Pharmaceutical Sciences Asia

Pharm Sci Asia 2025; 52(2), 230-239

DOI:10.29090/psa.2025.02.24.3254

Research Article

Development of Psoriasis-Like 3D Skin Models Using the HaCaT Keratinocyte Cell line as Potential Tools in Psoriasis Research

Intira Pathtubtim[1,3], Celine Viennet[2], Thanchanok Muangman[1,*], Jarupa Viyoch[3,*]

[1] Expert Centre of Innovative Herbal Products, Thailand Institute of Scientific and Technological Research (TISTR), Techno Polis, Klong Luang, Pathum Thani 12120, Thailand
[2] UMR 1098 RIGHT INSERM EFS FC, DImaCell Imaging Resource Center, University of Marie&Louis Pasteur, Besançon 25000, France
[3] Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok 65000, Thailand

ABSTRACT

This study focused on developing a psoriasis-like 3D skin model (RHE-Pso) utilizing HaCaT cells, which were treated with cytokine cocktails to serve as a platform for identifying anti-psoriasis agents. Reconstructed HacaT cells on high calcium condition were induced with cytokine cocktails on day 11 of culture We compared the morphology, epidermal protein, and cytokine release between the RHE-Pso and a control)RHE) that was not exposed to cytokines. The RHE-Pso model was subjected to two different cytokine formulas, which are known to be associated with psoriasis. Our findings revealed that the F1 formula (F1: 30 ng/mL TNF-α, 30 ng/mL IL-1 and 50 ng/mL IFN-γ) significantly altered the histological structure of the model more than the F2 formula (F2: 30 ng/mL IL-17 and 30 ng/mL IL-22), and resulted in lower filaggrin protein expression. Additionally, the RHE-Pso model induced with the F1 formula showed a markedly higher production of pro-inflammatory cytokines, including IL-1, IL-6, IL-8, IFN-γ, and TNF-α. Treatment of the RHE-Pso model with clobetasol propionate, an anti-psoriasis medication, effectively reduced tissue damage and decreased levels of inflammatory cytokines compared to the untreated model. This study demonstrates the potential of the RHE-Pso model as a valuable tool for exploring and validating new therapeutic compounds for psoriasis.

Keywords:
HaCaT; Psoriasis-like 3D skin model (RHE-Pso); Pro-inflammatory; Cytokine cocktails; Clobetasol propionate

1. INTRODUCTION

Psoriasis, a condition often linked to genetic predisposition[1], emerges in about one-third of global cases during childhood, triggered by external factors like skin trauma or chemical irritants, which can exacerbate symptoms. It's characterized by persistent inflammation, presenting as dry, red skin lesions with silver scales. These features arise from hyperproliferation and abnormal differentiation of keratinocytes, pivotal cells in the epidermal layer, leading to abnormal thickening of epidermal layer. This abnormality results from chronic inflammation driven by an abundance of immune cells, including conventional T cells and innate-like T cells, releasing numerous cytokines that activate keratinocytes. While the stimulation of conventional T cells, such as $CD4^+$ helper T cells, involves the recognition of peptides presented by major histocompatibility molecules on antigen-presenting cells, innate T cells like natural killer T cells by pass this recognition but can initiate a rapid immune response. Several studies suggest the efficacy of treating psoriasis with substances that inhibit cytokine production by helper T cells[2,3,4], underscoring the importance of these cytokines in psoriasis research and treatment.

***Corresponding author:**
* Thanchanok Muangman Email: Thanchanok@tistr.or.th
* Jarupa Viyoch Email: jarupav@nu.ac.th

Studies have reported that CD4+ helper T cells play a pivotal role in the initial phase of psoriasis by infiltrating the epidermal layer and subsequently triggering inflammation and hyperproliferation of keratinocytes through the release of various cytokines[5]. These cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor- α (TNF-α), interleukin-17 (IL-17), and IL-22, are closely associated with psoriasis development. They are predominantly released from T helper 1 (Th1) and Th17 cells, subtypes of $CD4^+$ helper T cells. IFN-γ is primarily released from Th1 cells, while IL-17 and IL-22 are released from Th17 cells. However, TNF-α can be produced by both Th1 and Th17 cells. Additionally, IL-23 produced by macrophages, Langerhans cells, and keratinocytes were facilitated by the function of Th17 cells, which are activated by the pro-inflammatory cytokine IL-1 released in response to stimuli triggered by keratinocytes. Moreover, there is evidence indicating a high concentration of IL-1 at the site of psoriasis lesions[6,7]. These findings suggest that IL-1 also plays a crucial role in the inflammation associated with psoriasis. As previously discussed, efforts to identify substances for treating psoriasis focus on targeting cytokines released from CD4+ helper T cells as biomarkers. In addition to in vivo models, in vitro cell or tissue-based models serve as effective tools for screening and discovering efficient psoriasis treatments and advancing our understanding of psoriasis molecular biology. Over the years, in vitro psoriasis models have been developed to deepen our understanding of the condition's biology and explore novel therapeutic candidates. Cells such as keratinocytes and fibroblasts, or tissues from psoriasis patients, prove beneficial for in vitro studies in terms of mimicking psoriasis conditions. However, limitations like insufficient growth and survival, and cell heterogeneity, hinder the use of patient-derived cells or tissues for in vitro studies. Alternatively, immortalized keratinocyte cell lines, such as HaCaT cell, is used to be induced as psoriasis by adding mixture of cytokines like IL-17, IL-22, IL-1α, TNF-α and oncostatin-M, or an immunomodulatory agent like imiquimod in the culture medium[8]. HaCaT cells are human epidermal keratinocytes that have undergone spontaneous immortalization[9]. These cells exhibit characteristics similar to normal keratinocytes in terms of differentiation ability, cytokine release, and transcriptional expression of protein markers such as keratin 10 (KRT10), keratin 14 (KRT14) and filaggrin, comparable to those in normal keratinocytes[10,11]. HaCaT cells are extensively used in keratinocyte monolayer culture models[12]. However, recent efforts have focused on constructing epidermal tissue from HaCaT cells to achieve a skin-like phenotype and responsiveness to external stimuli for toxicity testing of substances of interest.

