CHAPTER I – INTRODUCTION

1.1. Research Background

Skin is the largest organ of the body that serves as the vital barrier against routine or extreme noxious stimuli (De Benedetto, Kubo & Beck, 2012; Lin, Zhong & Santiago, 2017). The barrier function of the skin mainly resides in the epidermis outermost layer, which is the stratum corneum (SC) layer. SC plays an important role in protecting the body from environmental conditions changes, preventing allergen and microbial penetration, inhibiting transepidermal water loss (TEWL), and maintaining the skin pH and water content (Kim & Leung, 2018). The permeability of the skin is shown to be increasing upon the removal of SC, which highlights the importance of the SC layer as a skin barrier (De Benedetto, Kubo & Beck, 2012).

Various structures and components maintain these barrier functions of the skin. One of the essential structures involved in the skin barrier function is the Intercellular lipid lamellae, which is considered to be the specialized structure found in the SC layer. Intercellular lipid lamellae mainly consist of ceramides, free fatty acids (FFA), and cholesterol, which are produced by keratinocytes and are essential in maintaining the barrier function together with preventing TEWL (Hirabayashi et al., 2017). It is reported that the lipid lamellae composition alteration correlates with atopic dermatitis (AD) emergence (Kim & Leung, 2018). Ceramides as the main lipid lamellae composition function in maintaining the skin integrity by acting as the bridge for the corneocytes to the intercellular matrix and resulting in the TEWL reduction. The importance of normal ceramides amounts and organizations was proven by various skin disorders associated with skin barrier impairment due to the defect of ceramides, including AD. It was reported ceramides content was decreased by 52% in AD, in which the lesioned region is found to have lower ceramides content, while the ceramidase metabolism pathways are overactive in the epidermis layer. The ceramides' value alteration also increased the severity of the acne problems and dry skin. Ceramides, together with other lipid components, are essential in skin moisture balance and TEWL maintenance (Kahraman et al., 2019). Therefore, it can be concluded that ceramides have a significant role in maintaining the skin barrier

and integrity. Hence, the ceramides-related gene expression analysis is necessary to be conducted to reveal the skin barrier condition and integrity.

Filaggrin also plays an important function concerning the skin barrier through the enhancement of the moisture content and the maintenance of epidermis acidity. Filaggrin is also functionally linked with other proteins to sustain the mechanical stability of the keratin structures. Filaggrin can induce lipid envelope formation and antimicrobial peptide production. Hence, the impaired function or production of filaggrin contributes to skin barrier defects, such as AD (Hänel et al., 2016). Based on the paper written by Kim and Leung (2018), it was stated that the loss of filaggrin function leads to enhanced skin sensitivity towards irritants. Filaggrin deficiency affects various skin barrier-related functions, such as decreased water retention, increased infection susceptibility, and corneocyte integrity defect (Tsakok et al., 2018). Armengot-Carbo *et al.* (2015) mentioned that filaggrin loss affects the keratin filament organization, reduces the tight intercellular junction density, and induces the abnormal architectures of lipid lamellae. Other than AD, filaggrin impairment also resulted in the predisposition of ichthyosis Vulgaris. Therefore, the expression of filaggrin is essential to be analyzed in the field of skin barrier-related studies.

Hyaluronic acid (HA) also contributes to skin barrier integrity through its humectant properties to maintain skin hydration. HA is a glycosaminoglycan that can attract and hold a large amount of water from its surroundings (Draelos, 2012). It was reported that HA was also involved in the proliferation of the keratinocytes, extracellular structures synthesis, and elasticity maintenance together with the modulation of epidermal cells' contact with its environment. HA also supports skin barrier integrity by preventing infections and reducing the chance of allergic phenomena (Jegasothy et al., 2014; Nashchekina & Raydan, 2017). Hon and colleagues (2013) mentioned that HA-based treatment can support the skin barrier repair by providing maximum humectant and normalizing TEWL for a suitable barrier repairment environment, such as the application of HA-based treatment is found to improve the severity of AD rapidly. HA is also included in a study done by Barco and Giménez-Arnau (2008) as the hydrating agent and provides the physical support of the epithelial

barrier to combat and treat xerosis. Since skin hydration is strongly correlated to the integrity of the skin barrier, HA expression and content are commonly evaluated in relation to the skin barrier strength.

The skin barrier impairment leads to various skin-related disorders predisposition. Skin barrier defect is reported to be involved in the predisposition of AD, in which the impaired skin barrier increases the epidermal permeability, inflammation, and allergen sensitization of the skin. The reduced quality of life resulting from AD is comparable to type I diabetes with the annual cost burden reaching approximately US$5 billion in the USA (Tsakok et al., 2018). This issue highlights the importance of skin barrier protection and maintenance.

