CHAPTER 1: INTRODUCTION

1.1. PROJECT BACKGROUND

Skin is the largest organ in the human body that is composed of three main layers: the epidermis layer, dermis layer, and subcutaneous layer. An epidermal junction is found in between the epidermis and dermis layer. This junction plays a role in providing mechanical support and in acting as a partial barrier against large molecules. The epidermis layer is made up of stratified epithelium that is composed of keratinocytes, melanocytes, and macrophages. The dermis layer contains dermal fibroblasts that generate connective tissue and are responsible for skin recovery after an injury. At the base of the dermis layer, an extracellular matrix (ECM) containing polysaccharides and proteins can be found (Griffiths et al., 2016). The dermal fibroblasts are also responsible for the secretion of ECM components (Kular et al., 2014; Kusindarta & Wihadmadyatami, 2018; Tracy et al., 2016; Varani et al., 2006).

The ECM is a highly dynamic non-cellular structure that is divided into fiber-forming structural molecules, non-fiber-forming molecules, and matricellular (Järveläinen et al., 2009). The major fiber-forming molecules are collagen (COL) and elastin (ELN), while the major non-fiber-forming molecules are proteoglycans and glycosaminoglycans (GAGs). COL is responsible for providing the skin with tensile strength and for regulating cell adhesion, while ELN forms a network of elastic fibers that provides the skin with elasticity and flexibility. On the other hand, GAGs play a role in the skin's hydration as they attract large amounts of water. The major GAGs found in the skin are hyaluronan or hyaluronic acid (HA), which play a role in chemical signaling among skin cells (Kular et al., 2014).

The skin, just like any other organ, is susceptible to aging. However, skin aging is a complex process involving intrinsic and extrinsic factors (Zhang & Duan, 2018). The intrinsic factors that contribute to skin aging include genetics, cellular metabolism, hormonal processes, and metabolic processes. Meanwhile, extrinsic factors contributing to skin aging include pollution, chemicals, toxins, chronic light exposure, and ionizing radiation (Ganceviciene et al., 2012). Altogether, these factors cumulate structural alterations that cause physiological characteristic changes in the skin, resulting in skin

thinning, drying, wrinkling, loss of elasticity, and dermal atrophy (Ganceviciene et al., 2012; Zhang & Duan, 2018).

During skin-aging, the constituents of dermal ECM change. The amount of COL in the ECM is significantly reduced due to the constant degradation by matrix metalloproteinases (MMPs) and other enzymes, as well as reduced production of COL by fibroblast. For instance, collagen type I (COL1) and III (COL3) production have been associated with skin aging, in which their synthesis is reduced as the dermal fibroblast receives less stimulation in older skin. The reduction in the levels of COL causes a weakening of the integrity and strength of the skin. Moreover, the COL is more susceptible to unnecessary cross-linking, and the arrangement of both COL1 and COL3 becomes disorganized and fragmented, which causes skin stiffness (Sparavigna, 2020). The degradation of ELN and GAGs by MMPs during skin-aging also causes alterations to the ECM, and the skin loses its elasticity and strength. With that, wrinkles and skin sagging are likely to occur (Ganceviciene et al., 2012).

The three primary protein components of the skin ECM that are mainly altered during skin-aging are COL, ELN, and HA levels. Therefore, these ECM constituents have been the focus of many skin-aging and anti-aging studies (Frantz et al., 2010; Ganceviciene et al., 2012; Sparavigna, 2020; Zhang & Duan, 2018). Anti-aging studies have suggested that preventing the skin's primary structural constituents from degradation is crucial to prevent the formation of wrinkles and sagging of the skin (Ganceviciene et al., 2012). With that said, an alternative strategy is to use cosmetics that can increase collagen, elastin, and hyaluronic acid levels. Such cosmetics have been increasingly popular nowadays as more people are becoming aware of the aesthetic value of looking young. In this research project, the anti-aging activity of Essence is investigated by measuring the extracellular matrix collagen type I, collagen type III, hyaluronic acid, and elastin expression in primary human dermal fibroblast cells (HDF). These proteins were chosen as they are the primary constituents of dermal ECM.

1.2. OBJECTIVES

This thesis project aims:

1. To test the cytotoxic effect of Essence on primary human dermal fibroblast cells.
2. To investigate the anti-aging activity of Essence by measuring the ECM expression of the treated primary dermal fibroblast cells.
3. To investigate the effect of Essence on the level of collagen type I, collagen type 3, hyaluronic acid, and elastin production in primary human dermal fibroblast cells.

1.3. PROBLEM FORMULATION AND PROPOSED SOLUTION

The global anti-aging market has been growing throughout the years as usage of anti-aging cosmetics continues to grow, making the anti-aging research industry one of the most popular research areas. People are becoming more aware of the positive values of looking young, such as societal values. Therefore, many people are willing to pay huge amounts of money to look young by purchasing anti-aging cosmetics or undergoing anti-aging procedures. Like supplements and drugs, cosmetics testing is also necessary to validate their safety and claimed effects. The main focus of most anti-aging cosmetic products is to increase the levels of COL, ELN, and HA expression, as these proteins have been associated with anti-aging. Therefore, in this research project, the cytotoxicity and anti-aging activity of Essence will be investigated by conducting 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, and measuring the levels of ECM COL3, COL1, HA, and ELN. These proteins were chosen as they are the primary constituents of dermal ECM.

1.4. RESEARCH SCOPE

The scope of this thesis project is investigating the anti-aging properties of Essence, which consists of a miscibility test to make sure that the treatment groups are soluble in Dulbecco's Modified Eagle Medium (DMEM), MTS assay to test the cytotoxicity of the treatment groups, primary HDF cell culture, and treatment, as well as enzyme-linked immunosorbent assay (ELISA) to quantify the secreted proteins after treated with the treatment groups. It was hypothesized that Essence Product (EP), Essence Base (EB), and Essence Active Pharmaceutical Ingredient (EAPI) are not cytotoxic toward primary HDF cells, and are able to significantly increase the expression of ECM COL3, COL1, HA, and ELN in primary HDF cells following 72 hours of treatment.