CHAPTER I: INTRODUCTION

1. Background

The skin is one of the largest and most important organs in the body. It contributed to 16% of the total body weight of a human (McKnight et al., 2022). The skin functions as a waterproofing material, and thermal insulator, and also protects the body against environmental insults and/or pathogens. Essentially, the skin functions as a barrier to environmental noxious stimuli. There are several layers that make up the skin which is (in the descending order of depth from the outermost layer) the epidermis, dermis, and hypodermis (Gilaberte et al., 2016, p.1; Subramanian et al., 2011)). The epidermis can be further divided into several layers again, which are the (in the same descending order of depth from the outermost layer) *stratum corneum* (SC), *stratum lucidum* (SL), *stratum granulosum* (SG), *stratum spinosum* (SS), and the *stratum basale* (SB) layer (Nafisi & Maibach, 2018). The epidermis is perpetually regenerating, a process termed cornification. Keratinocytes from the SB layer migrate upwards, slowly maturing into terminally differentiated keratinocytes that form the outermost SC layer of the epidermis, and subsequently the skin (Gilaberte et al., 2016, p.2).

To function as a protector, the skin has a wide array of mechanisms such as antimicrobial peptides, hormones, neuropeptides, and cytokines to respond to noxious stimuli (Gilaberte et al., 2016, p.1). These innate epidermal defense mechanisms are activated in the event of penetration by microbes, allergens, and/or irritants (Kim & Leung, 2018) into the SS layer of the epidermis, where immune cells such as Langerhans cells (LCs) could be activated by microbes and allergens and subsequently trigger the adaptive immune response in the form of antibody formation and T-cell activation (Clayton et al., 2017). Keratinocytes of the SS layer, in and of itself, are also part of the innate immune system. They can release damage-associated molecular patterns (DAMPs) as pro-inflammatory signals to attract more immune cells to the site of infection or damage, thereby kickstarting the process of inflammation. It is therefore pertinent to highlight the importance of keratinocytes as the first-line responder in the immune response against extracellular insults, be it

against microbes, allergens, or irritants since they express different pattern recognition receptors (PRRs) on their surface to allow recognition of various external noxious stimuli and initiate immune responses (Chieosilapatham et al., 2021).

Normally, the skin itself is able to prevent the penetration of allergens and microbes, inhibiting transepidermal water loss (TEWL), and maintaining the skin pH and water content due to the SC layer of the epidermis (Kim & Leung, 2018). However, there are skin conditions where the skin barrier integrity is compromised and the penetration of these foreign substances is increased, such as seen in people with atopic dermatitis (AD), psoriasis, and, to an extent, sensitive skin syndrome (SSS) (Montero-Vilchez et al., 2021; Guerra-Tapia et al., 2019). It is due to this compromised barrier integrity that the skin is more susceptible or sensitive to irritants (soaps, shampoos, detergents, and cosmetics), environmental factors (cold, dry weather, and humidity), and allergens (house dust mites, pet fur, pollen, and molds) which will lead to inflammation (Kolb & Ferrer-Bruker, 2022).

In the event of inflammation, one of the pro-inflammatory cytokines produced by keratinocytes is IL-1α. It is constitutively expressed in a cell, meaning that there is a baseline expression of the gene responsible for IL-1α (*IL1A*). Noxious substances will cause cell death and these necrosed cells will release IL-1α into the environment, acting as the aforementioned DAMPs that will increase the expression of IL-1α in neighboring keratinocytes (Di Paolo & Shayakhmetov, 2016) while also promoting the infiltration of inflammatory immune cells to the site of inflammation by inducing the expression of adhesion molecules on endothelial cells (Gabay et al., 2010). This highlights the importance of IL-1α as an initiator of inflammation in the skin. Another inflammatory cytokine produced by keratinocytes, IL-1β, also functions in mediating inflammation and has similar roles to IL-1α since they both bind to the same receptor (Gabay et al., 2010). Tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine that can be produced by keratinocytes also plays an important role in the initiation of inflammation as it can also induce the expression of IL-1 (both α and β), IL-6, IL-8, and IL-33 to exacerbate the

inflammation (Mizuno et al., 2015). Apart from inflammation, the skin is also able to elicit neurogenic inflammation. This is because keratinocytes, like neurons, are derived from the ectoderm and express a variety of neurochemical properties and one of them is the ability to express calcitonin-gene related peptide (CGRP), a neuropeptide that can cause vasodilation, which further helps in the migration of immune cells into the site of inflammation (Hou et al., 2011).