For the discovery of anti-psoriasis substances intended for topical application on skin lesions, employing a 3D model offers advantages over 2D in vitro model. Unlike 2D models where tested substances must be dissolved in the culture medium, non-soluble substances or dosage forms can be directly applied to the surface of 3D reconstructed tissues, mimicking real usage conditions. In this study, our aim was to develop a psoriasis-like 3D skin model (RHE-Pso) using HaCaT cells cultured with two cytokine cocktails formulations (Formula 1 containing TNF-α, IL-1, and IFN-γ, and Formulation 2 containing IL-17 and IL-22). We observed the morphology, expression of filaggrin (a marker indicating the differentiated status of the epidermis), and the release of cytokines from the reconstructed tissue. Moreover, we examined the effect of clobetasol propionate, a topical glucocorticoid widely used for psoriasis treatment, on tissue morphology and the level of cytokines released. This was compared to a psoriasis-like 3D skin model not treated with clobetasol propionate. This study represents the first report of reconstructing psoriasis-like 3D skin models utilizing the HaCaT keratinocyte cell line induced by cytokines.

2. MATERIALS AND METHODS

2.1 Materials

The human skin keratinocyte cell line (HaCaT) was purchased from CLS Cell Lines Service GmbH, Germany (lot no. 3004934619). Dulbecco's Modified Eagle Medium (DMEM), DMEM/Nutrient Mixture F-12 (DMEM/F12), fetal bovine serum (FBS) and penicillin-streptomycin were purchased from GIBCO®, New York**,** USA. Keratinocyte growth kit from ATCC, Virginia,USA .Transwell® culture insert (24-well insert) was purchased from Corning, Arizona, USA. Paraformaldehyde solution was purchased from Sigma-Aldrich, Missouri, USA. FSC-22 clear frozen section compound was obtained from Leica, Illinois, USA. Anti-cytokeratin-10 (KRT10) rabbit monoclonal primary antibody, and anti-cytokeratin-14 (KRT14) mouse monoclonal primary antibody were purchased from Abcam, Cambridge, UK. Anti-filaggrin (FLG) mouse monoclonal primary antibody was purchased from Invitrogen, Carlsbad, USA. Goat anti-mouse IgG (Alexa Fluor 488(and goat anti-rabbit IgG secondary antibodies (Alexa Fluor 568) were purchased from Abcam. IL-1, IL-6, IL-8, IFN-γ and TNF- α ELISA kits were purchased from Abcam. Clobetasol propionate was purchased from Sigma, Saint louis, USA**.**

2.2 Cell culture

The human skin keratinocyte cell line, HaCaT was cultured in 75 cm^2 culture flasks using DMEM supplemented with 10% (v/v) FBS, and 1% (v/v) penicillin-streptomycin. Culturing took place within a humidified atmosphere incubator with 5% CO_2 at 37°C to facilitate cell proliferation.

2.3 Preparation of reconstructed human epidermis (RHE) utilizing HaCaT

HaCaT cells (passages 10-15) were seeded onto Transwell® culture insert (24-well insert at a density of $2x10^5$ cells/well. These cells were cultured in a medium composed of DMEM/F12 supplemented with a keratinocyte growth kit. The cell cultures were maintained at 37°C with 5% CO_2. On day **3** of culture, the old medium was replaced with fresh medium containing 2 mM $CaCl_2$ (high calcium media). The cells were then cultured for an additional **4** days. On day 7, the culture conditions were changed to an air-liquid interface, as shown in **Figure 1a**, and the cultured cells were maintained in this condition for 12 more days to induce epidermal differentiation. The high calcium medium was changed on days 9, 12, 15, and 18. The total cultivation period lasted for 19 days. The timeline of the tissue construction is shown in **Figure 1b**. The study was conducted in triplicate.

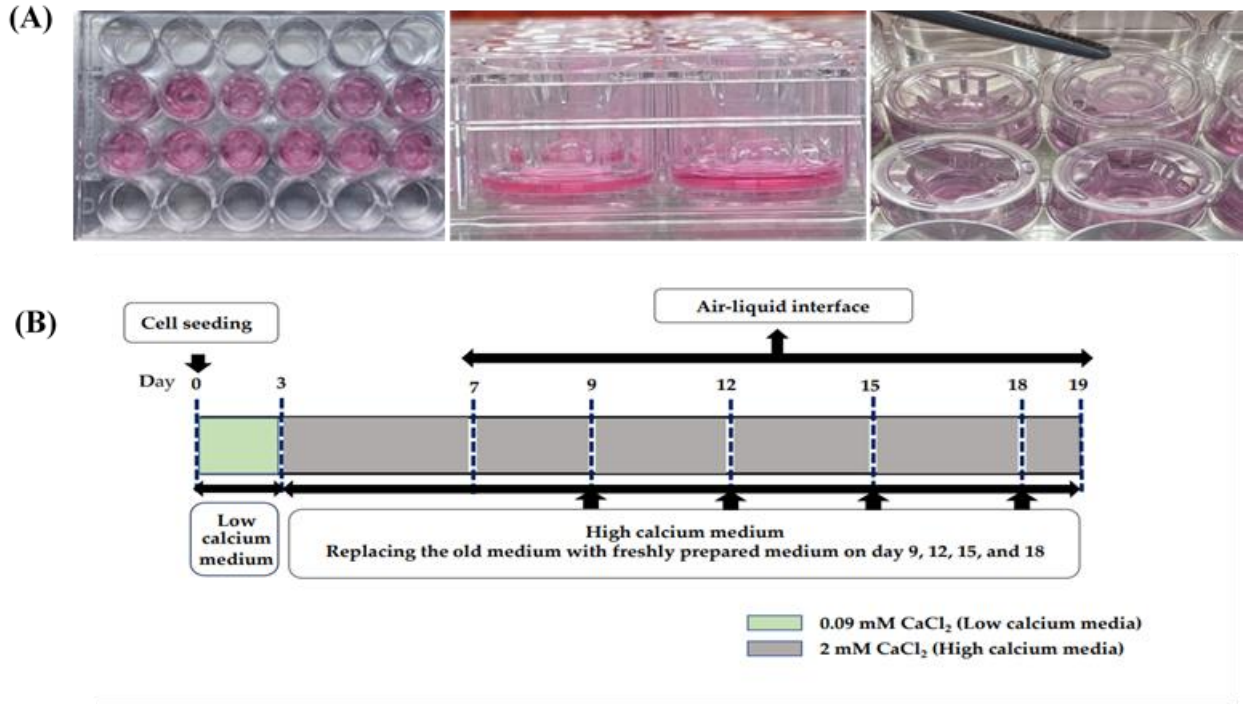

Figure 1 HaCaT keratinocyte cell line cultured on a 24-well insert under air-liquid interface conditions (A), along with a diagram illustrating the construction of the epidermis (RHE) (B).

