutic activities by secreting paracrine factors known as secretomes. Secretomes refer to the entire collection of molecules, including proteins, cytokines, immunomodulatory substances, and hormones that originate from stem cells and are released into the environment (Pinho et al., 2020). Secretomes have the potential to be used in a variety of therapies, particularly skin applications, since they can support skin repair and regeneration by promoting cell migration and proliferation through the release of several growth factors such as VEGF, EGF, and FGF (Damayanti et al., 2021). However, the use of secretome in a clinical setting is limited due to the instability of the active compound and the protein content.

Several investigations have suggested that secretomes could also be used as an anti-aging agent in cosmeceuticals. As a skincare therapy, secretomes could aid in the prevention of different aging factors like photoaging and reduce signs of skin aging (Damayanti et al., 2021; Kerscher et al., 2022). Therefore, understanding the underlying processes of MSC secretomes along with dermal aging is crucial as it will open up new avenues for extracting these substances as potential anti-aging agents.

Furthermore, manufacturing MSC secretomes for clinical uses necessitates efficient and good manufacturing practice (GMP) methods. Several concerns have prevented secretome from being widely used in a clinical setting. The major concern relies on the instability of the compound, the short life of the protein content inside the secretome, as well as the need for cold chain distribution. Therefore, there is a need to find a method to improve the secretomes' stability and shelf life. One method might be employing a freeze-drying technique with cryoprotectants (Koganti et al., 2011). Nevertheless, studies have revealed that various freeze-drying parameters can impact product potency. Greater moisture, for example, could result in greater potency. Moreover, fluctuations in the shelf temperature beyond the collapse temperature may also cause the product to collapse completely (Mutukuri et al., 2021). Keeping this in mind, the safety and potency of freeze-dried secretomes should be assessed using various freeze-drying parameters. This could be accomplished *in vitro* by conducting cytocompatibility, oxidative damage protection, and cell migration assay. These assays can be used to assess the anti-aging potential of freeze-dried secretomes through the measurement of cellular metabolic activity, which can detect cell viability, proliferation, and toxicity, as well as through the observation of basic cellular migration during the wound healing process.

1.2 Objective

This study aims to examine how various freeze-drying parameters affect the anti- aging potency of secretomes *in vitro* using HaCaT cells. The findings of this study will help to establish the upscale manufacturing of stem cell-based therapies in skin disease treatment, which will benefit the community as a promising anti-aging agent.

1.3 Hypothesis

This study hypothesized that the freeze-drying process would not negatively affect the anti-aging potency of secretomes. In addition, this study also hypothesized that temperature would be the critical process parameter during the freeze-drying process of secretomes that will eventually affect the secretomes' anti-aging potency. Higher temperatures during the primary drying process are hypothesized to have lower anti-aging potency.