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"An active perfume ingredient could stimulate the olfactive receptors of the human skin keratinocytes"

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INTRODUCTION

Olfactory receptors (ORs), members of the G protein-coupled receptor (GPCR) superfamily, were originally identified for their role in odor detection within the nasal olfactory epithelium. These receptors detect volatile compounds and convert the chemical signals into neuronal activity, ultimately resulting in odor perception. However, over the past two decades, the traditional view of ORs as limited to olfaction has changed radically. A growing body of literature has revealed the ectopic expression of ORs in a wide variety of non-olfactory tissues, including the liver, intestine, heart, testis and, notably theskin. This ectopic expression has led researchers to explore their broader physiological roles than olfaction, such as tissue homeostasis, cellular communication and regeneration [1–4] . In the skin, ORs are expressed in various cell types, including melanocytes, dermal fibroblasts and epidermal keratinocytes. . Among these, keratinocytes are the most abundant cells in the epidermis, serve as an ideal model for studying the functionality of ORs in skin physiology. Recent studies have identified a subset of skin-specific ORs involved in key processes such as cell migration, proliferation, and wound healing. Notable examples include OR2AT4, OR51B5, and OR2A4/7.

For instance, synthetic sandalwood odorants have been shown to activate OR2AT4, so enhancing re-epithelialization via cAMP signaling pathways [4,5]. Similarly, OR51B5 expression correlates with increased cell motility and tissue remodeling [1,6], while OR2A4/7 seems to be involved in cellular proliferationpathways [7]. These discoveries have changed the field of dermatological science , prompting new strategies for targeting ORs in regenerative medicine and skincare field.

At the interface between dermatology and cosmetic science, dermocosmetics typically combine bioactive molecules that interact with cellular signaling pathways to protect and moisturize the skin. Some perfume components traditionally selected as fragrances are now being re-evaluated to investigate their biological activity, particularly as potential modulators of ORs in the skin [8]. This development opens up an exciting approach where topical formulations could stimulate OR-mediated signaling pathways to enhance skin renewal and repair.The aim of this study is to assess the biological activity of a dermocosmetic healing cream (containing, VITAMIN B3, UREA ,Polysaccharide-complex and an active perfum) . In particular,we investigated its effect on the expression and intracellular localization of OR2AT4, OR51B5, and OR2A4/7 in cultured human epidermal keratinocytes. Additionally, using a wound healing assay, we also determined the functional impact of the cream on keratinocyte migration and healing potential. In this way, we aimed to close the gap between molecular OR signaling and macroscopic results of skin regeneration.

MATERIALS AND METHODS

Human epidermal keratinocytes-cell Culture

Primary human epidermal keratinocytes (HEKp) were purchased from CELLnTEC (Switzerland). These cells are basal-layer progenitor keratinocytes pooled from multiple donors and were obtained to ensure physiological relevance and reproducibility. Cells were maintained in CnT-PR medium (CELLnTEC) and culture flasks were kept at 37 °C in a humidified condition with 5% CO_2. Four days after the initial culture, the cell medium was changed and subsequently replaced twice a week using Celloneer KC/CC medium (a base medium for keratinocytes).**Product testing and Dilutions**

The tested dermocosmetic healing cream (containing, VITAMIN B3, UREA ,Polysaccharide-complex and an active perfum) was diluted in culture medium to final concentrations of 0.05%, 0.1%, 0.5%, and 1%. These dilutions were selected based on preliminary cytotoxicity testing and to reflect physiologically relevant topical concentrations. Prior to application, the cream was thoroughly mixed with the culture medium to ensure uniform dispersion.**Cytotoxicity**

Assessment (MTT Assay)

To determine maximum non-cytotoxic concentrations of the test cream, keratinocytes were seeded and treated for 24 hours with serial dilutions of the cream (ranging from 0.005% to 2%). Cell viability was evaluated using the MTT assay. Briefly, after treatment, MTT reagent was added and cells incubated, then absorbance was read using a FLUOstar OPTIMA

fluorometer (BMG Labtech, France). All values were normalized to untreated controls. Experiments were carried out in triplicate.**Immunofluorescence for Olfactory Receptor**

Detection

HEK cells were seeded in multiwell plates, treated with the cream dilutions for 24 hours, then fixed with 4% paraformaldehyde for 10 minutes. After PBS washing, cells were permeabilized with 0.2% Triton X-100 in PBS for 10 minutes at room temperature and blocked with 1% BSA + 0.1% Tween-20 for 60 minutes at 37 °C..

During this experiment we used different primary antibodies such as OR2AT4 (Invitrogen, PA5-39811), OR51B5 (Invitrogen, PA5-102273) and OR2A4/7 (Invitrogen, PA5-102168). Antibodies were applied overnight at 4 °C in blocking buffer. The next day, three washes with 1X PBS were applied and then Alexa Fluor® 488 goat anti-rabbit IgG (Invitrogen, A11008) was used as the secondary antibody for 2 hours at room temperature. DAPI (Invitrogen, D3571) was added for nuclear counterstaining during 10 min at room temperature. Imaging was performed using a fluorescence microscope, and fluorescence intensity was quantified using a FLUOstar OPTIMA plate reader.