2.4 Determination of physical and biological properties of RHE

2.4.1 Measurement of transcutaneous electrical resistance (TER)

TER values across the RHE were measured using a chopstick-like electrode connected to a Millicell-ERS (Electrical Resistance System, Millipore, Massachusetts, USA). The TER values were recorded as the measured resistance in ohms (Ω), with the resistance value of the tissue free-Transwell® subtracted. TER measurements were conducted continuously during the RHE formation period from day 7 to day 19.

2.4.2 Determination of RHE morphology

The RHE samples were immersed in a 4**%** paraformaldehyde solution for 15 min at room temperature. Subsequently, they were rinsed three times in 1X phosphate-buffered saline (PBS) for 5 min each and then embedded in FSC-22 clear frozen section compound to create 5 µm-thick vertical slides. The sectioned samples underwent staining with hematoxylin and eosin (H&E) to facilitate the observation of tissue morphology using an inverted microscope. Additionally, they were prepared for immunofluorescence analysis.

2.4.3 Determination of keratin 10, keratin 14, and filaggrin expressions

These sectioned samples obtained as mentioned above underwent immuno-fluorescence analysis for keratin 10 (KRT10), keratin 14 (KRT14), and filaggrin (FLG). Nonspecific antigens were blocked by incubating the sample in 10% bovine serum albumin (BSA) in PBS within a humidified chamber at room temperature for 60 min. Subsequently, the samples were incubated overnight at 4°C with the primary antibodies including rabbit anti-cytokeratin-10, mouse anti-cytokeratin-14 (Abcam), and mouse anti-filaggrin. After three rinses in PBS for 5 min each, the samples were incubated with secondary

antibodies including goat anti-mouse IgG (Alexa Fluor 488**)** or goat anti-rabbit IgG (Alexa Fluor 568) for 2 hr at room temperature in the dark. Immuno-fluorescence images were captured using fluorescence microscopy.

2.4.4 Induction of RHE into a psoriasis-like condition (RHE-Pso)

HaCaT cells were cultured as previously described. On the 8th day of cultivation, one day after transitioning to the air-liquid interface culture condition, psoriasis-associated cytokines were introduced into high calcium medium. Specifically, a cocktail of cytokineformulas was utilized, comprising cytokines formula 1 (F1: 30 ng/mL TNF-α, 30 ng/mL IL-1 and 50 ng/mL IFN-γ) or formula 2 (F2: 30 ng/mL IL-17 and 30 ng/mL IL-22), which were incorporated into the medium. The selection of each cytokine type and concentration was based on previous reports**.** The concentration was adapted from Hanna et al.[13]. and Smits[14]. with modifications to improve the accuracy of the results**.** The samples were then incubated with the cytokine-enriched high calcium medium for 3 days, with daily replacement of the medium using freshly prepared cytokine-enriched medium during this period. The total cultivation duration extended to 11 days, as outlined in **Figure 2**. The study was conducted in triplicate.

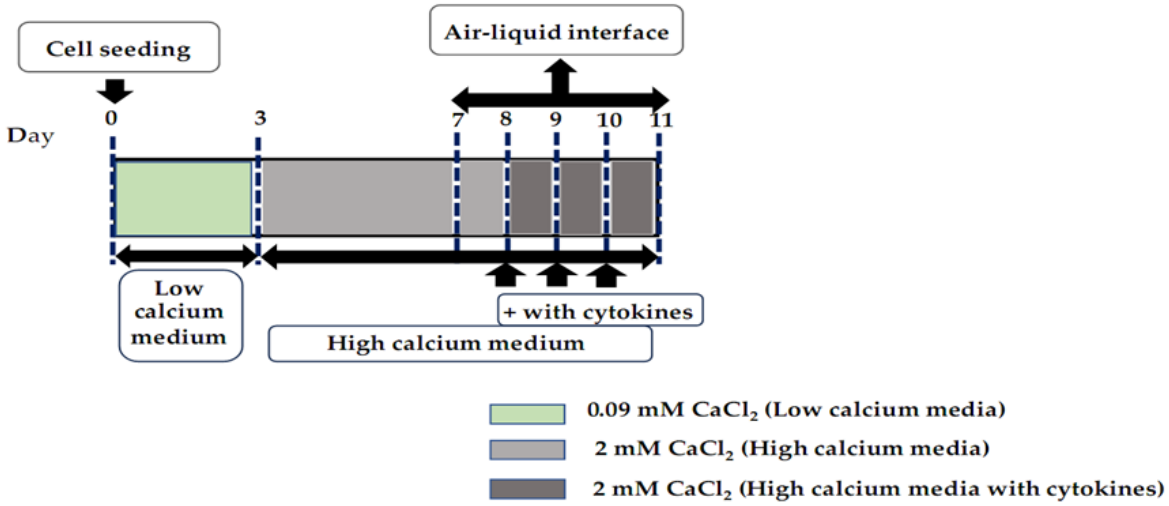

Figure 2 Diagram illustrating the construction of psoriasis-like epidermal tissue (RHE-Pso) utilizing the HaCaT keratinocyte cell line.

2.5 Evaluation of morphology and biological properties of RHE-Pso

2.5.1 Determination of RHE-Pso morphology

The RHE-Pso tissues were sectioned following the previously described above. The sectioned samples were stained with H&E to observe their morphology under an inverted microscope and used for immunofluorescent analysis.

2.5.2 Determination of filaggrin expression

The sectioned tissues were incubated in blocking buffer for 60 min before undergoing an overnight incubation with primary antibody at 4°C. This was followed by three 5-min washes in PBS. Subsequently, the washed sections were exposed to the secondary antibody, goat anti-mouse IgG (Alexa Fluor 488) for 2 hr at room temperature in the dark. Immunofluorescence images were captured using fluorescence microscopy.

2.5.3 Determination of inflammatory mediators

After 24 hr from the last induction, the cultured medium was collected to determine the levels of inflammatory cytokines, including IL-1, IL-6, IL-8, IFN-γ, and TNF-α. This analysis was performed using ELISA kits, following the recommended procedures provided by the manufacturer.

