CHAPTER I: INTRODUCTION

1.1. Background

The process of aging is characterized by the gradual loss of physiological structures and functions as a result of a combination of intrinsic and extrinsic factors. (Kwon et. al., 2015). As one of the most voluminous and outermost organs of the body, skin shows evident and apparent signs of aging (Vierkotter & Krutmann, 2012). During skin aging, the constituents of the extracellular dermal matrix (EDM) change. Eventually, it will undergo structural alterations and changes in the physiological characteristics of the skin including wrinkling, loss of elasticity, and dermal atrophy (Ganceviviene et. al., 2012). The accumulation of reactive oxygen species (ROS) results in oxidative stress, which is one of the main factors leading to skin aging damage. It has been demonstrated that excessive ROS can cause an increase in collagen breakdown and a decrease in collagen synthesis (Sárdy, 2009). Collagen type 1 as one of the major scaffold proteins found in the skin accounts for more than 70% of the skin collagen (Kim et. al., 2018). However, decreased levels of collagen are known to be associated with aging and cause a weakening of the integrity and strength of the skin (Szychowski & Skora, 2021; Kim et. al., 2018; Onar et. al., 2012). Hence, as one of the major protein components in EDM, an increase in collagen type 1 level can act as an indicator of a good anti-aging product.

Mesenchymal stem cells (MSCs) are stromal cells with multipotency that have the ability to differentiate into various types of cells, including osteoblasts, adipocytes, and chondrocytes (Liu et. al., 2022). It has been demonstrated that there is a new method for investigating the therapeutic potential of stem cells without the necessity for cell transplantation. Instead of using a therapeutic drug directly, this method—known as cell-free therapy—uses stem cells as a source of therapeutic molecules (Evangelista et. al., 2019). This approach could eliminate the risk of tumor formation, immunological reactions, and undesired differentiation of MSCs that are

commonly found in stem cell-based therapy. Interestingly, MSCs can produce proteins such as growth factors, cytokines, and chemokines, into their surroundings, which are collectively known as secretomes. Secretomes may trigger intracellular mechanisms in cell viability, proliferation, and angiogenesis toward their neighboring cells (Bogatcheva & Coleman, 2019; Meiliana et. al., 2019). MSCs lysate has also been considered as an alternate cell-free option of treatment for various diseases, in which it was shown to be able to upregulate angiogenesis and proliferation in inflamed tissue (Malik et. al., 2023). Currently, there has been a lack of studies on the anti-aging activity of MSCs lysate using human keratinocytes cells, where there are only available studies done using human and mouse fibroblast cell lines (Malik et. al., 2023). One of the sources of MSCs, which is the umbilical cord, has the ability to proliferate and produce differentiated cells that can replace the injured tissue in the targeted area (Nurkovic, 2016). Studies have shown that mesenchymal stem cells derived from human umbilical cord blood and tissue are efficient skin moisturizing and anti-wrinkle agents, where they were shown to stimulate the rejuvenation of the skin (Nakajima et. al., 2012; Kim et. al., 2018). Therefore, this study will compare the anti-aging activity of umbilical cord-derive MSC secretome and lysate towards human keratinocytes cell lines that have been exposed to H_2O_2.

1.2. Objectives

This research project aims to investigate and compare the anti-aging activity of umbilical cord-derived mesenchymal stem cells secretome and cell-free lysate by measuring the expression of collagen type 1 in H_2O_2-exposed human keratinocytes cells.

1.3. Hypothesis

This study hypothesizes that umbilical cord-derived mesenchymal stem cell's secretome and lysate are able to increase the expression of collagen type 1 in human keratinocytes cells that have been exposed to H_2O_2, in which the lysate has better anti-aging capacity than the secretome.

1.4. Research scope

This research project encompasses the culturing of human keratinocytes cells (HaCaT) and umbilical cord-derived mesenchymal stem cells, followed by the collection of the stem cells conditioned medium (secretome) and cell-free lysate preparation. Oxidative stress was induced to mimic skin aging by treating the HaCaT cells with H_2O_2. Later, the H_2O_2-exposed cells were treated with different concentrations of the secretome and lysate. The anti-aging activity measurement will be performed by quantifying the secreted collagen type 1 protein using an ELISA kit on the previously treated HaCaT cells.