Treatment options for these skin barrier dysfunction disorders are limited to topical application of prescriptions such as corticosteroids that could alleviate the inflammation, thereby allowing the skin to heal (Gabros et al., 2022; Kapur et al., 2018). Topical corticosteroids do not address the underlying skin barrier defect itself when in fact, treatment of skin barrier defect disorder management should focus on the skin barrier itself (Elias et al., 2019). Therefore, the skin barrier repair should precede immunomodulation, which is why attempts have been made to create topical prescriptions of barrier repair therapies (BRT) that supposedly promote skin barrier integrity repair while at the same time containing anti-inflammatory agents that will reduce the inflammation that manifests in the form of soothing sensation on the applied area. This is in line with the management of skin barrier dysfunction disorder explained by Boguniewicz et al. (2018).

Be that as it may, it is also pertinent to evaluate the safety of the BRT to support the efficacy of the product since a product will be as good as it is safe since it will heavily determine whether the product will reach the hands of a consumer to prevent the situation of doing more harm than good. This is because irritants may be contained in the BRT or any topical prescriptions (Tasic-Kostov et al., 2014) and may invoke undesirable adverse effects, depending on the composition of the product, a condition termed contact dermatitis. This was the case in 1933, with a cosmetic product under the name ‘Lash Lure’. It is an eyelash and eyebrow dye that once caused severe dermatitis of the eyelids and surrounding skin and exuberant conjunctival edema that began almost immediately after application. It caused blindness in one person, and even death in another. The culprit, Paraphenylenediamine (PPD) appeared to be able to cause an

allergic reaction in 1.5-6% of Europeans alone (Bacharewicz-Szczerbicka et al., 2019). In addition, the fact that the product is intended to be applied to the area of the skin that has the thinnest layer of SC, it further promotes adverse reactions since the SC layer is the most important layer of the epidermis in terms of preventing environmental stimuli penetration deep into the skin (Kim & Leung, 2018). This is analogous to skin barrier dysfunction disorders whereby the epidermis has an impaired skin barrier function due to a disrupted SC layer.

Taken together, this study aims to evaluate the inflammatory potential of a BRT in the form of a topical moisturizer that is intended to repair the skin barrier integrity while at the same time containing anti-inflammatory agents that will give a soothing sensation to the skin upon application. The study employed an *in vitro* testing whereby a Human Keratinocyte (HaCaT) cell line was treated with a moisturizer and a cytotoxicity assay was employed by means of an MTS assay. Previous studies have been conducted that evaluated the cytotoxic and inflammatory effects of chemical products or plant extracts and subsequently monitored their inflammatory capabilities or lack thereof by measuring the expression of proinflammatory cytokines on HaCaT cell lines. Choi & Hwang (2019) evaluated the anti-inflammatory properties of a plant extract by measuring the expression of TNF-α, IL-1α, IL-1β, IL-6, IL-8, and COX-2. Lee et al. (2016) evaluated the anti-inflammatory properties of a topical moisturizer by measuring the expression of human CGRP. To evaluate whether the moisturizer can cause inflammation, RT-qPCR was conducted to evaluate the expression of *IL1A*, *IL1B, CALCB,* and *CXCL8*. There are two forms of IL-1 and CGRP and both are encoded by separate genes (*IL1A* and *IL1B*; *CALCA* and *CALCB*). This study measured the expression of *IL1A, IL1B, CALCB,* and *CXCL8*.

2. Research Objectives

- To evaluate the cytotoxicity of a moisturizer product (Moisturizer-0921-F), base (Moisturizer-0921-G), API (Raw Mat-0921), and the positive control (SDS) using an MTS assay.

- To evaluate the potential inflammatory effect of a moisturizer product *in vitro* using a HaCaT cell line by RT-qPCR to measure the expression of *IL1A*, *IL1B*, *CALCB*, and *CXCL8*.

3. Scope of Work

- The scope of work for this thesis will focus on mammalian cell culture, the treatment thereof, and gene expression analysis. The type of work done in this project will be
    a. HaCaT cell culture
    b. Miscibility Test
    c. Cytotoxicity Test:
        i. MTS assay
    d. Gene expression analysis:
        i. Primer design
        ii. Total RNA Extraction
        iii. cDNA synthesis
        iv. Real-time RT-PCR
        v. Melting curve analysis
        vi. Agarose gel electrophoresis