2.5.4 Determination of the effects of clobetasol propionate (topical corticosteroid for psoriasis treatment) on the levels of inflammatory mediators released from RHE-Pso

To assess the suitability of RHE-Pso as a model for investigating psoriasis treatment with a specific compound of interest, clobetasol propionate, a common drug for psoriasis treatment, at a concentration of 10 ug/mL, was introduced into the medium along with F1 or F2 cytokine formula. The mixture was used to incubate the HaCaT- reconstructed human epidermis during the

induction period for 3 days, with daily replacement of the medium containing cytokines and clobetasol propionate**.** Following this incubation period, a morphological evaluation of the tissues was conducted using H&E staining. Additionally, the culture medium was collected to analyze the levels of inflammatory mediators including IL-1, IL-6, IL-8, IFN-γ, and TNF-α using ELISA assay.

2.6 Statistical Analysis

The data are presented as mean ± SD and analyzed using one-way analysis of variance (One way ANOVA). The results are considered statistically significant when the *p*-value is ≤ 0.5.

3. RESULTS

3.1 Physical and biological properties of RHE

According to TER measurement, there was a significant increase in TER values from day 7 (156.56 ± 1.19 Ω/cm^2) to day 11 (174.99 ± 5.76 Ω/cm^2), followed by a subsequent decline to 144.91 ± 2.12 and 139.39 ± 6.03 Ω/cm^2 by day 15 and 19, respectively. Notably, these fluctuations in TER values were observed in conjunction with the development of tissue structures, as shown in **Figure 3**. Specifically, on day 11, the presence of a suprabasal layer is evident, along with the outermost layer containing cells lacking nuclei. The tissue thickness on day 11 was 218.75 ± 24.16 µm. Turning attention to cellular differentiation, on day 11, four days of post-air-liquid interface exposure, cells above the basal layer exhibited larger nuclei compared to those in the basal layer. Above this layer, cells flattened, and at the outermost layer, nuclei were no longer visible. However, by day 15 and 19, such distinctive appearance and integrity of the tissue layer were lost.

Based on the results obtained from fluorescence microscopy imaging of skin tissue, as shown in **Figure 4**, it is evident that both KRT10 and KRT14 proteins exhibited staining within the skin tissue. Notably, on day 7, the intensity of KRT14 staining surpassed that of the KRT10 protein. Conversely, from day 11 to day 19, there was a shift in the staining pattern, with the intensity of KRT14 protein staining being lower compared to that of the KRT10 protein. For the protein marker FLG, its fluorescent staining was clearly observed on the day 11 of cultivation.

3.2 Morphology and biological properties of RHE-Pso

The 11-day cultivation system with cytokine formulas, F1 and F2, provided the tissues with different histological characteristics as compared to the tissues obtained from the cultivation system without cytokine induction, according to H&E staining in **Figure 5**. In the case of the cultivation system stimulated with the F1 cytokine formula, the histological structure exhibited features indicative of incomplete tissue integrity and detachment of the epidermal layer. Conversely, the skin tissue resulting from the induction with the F2 cytokine formula showed comparatively lower disruption of the formed tissue compared to the F1-stimulated system. Examining the expression of FLG protein, a lower staining intensity was observed in the skin tissue induced by both F1 and F2 cytokine formulas compared to the control group. Furthermore, it was noteworthy that in the skin tissue stimulated with the F1 cytokine formula, the intensity of FLG protein staining was the lowest among the conditions. Psoriasis-like 3D skin model (RHE-Pso) was significant increasing the inflammatory cytokine levels compare with RHE **(Table 1).**

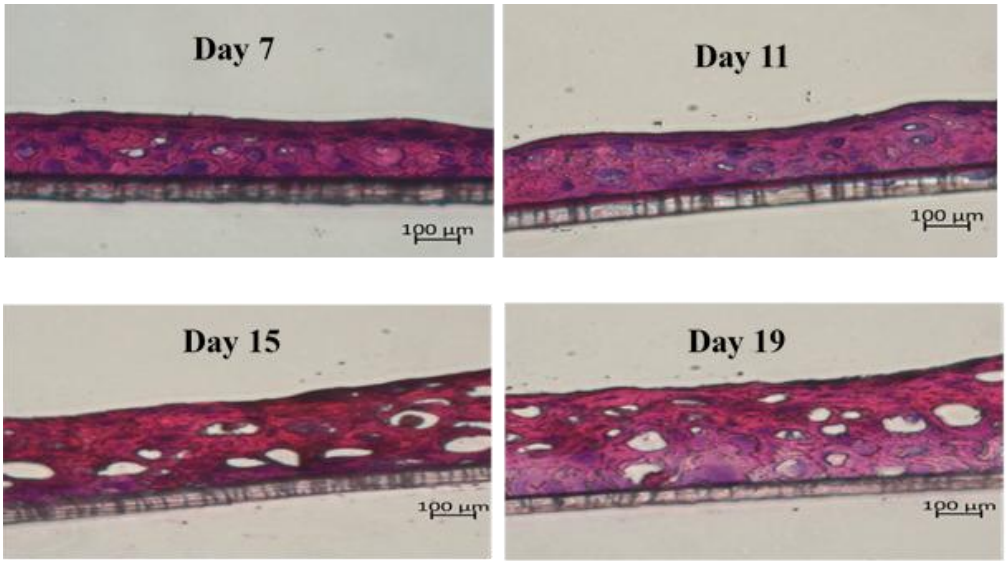

Figure 3 H&E staining images of the reconstructed human epidermis (RHE) utilizing the HaCaT keratinocyte cell line on days 7 (the first day of air-liquid interface cultivation), 11,15, and 19.