Wound Healing (Scratch) Assay

Keratinocytes were seeded in 96-well plates and grown to confluence. A uniform scratch wound was introduced using the 96-Well WoundMaker™. After PBS rinsing to remove detached cells and debris, fresh media containing designated concentrations of healing Cream were added.

Cell migration into the wound area was monitored using IncuCyte® live-cell imaging over a 24-hour period. Images were captured every hour. Wound closure rate was calculated based on the area covered at the time of 50% closure in control wells. The migration behavior was compared across treatment groups. Visual confirmation was also performed via video-microscopy at key time points.

Statistical Analysis

Quantitative data from the MTT viability assay and immunofluorescence-based receptor expression analyses were evaluated using one-way ANOVA. A p-value of less than 0.05 was considered statistically significant. Data are presented as the mean ± standard deviation (SD) from at least three independent experiments.

RESULTS

1. Cytotoxicity of the healing Cream on Human Keratinocytes

To assess the biocompatibility of the dermocosmetic formulation, HEK cells were incubated for 24 hours in a series of healing Cream concentrations (0.005%–2%), followed by MTT viability measurement. All tested concentrations demonstrated no significant cytotoxic effects compared to untreated controls (fig. 1). Based on these data, concentrations of 0.05%, 0.1%, 0.5%, and 1% were selected for subsequent mechanistic assays.

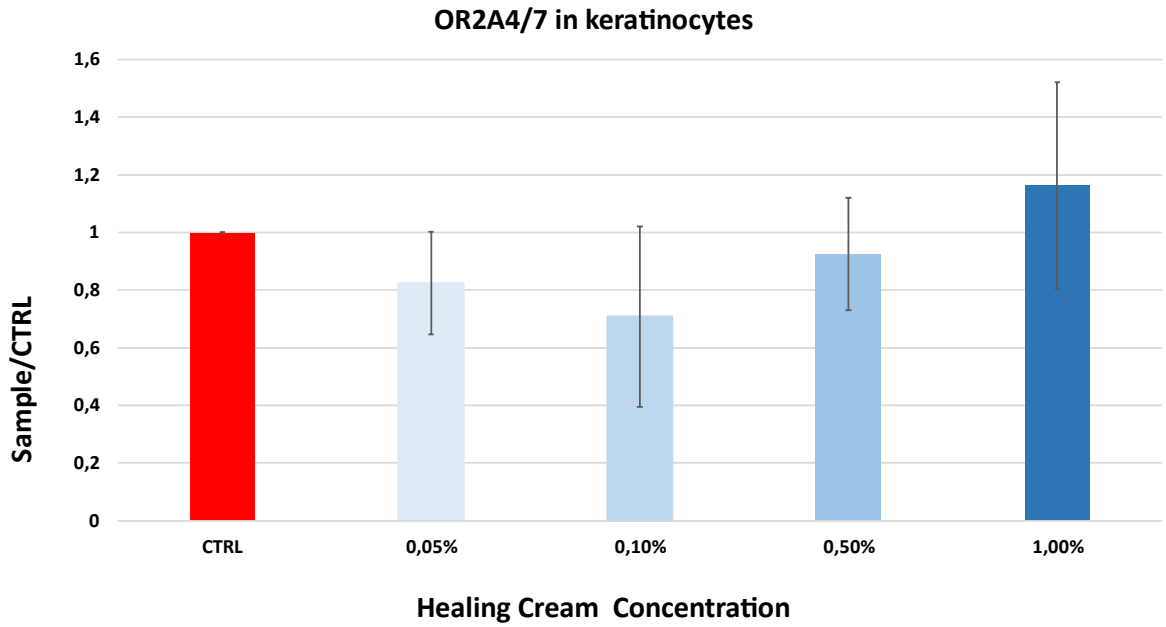

Figure 1: Effect of Healing Cream on HEK viability after 24h exposure, expressed as percentage of control (MTT assay).

2. Expression level and Localization of OR2AT4

To examine the variation in OR2AT4 expression between control and treated keratinocytes, a quantitative fluorescence assay was conducted. The results showed no significant change in receptor expression across the tested concentrations when compared to untreated controls (Figure 2).

