CHAPTER 1: INTRODUCTION

1.1. Project background

Skin also referred to as the cutaneous membrane, is the largest organ in our body and covers the external surface of the body. Skin segregates the body from the harsh environment while also serving as a sensor for various environmental stimuli. Common adult skin covers an area of 2 m^2 with a weight of 4.5-5 kg and has a variable thickness throughout the body. The skin comprises the epidermis, the dermis, and the subcutaneous layer (Tortora & Derrickson, 2017; Tobin, 2017).

The epidermis layer consists of the keratinized stratified squamous epithelium of multiple layers. It provides tough protection against skin abrasion, heat, microbes, and chemicals, while also secreting water-repellent chemicals that prevent water transfer and limit the entry of foreign materials. The dermis layer enveloped an extracellular matrix of dense irregular connective tissue containing collagen and elastic fibers.

As the organ is exposed to the environment, skin also has a cosmetic role in which clean and beautiful skin is perceived as a symbol of beauty and may positively influence the social behavior of surrounding people and symbolize the perceived "health" of an individual. However, being exposed to the environment also made the skin vulnerable to damage, which resulted in wrinkles, loss of elasticity, dry skin, thickened epidermis, skin darkening, and discoloration. These conditions are also referred to as skin aging, and many researchers have now considered skin aging as a separate disease that would require more attention (Tortora & Derrickson, 2017; Tobin, 2017; Mohiuddin, 2019).

Two main factors that contribute to skin aging, which are intrinsic and extrinsic factors. Intrinsic factors are mostly related to an individual's age and the genetic makeup, such as hormonal and metabolic factors and cellular metabolism. Intrinsic factors generally affect aging slower than extrinsic factors and vary widely between individuals with different ethnicities.

Meanwhile, extrinsic factors are mostly related to radiation, chemicals, pollution, and toxins exposure and are receiving more attention as skin aging prevention strategies. One of the most common extrinsic factors of skin aging is UV light radiation, sometimes called photoaging. The highly energetic UV rays, such as UVA (400-315 nm) and UVB (315-280 nm) that penetrate deeply through the epidermis layer might change the mechanical properties of the epidermis layer and alter the molecular structure of cell proteins and lipids (Tobin, 2017; Mohiuddin, 2019). The most damaging effects of UV radiation result from DNA damage on cells, which can induce apoptosis, followed by inflammatory responses. These inflammatory reactions result in the overproduction of enzymes that degrade the extracellular matrix content, such as matrix metalloproteinases (MMPs). Several other environmental factors, such as pollution, smoking, and various chemicals, might result in the production of excessive reactive oxygen species (ROS) that also induce inflammatory responses and result in further degradation of extracellular matrix content (Tobin, 2017; Mohiuddin, 2019; Kular *et al.*, 2014).

The combination of damaging processes from UV radiation, other environmental factors, and the genetic makeup of an individual results in a low level of extracellular matrix content, particularly for collagen, elastin, and hyaluronic acid. Prolonged exposure to these factors further increases the damage to the skin, resulting in the appearance of skin aging signs such as wrinkles, improved skin stiffness, decreased elasticity, dry skin, thickened epidermis, skin darkening, and discoloration (Tobin, 2017; Mohiuddin, 2019; Frantz *et al.*, 2010; Kular *et al.*, 2014). Therefore, many attempts have been made throughout history to preserve skin's beauty and youthful appearance and slow down the skin aging process. Skin aging prevention involves the development of various cosmetics that protect against damaging UV radiation and ROS build-up. The development of anti-aging cosmetic products is of particular interest due to the growing interest in having beautiful and healthy skin and maintaining healthy skin (Tobin, 2017; Mohiuddin, 2019). In particular, various cosmetics also induce extracellular matrix content synthesis to replenish extracellular matrix (ECM) content that has been lost; including some of the major components of the ECM that are involved in the skin aging process include collagen, elastin, and hyaluronic acid (Tobin, 2017; Mohiuddin, 2019; Kular *et al.*, 2014).

Collagen fibers, mainly collagen I and III, provide resistance to pulling and stretching forces, regulate cell adhesion, and provide structural integrity in the tissue. Meanwhile, elastin fibers provide the ability of the skin to recover from continuous stretching and provide skin elasticity. In addition, the dermis layer also contains a considerable amount of proteoglycans such as hyaluronic acid, which also provides a skin moisturizing and brightening effect and serves as an antioxidant to prevent oxidative damage to the skin. Collagen, elastin, and hyaluronic acid could be assessed to determine anti-aging activity, as these substances contribute heavily to the process of anti-aging, thus will be the main observation focus in this study (Tortora & Derrickson, 2017; Tobin, 2017; Frantz *et al.*, 2010; Kular *et al.*, 2014).

In this study, an anti-aging cosmetic from a well-known cosmetic company will be tested to examine its capability to induce the expression of collagen I, collagen III, elastin, and hyaluronic acid in primary HDF cells. This cell line resembles the dermis layer of human skin. Therefore, it is possible to see whether the product can increase the production of collagen I, collagen III, elastin, and hyaluronic acid necessary for preventing skin aging

1.2. Objectives

This study aims to:

- Determine the cytotoxicity of anti-aging toner products on primary HDF cells
- Investigate the anti-aging properties of cosmetic toner products by observing the level of collagen I, collagen III, elastin, and hyaluronic acid expression in primary HDF cells treated with toner products, base, and API.

1.3. Hypothesis

The cosmetic product shows a significant difference in collagen, elastin, and hyaluronic acid expression in primary HDF cells compared to untreated cells

1.4. Scope of Work

The scope of work of this project is:

- Determine the minimum amount of media required to dissolve the recommended amount of product using a miscibility test

- Evaluate the maximum working concentration that is not toxic towards primary HDF cell culture using MTS assay on primary HDF cells
- Determine the level of collagen I, collagen III, elastin, and hyaluronic acid expression using ELISA