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3-D Skin Model Study of UV-Induced Sebum Oxidation and Antioxidants

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1. Introduction

The scalp and hair are frequently subjected to a myriad of external factors that can lead to significant damage and irritation. [1] Among these factors, the oxidation of sebum stands out as a critical concern, particularly in the context of ultraviolet (UV) exposure. Sebum, an oily substance produced by sebaceous glands, plays a vital role in maintaining the health and integrity of the scalp and hair. However, its oxidation can initiate a cascade of negative effects that compromise scalp health and overall hair quality. [2] Research indicates that oxidized sebum can lead to a range of adverse outcomes, including scalp irritation, the development of unpleasant odors, increased greasiness, and potential damage to hair follicles. These issues not only affect the aesthetic appearance of hair but can also contribute to conditions such as seborrheic dermatitis and other forms of scalp inflammation.

The process of sebum oxidation is particularly exacerbated by environmental stressors such as UV radiation, which can generate reactive oxygen species (ROS) that further exacerbate oxidative stress on the scalp. [3] ROS are highly reactive molecules that can damage cellular structures, including lipids, proteins, and DNA, leading to inflammation and impaired skin barrier function. Consequently, there is a growing interest in strategies aimed at minimizing sebum oxidation as a means to enhance scalp health and mitigate the detrimental effects of oxidative stress.

In light of these concerns, the present study aimed to develop a novel and robust method for measuring UV-induced sebum oxidation on both scalp and skin using a sophisticated three-dimensional (3-D) skin model. This innovative approach allows for a more accurate simulation of the human scalp environment, facilitating a better understanding of the complex interplay between sebum, oxidative stress, and environmental factors. The primary objectives of this research were twofold: first, to assess the contribution of sebum to the formation of reactive oxygen species (ROS) under UV exposure, and second, to evaluate the protective effects of

natural antioxidants, specifically rosemary extract, against oxidative stress induced by sebum oxidation.

Rosemary extract has garnered attention in recent years for its potential antioxidant properties, which may offer a protective effect against oxidative damage. By exploring the efficacy of this natural extract, the study aims to identify viable strategies for promoting scalp health and mitigating the adverse effects of sebum oxidation.

2. Materials and Methods

Rosemary was supplied from Hunan Health-Guard Bio-Tech (Yongzhou, Hunan China). Rosmarinic acid, squalene and Dihydrofluorescein diacetate (DHF-DA) were supplied from Sigma (Sigma Aldrich). MatTek EpiDerm™ EPI-200 cultures were purchased from MatTek (MatTek Corp., MA, USA).

MatTek Epiderm cultures were incubated for 18hrs in the provided buffer solution before treatments. A solution of Dihydrofluorescein diacetate (DHF-DA) was prepared in H2O/EtOH (1:1) (300 µM). For selected treatment legs Rosmarinic extract (RME) (3.4 mg, 0.17%) or rosemary extract (6% rosmarinic acid) was dissolved in 2 mL of the DHF-DA solution. An aliquot of the solution of fluorescence probe and antioxidant (20 µL) was added onto the pre-warmed (37 C) 3-D skin surface. The skins were incubated at 37 ℃, 5% CO2 for 30 minutes. Squalene (8 µL) was applied after incubation as a thin evenly spread layer on top of the skin. The sample was irradiated for 12 min at 110 mW/cm2 (10.7 BED) and analysed by Fluorimetry. The procedure was repeated by triplicate (Figure 1).

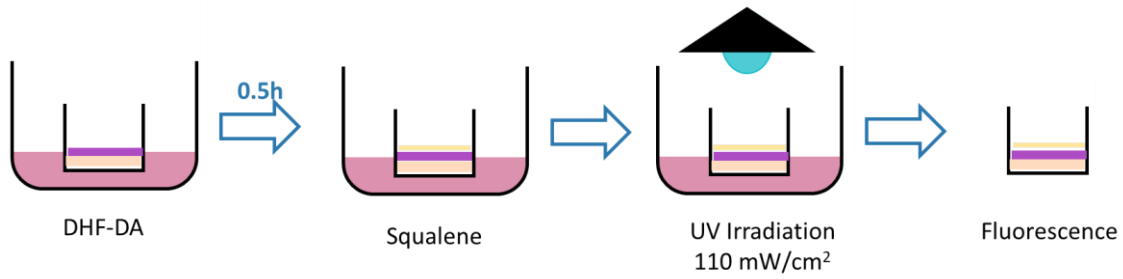


Figure 1. The schematic for how the MatTek Epiderm 3D Skin Models is performed.

3. Results and Discussion

The MatTek EpiDerm 3D skin model is a Reconstructed Human Epidermis (RHE) composed of normal, human-derived epidermal keratinocytes. It features a three-dimensional structure that includes organized and proliferative basal cells, spinous and granular layers, as well as cornified epidermal layers, all of which are both mitotically and metabolically active (Figure 2). This complex architecture allows for a more accurate assessment of how various materials interact with the skin, enabling researchers to draw conclusions that are more relevant to consumer experiences.

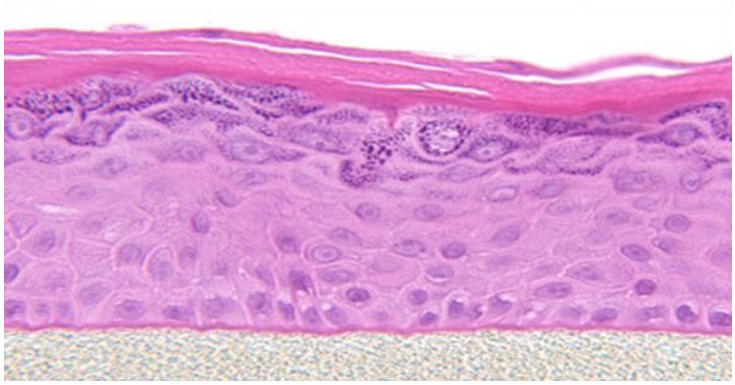


Figure 2. The skin structure of a MatTek EpiDerm 3D skin model.