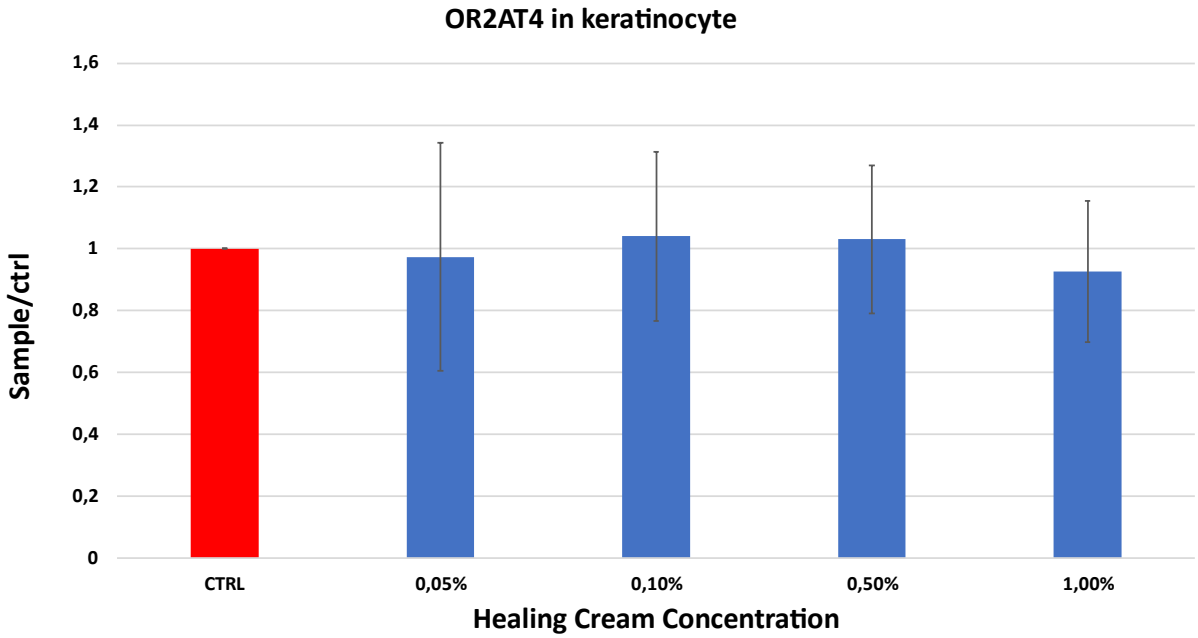

Figure 2: Quantitative analysis of OR2AT4 receptor expression after Healing cream application.

However, the immunofluorescence assay revealed a clear redistribution of the receptor's localization. In untreated cells, OR2AT4 was predominantly localized in the perinuclear region. In contrast, cells treated with healing Cream exhibited a broader staining pattern, including cytoplasmic and membrane-associated localization, , suggestive of receptor mobilization toward sites of functional activity (fig. 3).

This translocation may indicate receptor activation, even in the absence of increased overall protein expression levels. Such shifts in localization are consistent with G protein-coupled receptor (GPCR) trafficking dynamics observed upon ligand interaction in other systems.

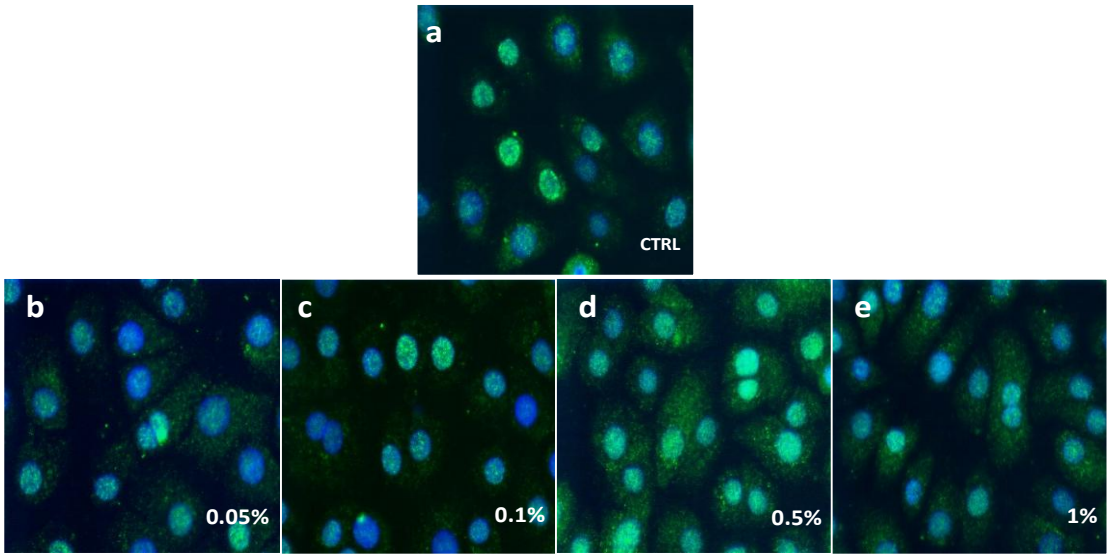

Figure 3: Immunofluorescence illustration showing OR2AT4 localization in control versus Healing cream treated cells. DAPI (blue) and OR2AT4 (green). Immunofluorescence illustration showing OR2AT4 localization in control cell (CTRL, a) and Healing cream-treated cells at different concentrations: 0.05% (b), 0.1% (c), 0.5% (d), and 1% (e). Nuclei are stained with DAPI (blue) and OR51B5 is shown in green.

3. Expression and Functional Enhancement of OR51B5

Among the three tested receptors, OR51B5 demonstrated a statistically significant increase by approximately 6% in the expression following treatment with 0.5% of healing Cream ($p = 0.01$). This effect was dose -dependent, with maximal expression observed at the intermediate dose (fig. 4).