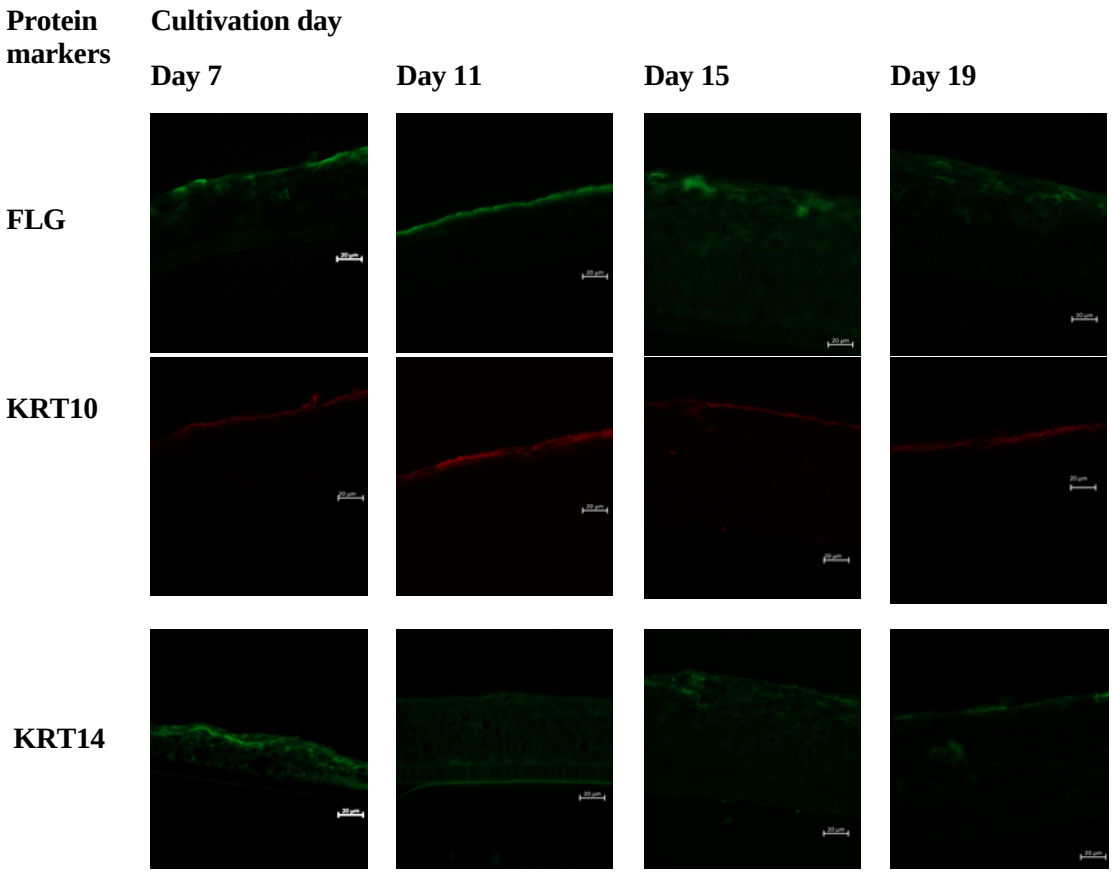

Figure 4 Expression of protein markers, including filaggrin (FLG, in green), Keratin 10 (KRT10, in red), and Keratin 14 (KRT14, in green) during the reconstruction of epidermal tissue (RHE)

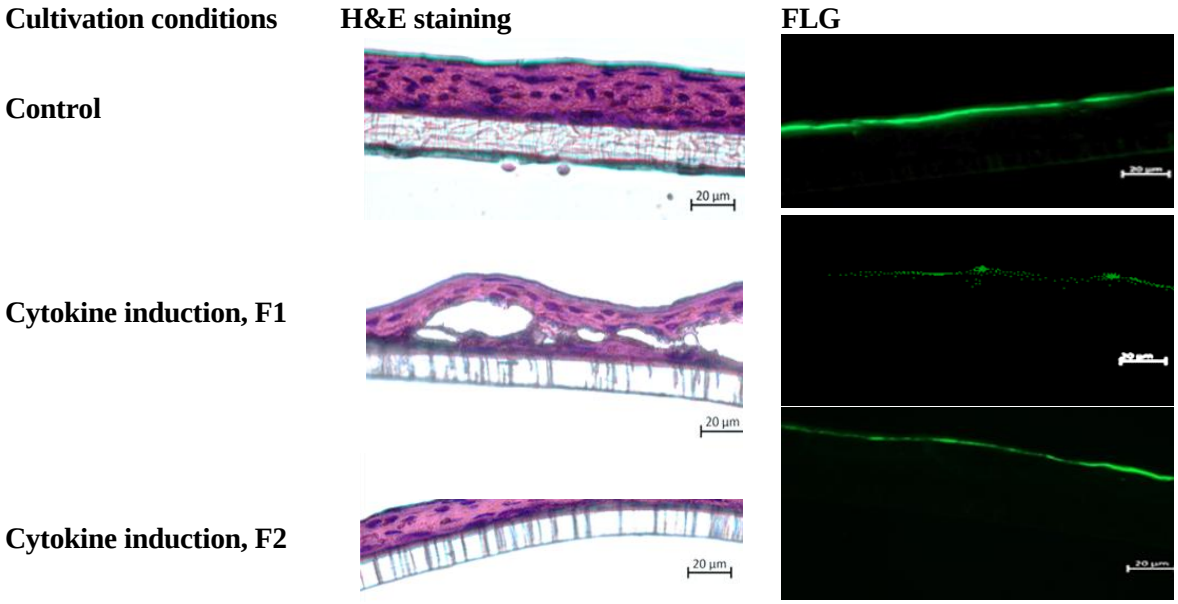

Figure 5 H&E staining images and immunofluorescent staining of filaggrin (FLG) protein expression of the reconstructed epidermal tissue utilizing HaCaT keratinocyte cells induced by cytokines (RHE-Pso), Formula 1 and Formula 2.

Table 1. Levels of inflammatory mediators released from reconstructed human epidermis (RHE) and tissues induced into a psoriasis-like condition (RHE-Pso). Data are presented as mean ± SD of n = 6. a**, b**, and c**, $p < 0.01$ as comparing the control with the formula 1 group, the control with the formula 2 group, and the formula 1 with the formula 2 group, respectively.