Sensitive skin or redefined as SSS is the enhanced reaction triggered by environmental factors, which may be caused by the skin barrier dysfunction and characterized by skin thickening, dryness, pruritus, tingling, and burning sensation on the skin after the contraction with external stimuli. The increasing number of studies related to SSS revealed the prevalence of sensitive skin, with a ranging number depending on the ethnicity and country (Duarte et al., 2017). Richters and colleagues (2014) reported that the prevalence of self-diagnosed sensitive skin reaches 50-61% in women, while 30-44% in the men population. Duarte *et al.* (2017) showed the epidemiology of sensitive skin across different countries, with Japan's population being found to have the highest sensitive skin prevalence, while Belgium has the lowest sensitive skin population. Although there was an increasing number of studies discussing sensitive skin, the consensus in defining sensitive skin has not been fully elucidated due to its high subjectivity regarding the symptoms (Richters et al., 2014). However, it was predicted that the main cause of sensitive skin is the impaired skin barrier together with the decreased corneal layer thickness and resulting in the increased epidermal permeability (Duarte et al., 2017). Duarte *et al.* (2017) also discussed the inverse relationship between TEWL and skin moisture content. The disruption of skin barrier integrity resulted in higher TEWL, which contributes to lower skin hydration. It was predicted that the reduced skin hydration may be caused by the change in filaggrin expression caused by impaired skin barrier function. The ceramides level

decreases also found in sensitive skin. Since sensitive skin has higher water permeability, TEWL measurement has been used to aid the sensitive skin diagnosis.

Currently, the study related to the treatment and management of skin barrier function is increasing significantly due to the higher awareness of its importance. Various topical products have been developed to treat or increase skin barrier integrity. One common example is the use of emollients or moisturizing creams. The moisturizer has been shown to be quite effective in inducing skin normalization through its support in skin barrier repair. Although there are only a few studies that tested the ability of moisturizers in repairing the skin barrier, it was mentioned that the skin barrier improvement at the early stage of barrier defects may prevent or delay the onset of skin barrier-related diseases. Moisturizer formulation also can reduce TEWL and skin penetration, while promoting barrier recovery (Loden, 2016). Concerning sensitive skin, moisturizer along with other topical prescriptions also has been used to provide hydration to the skin while also modulating the skin's inflammatory reactions. Therefore, the associated symptoms can be dampened, while also promoting the skin barrier repair process (Duarte et al., 2017). In this study, the formulation of moisturizer with several ingredients associated with skin barrier improvement, such as active A (skin barrier repair enhancement), active B (humectant and emollient), and active C (humectant) were evaluated to determine the ability of the moisturizer in enhancing the skin barrier integrity.

The various analysis of the products or compounds' ability in improving skin barrier integrity has been studied using gene expression analysis of the genes associated with the skin barrier. Several studies have been analyzing the effect of several compounds on skin barrier integrity using the qRT-PCR method. A study done by Takeda and colleagues (2021) analyzed the expression of ceramide biosynthesis-related genes to evaluate the effect of β-Sitosterol 3-O-D-glucoside on skin barrier integrity. Other studies also analyzed the filaggrin expression through gene expression analysis, such as the study conducted by Hanyu and colleagues (2012) and Grether-Beck and colleagues (2012). Kim and colleagues (2018) also analyzed the skin barrier integrity by analyzing the expression of

hyaluronic acid biosynthesis-related genes on the HaCaT cell line. Thus, gene expression analysis can be applied in measuring skin barrier integrity.

1.2. Objectives

The main objective of this study is to analyze the effect of the moisturizer formulation on the improvement of skin barrier integrity. The specific aims of this project are elaborated below:

- Evaluating the formulation cytotoxicity towards the HaCaT cell line.
- Determining the suitable amount of the samples for cell treatment.
- Analyzing the gene expression at mRNA level of ceramide-biosynthesis, filaggrin, and hyaluronic acid-biosynthesis genes.
- Testing the efficacy of moisturizer formulation in increasing skin barrier integrity.

1.3. Scopes of Work

Since this study proposes to analyze skin barrier improvement by measuring the gene expression, this project focuses on the expression of skin barrier-related genes towards HaCaT upon the treatment of cells with the ingredient's formulation of cosmetic Y. There are several relevant scopes of work that will be conducted, which are stated below.

- Homogeneity test to ensure the cosmetic product has properly cooperated with the cell growth medium.
- HaCaT cell culture will be conducted before the cell treatment.
- Cell treatment with moisturizer samples formulation.
- Cytotoxicity assay to determine the optimal concentration and duration for the cell treatment.
- Gene expression analysis using qRT-PCR methods.
- PCR product specificity validation using melting curve analysis and agarose gel electrophoresis.

1.4. Hypothesis

Several hypotheses are proposed based on the study, which are shown below.

- The amount of product that is recommended for the facial skin possesses low cytotoxicity.
- The recommended amount of product used on the face is suitable for cell treatment.
- The gene expression of the ceramide-biosynthesis, filaggrin, and hyaluronic acid-biosynthesis genes at the mRNA level is increased upon product treatment.
- The moisturizer formulation is effective in increasing skin barrier integrity.