Following immunofluorescence, unlike OR2AT4, no marked changes in subcellular localization were seen, and the receptor remained broadly cytoplasmic (fig. 5).

These findings suggest a true upregulation in receptor production rather than redistribution. Given the known role of OR51B5 in modulating the cell migration, this expression boost may contribute directly to the regenerative properties of the cream.

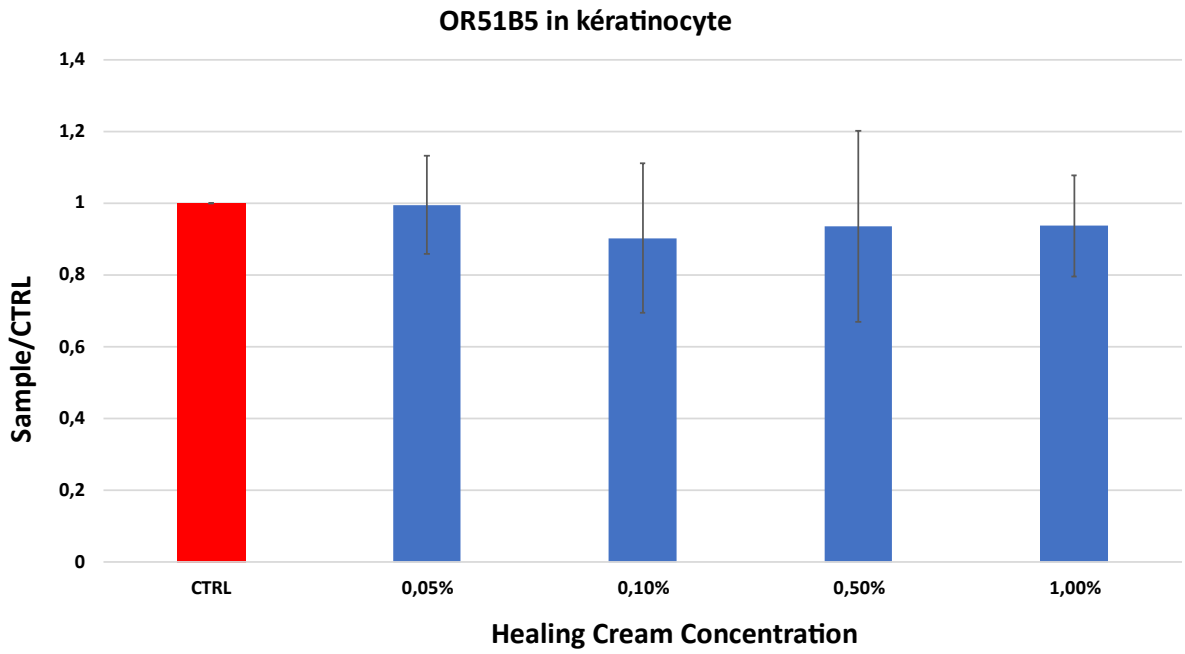

Figure 4 : Expression levels of OR51B5 across control and Healing cream treated groups.

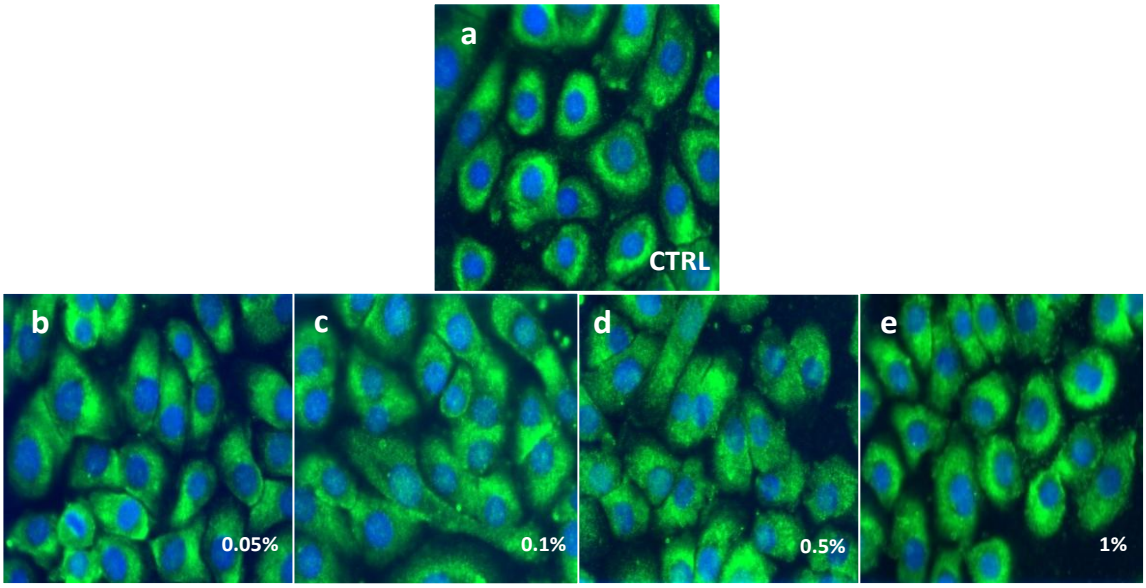

Figure 5 : Illustration of the effect of healing Cream on the expression and localization of OR51B5 receptors in cultured human keratinocytes. DAPI (blue) and OR51B5 (green). Immunofluorescence illustration showing OR51B5 localization in control cells (CTRL, a) and Healing cream-treated cells at different concentrations: 0.05% (b), 0.1% (c), 0.5% (d), and 1% (e). Nuclei are stained with DAPI (blue) and OR51B5 is shown in green.