Inflammatory cytokines	Level of cytokines (pg/mL)		
	RHE	RHE-Pso induced by	
	Control	Formula 1	Formula 2
IL-1	17.57±2.82	338.12±27.84^{a**}	252.79±13.03$^{b**, c**}$
IL-6	42.52±4.43	247.67±0.56^{a**}	111.24±22.33$^{b**, c**}$
IL-8	319.97±5.01	447.89±15.43^{a**}	426.04±13.08$^{b**, c**}$
IFN-γ	533.27±71.04	4,628.54±358.52^{a**}	2,885.00±528.57$^{b**, c**}$
TNF-α	118.69±2.14	431.23±26.54^{a**}	339.03 ± 8.46$^{b**, c**}$

3.3 Morphology changes and levels of inflammatory mediators released from RHE-Pso treated with clobetasol propionate

The RHE-PSo condition was utilized to explore the impact of clobetasol propionate, a commonly prescribed drug for psoriasis treatment, on both the morphology **Figure 6** and the levels of released cytokines, as depicted in **Table 2**. Our findings reveal that the introduction of clobetasol propionate into the medium, in conjunction with either F1 or F2 cytokine formula, led to a significant decrease in the levels of released cytokines compared to those induced by the F1 or F2 groups alone, without clobetasol propionate. Additionally, tissues treated with clobetasol propionate exhibited improved integrity and reduced epidermal detachment compared to those without clobetasol propionate in the RHE-Pso condition.

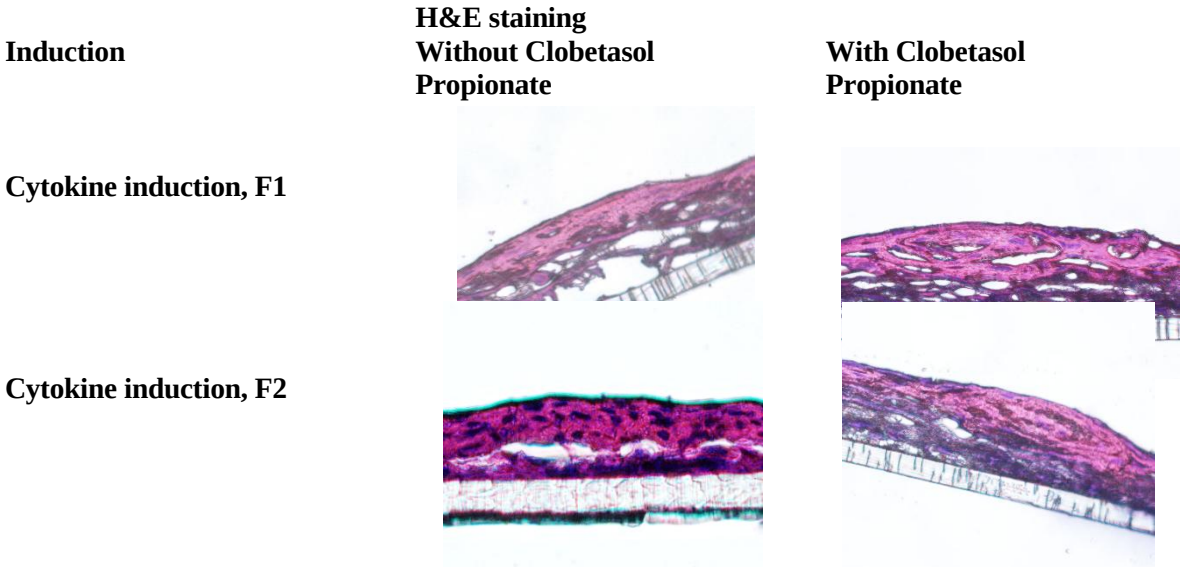

Figure 6 H&E staining images of reconstructed human epidermis induced into a psoriasis-like condition (RHE-Pso) using cytokine Formula 1 and Formula 2 both with and without the presence of clobetasol propionate.

Table 2 Levels of inflammatory mediators released from reconstructed human epidermis induced into a psoriasis-like condition (RHE-Pso), with or without incorporation of clobetasol propionate. Data are presented as mean ± SD of n =6 a** and b**, $p < 0.01$ when comparing between the groups with and without clobetasol propionate for formulas F1 and F2, respectively.

Inflammatory cytokines	Control	Level of cytokines (pg/mL)			
		Formula 1		Formula 2	
		Without Clobetasol Propionate	With Clobetasol Propionate	Without Clobetasol Propionate	With Clobetasol Propionate
IL-1	17.57 ± 2.82	338.12 ± 27.84	238.98 ± 8.16^{a**}	252.79 ± 13.03	241.50 ± 8.35^{b**}
IL-6	42.52 ± 4.43	247.67 ± 0.56	229.88 ± 1.41^{a**}	111.24 ± 22.33	37.98 ± 5.44 ^{b**}
IL-8	319.97 ± 5.01	447.89 ± 15.43	358.80 ± 0.60^{a**}	426.04 ± 13.08	379.60 ± 0.4^{b**}
IFN-γ	533.27 ± 71.04	4,628.54 ± 358.52	3,097.39 ± 7.84^{a**}	2,885.00 ± 528.57	1,167.65 ± 6.85^{b**}
TNF-α	118.69 ± 2.14	431.23 ± 26.54	178.15 ± 19.04^{a**}	339.03 ± 8.46	256.33 ± 4.52^{b**}

4. DISCUSSION

Due to limitations associated with using cells or tissues from psoriasis patients for in vitro cell culture, such as insufficient growth and survival, and heterogeneity, we have developed a psoriasis-like 3D epidermal tissue (RHE-Pso) using an immortalized keratinocyte cell line, HaCaT. This model serves as a valuable tool for investigating the efficacy of substances intended for psoriasis treatment.

Initially, HaCaT cells were cultured in DMEM/F12 supplemented with necessary growth and function factors under low calcium conditions to mimic the basal stratum environment conducive to cell proliferation[15]. Keratin 14 (KRT14) serves as a critical marker under this condition[16]. After 3 days of cultivation, the culture conditions were transitioned to high calcium levels, initiating differentiation. Calcium-regulated signaling pathways drive this differentiation, leading to morphological changes, development of cell-cell junction, and increased intracellular calcium levels, triggering the expression of epidermal protein markers such as keratin 10 (KRT10), involucrin, and filaggrin (FLG)[17,18]. Employing the air-liquid interface cultivation technique further enhances keratinocyte differentiation and the formation of suprabasal layers, resulting in the development of the outermost layer lacking nuclei. The complete formation of the epidermal layer, characterized by the highest TER (transepithelial electrical resistance) value, was typically observed by day 11 of cultivation (4 days of air-liquid interface cultivation), coinciding with increased expression of KRT10 and filaggrin. Subsequently, a decrease in TER values correlates with the onset of non-integrity formation in the epidermis at days 15 of cultivation. Our data indicate that, based on our cultivation condition, an 11-day cultivation period could provide the formation of an epidermal layer comprising crucial epidermal markers including KRT10 and FLG.