4. Trends in OR2A4/7 Expression and Membrane Clustering

To evaluate potential changes in the expression, a quantitative analysis of OR2A4/7 expression was carried out. Considering the level of alteration in the expression of other olfactory receptors after incubation with the healing cream, , the results revealed a trend towards an increase at the 1% concentration of the cream, though without reaching statistical significance ($p = 0.076$) (fig.6).

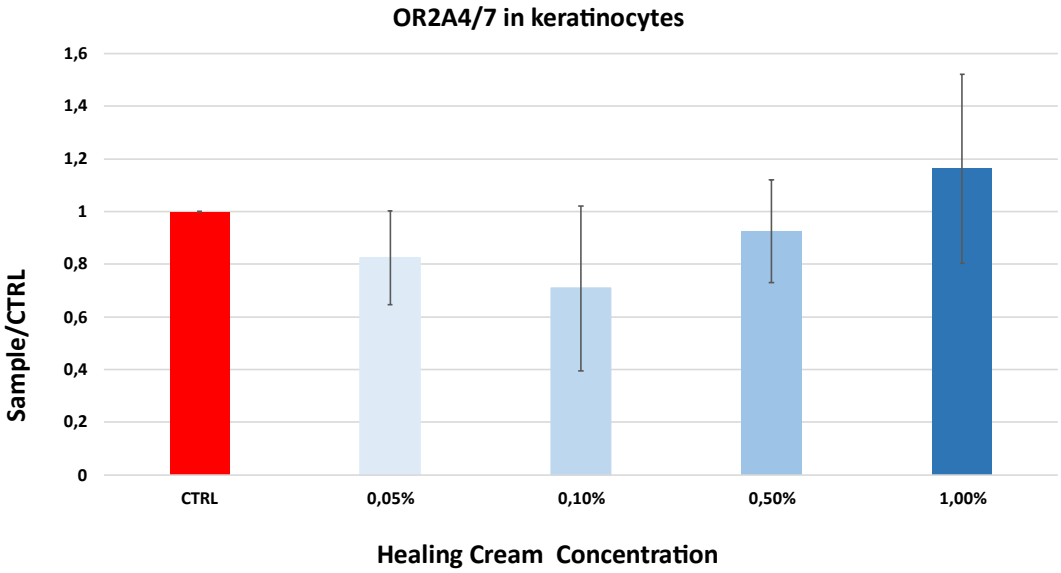

Figure 6: Quantitative measurement of the Effect of Tested Cream on OR2A4/7 expression. However, qualitative examination by fluorescence microscopy showed distinct clustering of OR2A4/7 receptors at the plasma membrane in treated cells. These clusters may reflect GPCR dimerization or multimerization processes, potentially indicative of receptor activation and the involvement of downstream signaling. Notably, these patterns were not observed in untreated cells, where OR2A4/7 appears to be diffusely localized (fig.7). The clustering effect is particularly compelling given this receptor's association with proliferative signalling pathways.

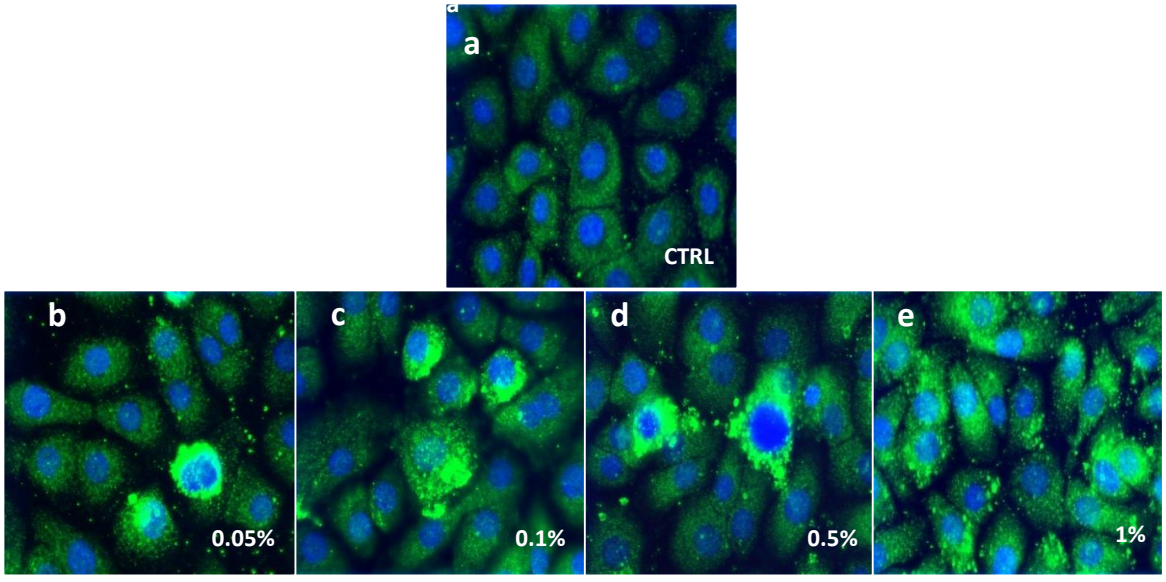

Figure 7: Membrane-associated receptor clusters in treated cells. DAPI (blue) and OR2A4/7 (green). Immunofluorescence illustration showing membrane-associated receptor clusters in control cells (CTRL, a) and treated cells at different concentrations: 0.05% (b), 0.1% (c), 0.5% (d), and 1% (e). Nuclei are stained with DAPI (blue) and OR2A4/7 is shown in green.

5. Wound Healing Assay Reveals Biphasic Migration Response

In order to evaluate the migration ability of keratinocytes after incubation with the tested cream, a scratch test was performed over 24 hours on a monolayer keratinocyte culture. After wounding, the cells were incubated in the presence of different concentrations of the test Cream and migration was measured with the Incucyte® live-cell imaging device or optically monitored with a video microscope.

Functional analysis of keratinocyte migration showed a biphasic effect of B3 Recovery Cream at low concentrations (0.05 % and 0.1 %). The keratinocytes showed accelerated wound closure during migration compared to the control before reaching 50% wound closure (from 49% for the control to 68% for the treated cells). This improvement was observed in the early phase (first 13 hours) of migration, with a higher percentage of the wound area covered at the half-closure time point (figures.8 ,9).

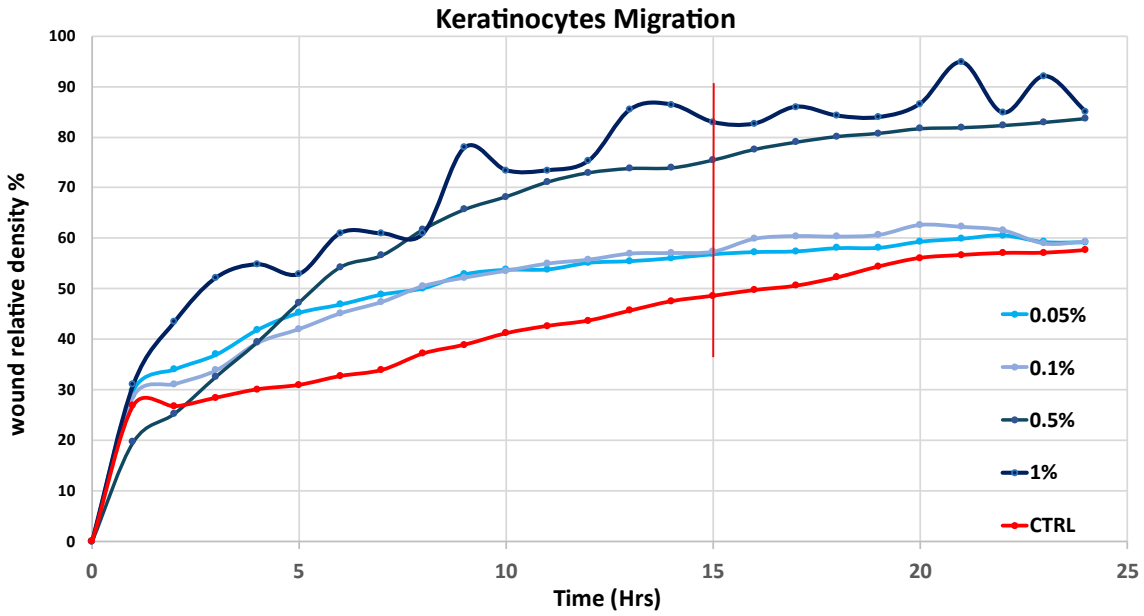

Figure 8 : Wound closure kinetics measured by IncuCyte. The wound closure rate is calculated at the point when 50% of the wound surface is covered by migrating cells in untreated control. The wound closure rates for different doses of the product are determined at ½ wound closure (red vertical bar).