The pathogenesis of psoriasis is complex, involving interactions among keratinocytes, immune cells, and various other skin cells. Recent research has emphasized the pivotal role of immune cells, particularly CD4+ helper T cells, in the early stages of psoriasis. Notably, TNF-α and IFN-γ are primarily released from T helper 1 (Th1) cells, while IL-17 and IL-22 are released from Th17 cells, both subsets of CD4+ helper T cells. These cytokines, including TNF-α, IFN-γ, IL-17, and IL-22, exert their influence on keratinocytes, thereby exacerbating inflammation in psoriasis by triggering the release of several cytokines, such as IL-1, IL-6, IL-8, IFN-γ, and TNF-α from keratinocytes[19]. This underscores the role of keratinocytes as amplifiers in psoriasis. Consequently, the development of a psoriasis-like model using the HaCaT keratinocyte cell line holds promise as a tool for psoriasis research and for exploring potential anti-psoriasis agents.

In our study, psoriasis-like 3D skin model (RHE-Pso) was generated by introducing cytokines into the culture medium. While acknowledging that psoriasis stems from chronic inflammation, it is important to note that incorporating cytokines into the culture medium may not entirely replicate the chronic inflammatory state driven by an abundance of helper T cells. Nevertheless, this approach can mimic the conditions observed in psoriasis lesions, where keratinocyte cells are exposed to numerous cytokines and become activated.

In our RHE-Pso cultivation protocol, cells were initially cultured under low calcium condition for 3 days, followed by a transition to high calcium conditions, and then to the air-liquid interface condition, loosely resembling the RHE culture conditions. One day after transitioning to the air-liquid interface, psoriasis-associated cytokines (F1: 30 ng/mL TNF-α, 30 ng/mL IL-1 and 50 ng/mL IFN-γ; F2: 30 ng/mL IL-17 and 30 ng/mL IL-22) were introduced into the high calcium medium. The total cultivation duration was extended to 11 days. We observed that the F1 cytokine formula induced incomplete tissue integrity and detachment of the epidermal layer more strongly than the F2 formula and also led to reduced FLG expression. Indeed, TNF-α, IFN-γ, IL-17, and IL-22 have the ability to activate keratinocytes, initiating various cell signaling pathways, which can ultimately lead to excessive proliferation of keratinocytes. However, IFN- γ represents the potent stimulus for keratinocyte, and TNF-α, IFN-γ, and IL-1 have been shown to provoke damage to the plasma membrane and tissue destruction. These actions may contribute to the observed epidermal layer detachment, incomplete formation of epidermal tissue, and low expression of FLG observed in the RHE-Pso induced by the F1 cytokine formula.

When examining the release of cytokines, such as IL-1, IL-6, IL-8, IFN-γ, and TNF-α, introducing either the psoriasis-associated cytokine formulas, F1 or F2, into the culture medium led to a significant increase in cytokine release, particularly IL-1 (14-19 times higher than the control), IFN-γ (5-8 times higher than the control), IL-6 (3-6 times higher than the control), and TNF-α (about 3 times higher than the control). Remarkably, the F1 formula elicited higher levels of cytokine release from the tissue compared to the F2 formula. Generally, TNF-α increases in pathogenic processes by promoting the production of inflammatory mediators such as IL-1, IL-6, and IL-8, which are released from keratinocytes[20,21], leading to tissue destruction. Additionally, TNF-α can potentiate the effect of IFN-γ, one of the key cytokines in the pathogenesis of psoriasis, in keratinocytes. ,TNF-α, IL-1, and IFN-γ are key stimulators that enhance the production of the

inflammatory cytokine IL-8 by activating the NF-κB and MAPK signaling pathways. IL-8 primarily functions to recruit neutrophils to the site of inflammation, leading to their accumulation in the epidermis, which serves as a significant indicator of psoriasis.

Although IFN-γ is mainly produced by Th1 cells, there have been reports indicating its production by keratinocytes. Collectively, these factors contribute to the substantial release of cytokines, particularly, IL-1, IL-6, TNF- α, and IFN-γ, in the culture system with F1 introduction, resembling the environment of psoriasis lesions characterized by elevated concentrations of IL-1, IL-6, TNF-α, and IFN-γ . Conversely, these cytokines serve as potential indicators for predicting the psoriasis inflammation of the skin tissue.

Clobetasol propionate, a potent topical glucocorticoid, is widely utilized for managing various skin conditions, including psoriasis, due to its robust anti-inflammatory and anti-proliferative activities. It has been reported that topical application of clobetasol propionate can alleviate psoriasis-related skin inflammation and abnormal hyperproliferation[22], while also reducing serum levels of IL-6 and TNF-α. These effects may arise from clobetasol propionate's ability to reduce HaCaT proliferation and suppress TNF signaling. Consequently, our study observed that introducing clobetasol propionate into the medium of RHE-Pso led to reduced epidermal detachment and improved tissue integrity, particularly noticeable in RHE-Pso treated with F1 plus clobetasol propionate, alongside a decrease in cytokine levels, notably TNF-α. Given TNF-α's role in tissue destruction, lowering its levels may mitigate inflammation and tissue abnormalities. Our findings collectively demonstrate that inducing a psoriasis-like condition in reconstructed tissue from HaCaT cells can be achieved by introducing a cytokine cocktail, particularly a combination of TNF-α, IL-1, and IFN-γ. Moreover, IL-1, IL-6, TNF-α, and IFN-γ hold potential as cytokine markers for indicating psoriasis inflammation in in vitro studies aimed at exploring anti-psoriasis substances.

5. CONCLUSIONS

The HaCaT cell line, a spontaneously immortalized human keratinocyte cell line due to its ability to respond to calcium levels (low and high), which facilitates the processes of differentiation. This capability is crucial for modeling calcium-driven differentiation, a key factor in understanding skin cell behaviors. Additionally, the strong response of HaCaT cells to inflammatory stimuli underscores their utility in developing sophisticated models, such as the psoriasis-like 3D skin model (RHE-Pso). Our project developed the RHE-Pso model using HaCaT cells, which were cultured for a total of 11 days. Three days before cultivation, the cells were induced with cytokine cocktails, specifically the F1 and F2 formulas. This led to morphological changes and alterations in cytokine release. The F1 formula has a strong effect on the detachment of the epidermal layer and leads to a significant increase in cytokine release; on the other hand, it reduces FLG expression. The clarity of this testing is further enhanced when conducting trials with Clobetasol Propionate and without Clobetasol Propionate show significantly different inflammatory responses. The consistent performance of these cells supports their ongoing use in dermatological research, particularly for exploring the pathophysiology of skin diseases and evaluating potential therapeutic interventions. In the next phase of the implementation plan, it is necessary to test the RHE-Pso model in conjunction with other drug types and standard substances that have therapeutic effects on psoriasis to gather data for confirming the model's validity in response to various medications.