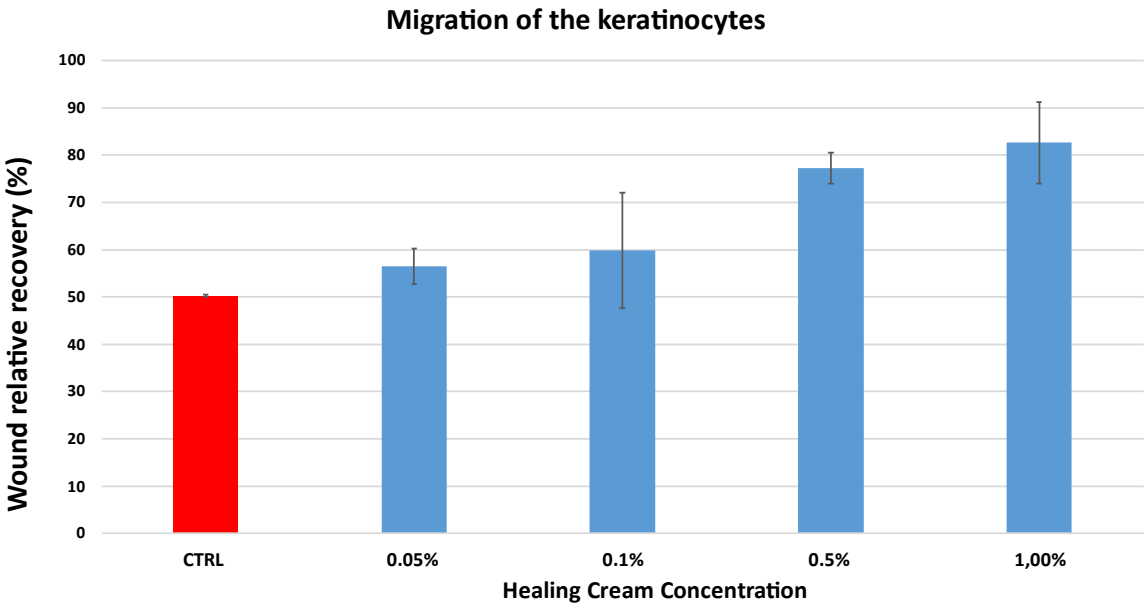

Figure 9: Quantitative measurement of the percentage of wound surface covered by keratinocytes at the time of ½ wound closure in control across cream concentrations.

However, once the measurement time exceeds the time of half wound closure, migration rates begin to decline. After 26 hours, the cells treated with the cream showed incomplete wound closure, while the untreated control cells achieved complete re-epithelialization. Microscopic imaging has indicated that excess cream may physically impede migration by forming a viscous layer in the wound gap, trapping cells and blocking further movement (fig. 10).

In addition, at concentrations above 0.5%, the cream interfered with IncuCyte® measurements by being falsely detected as part of the cellular monolayer, leading to biased readings toward the end of the experiment. This artifact may result from the device detecting cream deposits as occupied surface area.

Overall, while the cream appears to enhance initial keratinocyte migration, it may interfere with the monolayer wound healing model at later stages, either due to the physical presence of the formulation or due to limitations in surface detection by the imaging system.

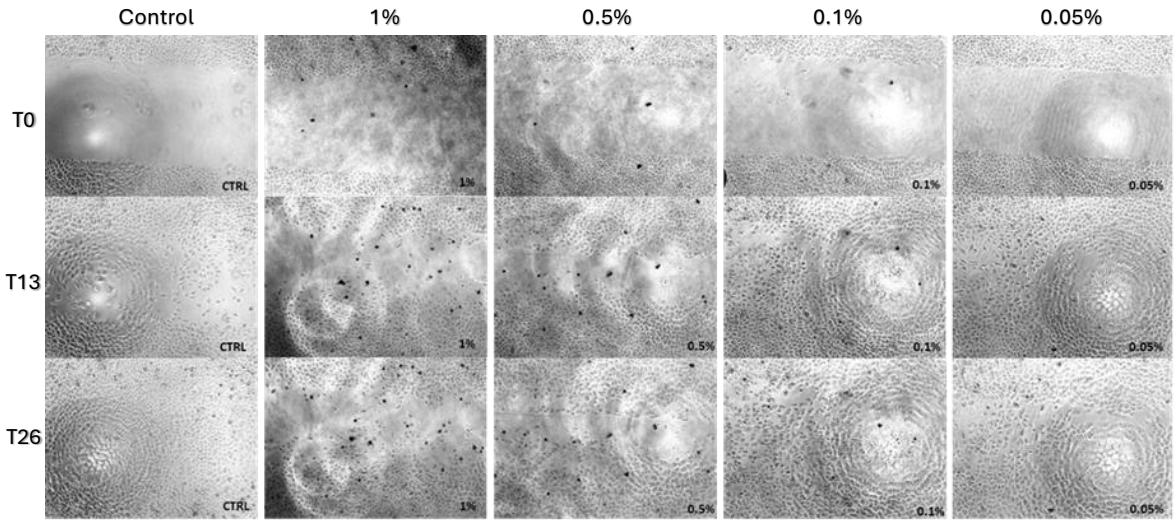

Figure10 : Microscopy time-lapse images showing the keratinocyte migration at 0h, 13h, and 26h with different concentration of 1%, 0.5%, 0.1% and 0.05%.

On the other hand, the cream, especially at concentrations above 0.5%, interferes with the measurements of the Incucyte® device at the site (detection of the cream by the device, which adapts to the surfaces occupied by the keratinocytes), leading to potentially biased measurements at the end of the experiment.

Although the healing cream showed to enhance the keratinocyte migration, the final results is missing because of the texture of the cream which interfered with the monolayer wound healing model, especially at later stages. This model is better adapted to the medications as there is not a heavy texture. The other probably reason could bedue to limitations in surface detection by the imaging system.

DISCUSSION

This study provides novel evidence that a dermocosmetic cream containing an active perfume ingredient can modulate the expression and intracellular localization of olfactory receptors in human epidermal keratinocytes, with functional consequences on wound healing behavior. These findings reinforce the evolving view that olfactory receptors are not restricted to sensory tissues, but instead play multifaceted roles in peripheral organs such as the skin [1,2].

In line with previous researches, the unrestricted level of OR2AT4 expression and a marked redistribution from perinuclear regions to the cytoplasm and plasma membrane, could suggest that receptor trafficking may biologically be as relevant as expression quantity. The previous publications demonstrated that by activating OR2AT4 with synthetic sandalwood odorants,

[6,9] the intracellular cAMP and calcium are elevatedleading to the stimulation of the keratinocyte migration and proliferation [9,10]. Although the second messengers were not measured in the present study, the observed subcellular mobilization supports the hypothesis of receptor sensitization or engagement by the active ingredient within the cream. This can address to an early stage of signaling readiness, potentially preceding measurable up-regulation [4,11].

In contrast, OR51B5 demonstrated a statistically significant up-regulation in response to 0.5% cream concentration. This dose-specific effect suggests a concentration window within which the perfume ingredient effectively interacts with the receptor or its regulatory transcriptional machinery. Although OR51B5 has been less extensively studied than OR2AT4, transcriptomic datasets have reported its expression in actively migrating keratinocytes and its involvement in cytoskeletal remodeling [3,7]. Its selective induction in our model supports a role in mediating the early migration-enhancing effects observed in our wound healing assays [12].

Moving to OR2A4/7, this olfactory receptor showed a dynamic spatial changes, with increased expression and membrane clustering observed at the 1% concentration. Clustering of GPCRs is a known hallmark of receptor activation, particularly in the context of ligand-induced signaling platforms at the cell surface [13,14]. Although the p-value for upregulation did not cross the significance threshold, the morphological evidence of clustering is compelling. OR2A4/7 has been implicated in pathways regulating keratinocyte proliferation and cellular renewal [4,7], and its behavior here may indicate that the cream influences proliferative signaling even without large quantitative changes.

A wound healing assay was performed to add a layer of relevance to our molecular findings on the active perfume ingredient studied. The cream promoted migration during the initial phase of re-epithelialization at low concentrations, reflecting the expected impact of OR activation. However, at higher concentrations, the cream's physical properties appeared to impede cell migration. This observation highlights an important aspect of dermocosmetic development: the formulation matrix can paradoxically offset or limit the bioactivity of otherwise effective ingredients. Similar biphasic responses have been observed in other topical studies, where increased viscosity or occlusion can trap cells or block directional movement despite initial stimulation [15,16].

These findings open up several possibilities for future research. Studies measuring second messengers such as cAMP, calcium or ERK phosphorylation could confirm that the changes observed in the ORs translate into functional signaling cascades. In addition, post-treatment transcriptomic or proteomic profiling could reveal the downstream genes regulated by these olfactory pathways. In the wider context of dermatological science and product innovation, this work adds to the growing recognition that skin ORs represent untapped pharmacological

targets. Their modulation by dermocosmetics introduces a mechanistic layer into topical skincare products that goes beyond hydration or barrier reinforcement.

CONCLUSION

This study demonstrates, for the first time, that a dermocosmetic formulation containing an active ingredient can modulate the expression and intracellular distribution of key olfactory receptors; OR2AT4, OR51B5, and OR2A4/7; in human epidermal keratinocytes. These molecular changes appear to influence fundamental processes such as cell migration and potentially proliferation, underlining the ability of topical formulations to engage receptor-mediated regenerative pathways in the skin.

While OR2AT4 showed no significant change in expression, its cytoplasmic and membrane redistribution suggests early-stage receptor engagement, consistent with priming for signal transduction. OR51B5 expression was significantly increased at an optimal concentration, reinforcing its proposed role in migration and tissue remodeling. Meanwhile, OR2A4/7 exhibited receptor clustering at the membrane, a morphological hallmark of activation, even in the absence of statistical upregulation.

Functionally, these molecular changes translated into enhanced keratinocyte migration in vitro, particularly during the early phase of wound healing. However, this beneficial effect was attenuated at higher cream concentrations, probably due to physical interference from the cream matrix.

Taken together, these results support a new paradigm in dermocosmetic development, in which bioactive fragrance compounds are not simply sensory additives but functional modulators of epidermal biology. As our understanding of cutaneous olfactory receptors deepens, they could become new targets for skin regeneration, barrier repair and anti-aging interventions. Future studies focusing on downstream signaling pathways, transcriptomic changes and 3D skin models will be essential to fully exploit this potential and refine ingredient formulation for maximum efficacy.

CONFLICT OF INTEREST

The study fees paid by Fillmed Laboratories. FF, SC and RB are the employee of FILLMED in the scientific department. The other authors declare no conflicts of interest related to this work.

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