6. ACKNOWLEDGEMENTS

This study was a part of the research project funded by the Ministry of Higher Education Science. Research and Innovation the Thailand Institute of Scientific and Technological Research *(*TISTR*)* and research funding of Franco-Thai Cooperation Programme in Higher Education and Research (Franco-Thai Mobility Programme / PHC SIAM) Year 2023 – 2024.

Ethics approval

This research does not involve human subjects

Conflict of interest

none to declare

Funding

This study was a part of the research project funded by the Ministry of Higher Education Science. Research and Innovation the Thailand Institute of Scientific and Technological Research (TISTR).

Author contribution

Intira Pathtubtim designed the study, analyzed data, and prepared the manuscript. Celine Viennet, Thanchanok Muangman, and Jarupa Viyoch suggested the experimental design. All authors contributed to the study and approved the final version.

Article info:

Received November 9, 2024
Received in revised form March 5, 2025
Accepted March 18, 2025

REFERENCES

1. Francesca C. The Genetic basis of psoriasis. Int J Mol Sci.2017;18,2526.

2. Peng Z, Yuwen S, Siying L, Hui C, Ruifang W, Haijing W. The Roles of T cells in psoriasis. Front.Immunol.2023;14,1081256.
3. Wenlu Y, Chumxiu L, Deju Z, Zhangwang L, Panpan X, Xiao L, et al. Advances in T cells based on inflammation in metabolic diseas. Cells. 2022;11,3554.
4. Lina S, Yanhong S, Anjun J, Xin W, Baojun Z. T cells in health and disease. Signal Transduct Target Ther.2023; 8-235.
5. Franziska G, Andreas K, Edgar S, Matthias G,Khalid M. Current Developments in the Immunology of Psoriasis.Yale J. Biol. Med .2020;93, 97-110
6. Erin F, Erin H, lliyana S, Stephen E.K, Andrew B. Pathophysiology of psoriasis: recent advances on IL-23 and Th17 Cytokines. Curr Rheumatol Rep. 2007;9(6): 461–7.
7. Yihua C, Feng X, Chen Q, Minye Q, Na L, Yuan Z, et al. Critical role of the IL-1β-IL-1R signaling pathway in skin inflammation and psoriasis pathogenesis. J Invest Dermatol. 2019;139(1), 146–56.
8. Sandeep R V, Thiyagarajan O S, Abheepsa M, Sunil P, Rafiq M, Rangesh P. Imiquimod-induced psoriasis inflammation in differentiated human keratinocytes: its evaluation using curcumin. Eur.J.Pharmacol .2017; 813,33-41
9. Petra B, Rule T P, Dirk B, Jiirgen H, Alex M, Norbert E. F. Normal Keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. Int. J. Biochem. Cell Biol. 1998;106,761-71
10. Takao H,Yuichi I, Masakazu K, Masukazu I, Ken-ichi H. Keratinization induced by air exposure in the reconstructed human epidermal model: an in vitro model of a cultured epithelial autograft. J. Biosci. Bioeng, 2014;118(3), 323-6
11. Irwin M F, Marjana T C, Mayumi K, Miroslav B. Keratins and the Keratinocyte Activation Cycle. J Invest Dermatol.2001;116,633-40
12. Boelsma.E, Verhoeven.M.C and Ponec.M. Reconstruction of a Human Skin Equivalent Using a Spontaneously Transformed Keratinocyte Cell Line (HaCaT)J Invest Dermatol. 1999;112:489– 98
13. Hanna N, Gijs R, Ivonne M J J, Diana R O, Piet E J, Patrick L J M,et al. Identification of keratinocyte mitogens implication for hyperproliferation in psoriasis and atopic dermatitis. JID Innov.2022; 2, 100066
14. Smits J P H, Niehues H, Rikken G, Vlijmen-Willems I, Zande G, Zeeuwen P, et al. Immortalized N/TERT keratinocytes as an alternative cell source in 3D human epidermal models. Sci. Rep.2017;7:11838
15. Esther B, Mary C H V, Maria P. Reconstruction of a Human Skin Equivalent Using a Spontaneously Transformed Keratinocyte Cell Line (HaCaT). J Invest Dermatol. 1999;112,489–98
16. Hunain A, Lalit S, Samrat T K, Sorab N. D, Milind M V. Novel function of keratins 5 and 14 in proliferation and differentiation of stratified epithelial cells. Mol Biol Cell.2011;22 (21) 4068–78
17. Mirentxu S, Jesu M P, Ana B, Angel R, Jose´L J. The Expression of Keratin K10 in the Basal Layer of the Epidermis Inhibits Cell Proliferation and Prevents Skin Tumorigenesis. J Biol Chem.2002;277,19122-30.
18. Steinert P M, Marekov L N. The proteins elafin, filaggrin, keratin intermediate filament, loricrin and small proline-rich proteins 1 and 2 are isodipeptide cross-linked components of the human epidermal cornified. J Biol Chem.1995; 270, 17702-11
19. Nograles K E, Zaba L C, Guttman E, Fuentes-Duculan J, SuarezFarinas M, Cardinale I, et al. Th17 Cytokines Interleukin (IL)-17 and IL-22 Modulate Distinct Inflammatory and Keratinocyte-Response Pathways. Br J Dermatol. 2008;159(5),1092–102.
20. Michelle A L, Mayte S F, James G K. Immunology of psoriasis. Annu Rev Immunol. 2014;32,227-55
21. Hakim B A, Claus J, Lars I. Key signaling pathways in psoriasis: recent insights from antipsoriatic therapeutics. Psoriasis: Targets Ther. 2021; 11, 83-97
22. Anroop B. N, Sunil K, Pooja D, Chahat N, Sweta D,Rekha R, et al. Novel Dermal Delivery Cargos of Clobetasol Propionate: An Update. Pharmaceutics 2022;14, 383.