To evaluate the damage inflicted on the skin and scalp by UV-induced oxidation, we utilized Dihydrofluorescein diacetate (DHF-DA) as a fluorescence probe. DHF-DA fluoresces in the presence of reactive oxygen species (ROS), thereby allowing for the detection of oxidative damage to the cells as ROS are generated during this process (Figure 3). While the MatTek 3D skin model is already established, we developed a specific method to incorporate squalene to investigate whether it contributes to increased cellular damage.

This incorporation is crucial for enhancing the model's relevance to the scalp, which naturally contains higher levels of sebum compared to other areas of the body. During the development of this method, we discovered that sebum should be added after the application of DHF-DA, and achieving a uniform coating on the surface was essential to ensure consistency across replicates.

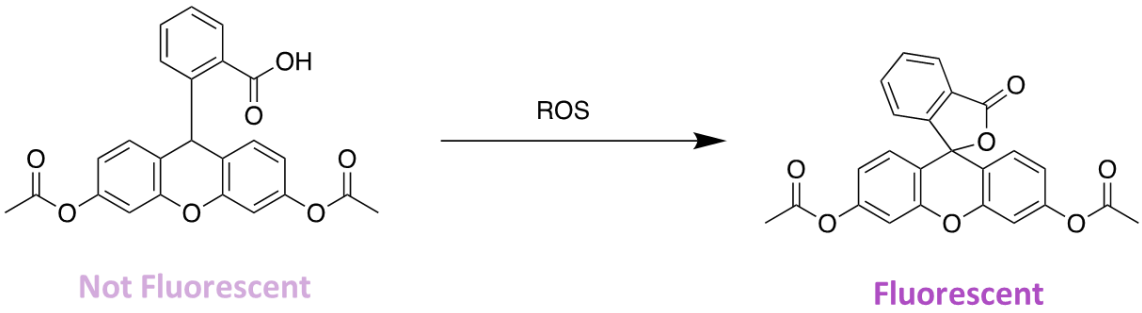
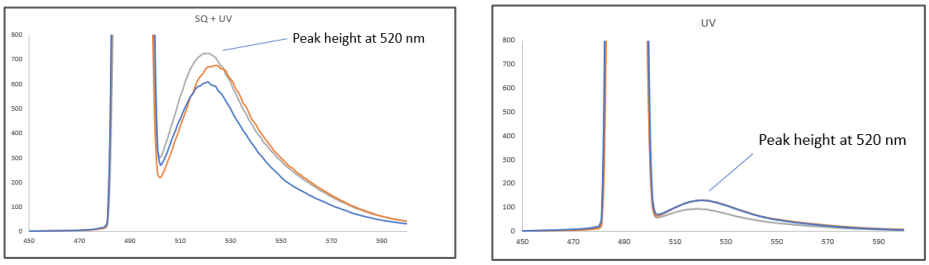


Figure 3. The structural shift that causes the DHF-DA to fluoresce when exposed to ROS.

Squalene was employed as a surrogate for sebum due to the variability in sebum composition among individuals, however squalene is typically conserved as the predominant component of sebum. The goal was to determine whether the presence of squalene leads to an increase in cellular damage compared to samples lacking squalene (see Figure 4). When the 3D skin model is exposed to UV radiation, it experiences cellular damage, which is quantified by measuring fluorescence at a peak of 520 nm—this peak corresponds to the oxidized form of Dihydrofluorescein diacetate (DHF-DA).

The data reveal that UV irradiation indeed induces cellular damage in the 3D skin model; however, the presence of squalene significantly exacerbates this damage, increasing it by

approximately 5.6-fold (Figure 4). This indicates that as previously known UV causes oxidative damage to the skin, in the presence of squalene the amount of oxidative damage is increased which is very relevant to scalp balance.



(a) **(b)**

Figure 4. The detection of the flourescent DHF-DA, when the skin mimic is exposed to UV with (a) and without (b) the presence of squalene.

To minimize oxidative damage to the skin, the potential of squalene antioxidants was investigated, focusing on rosemary and its primary phytochemical, rosmarinic acid. Rosemary is recognized for its strong antioxidant properties, as evidenced by its high Oxygen Radical Absorbance Capacity (ORAC) score, which measures the material's ability to neutralize free radicals. Additionally, it activates a biological antioxidant pathway through the upregulation of the Nrf-2 protein in a cellular reporter system. [4]

In this study, the protective effects of rosemary extract were evaluated against skin damage caused by squalene and UV exposure using the developed 3D skin model. The findings indicated that rosemary extract significantly protects the scalp from oxidative damage induced by sebum, reducing oxidative harm in the 3D skin model by approximately 4-fold (Figure 5). Rosmarinic acid, a key antioxidant phytochemical in rosemary, was also assessed for its protective abilities. Notably, it demonstrated even greater efficacy than rosemary extract, reducing oxidative damage by about 12-fold compared to the unprotected sample (Figure 5). Furthermore, rosmarinic acid was three times more effective than rosemary extract in protecting the skin. Overall, these botanical extracts exhibit remarkable protective properties for the skin and scalp, potentially enhancing resilience against everyday aggressors such as sebum and UV radiation.

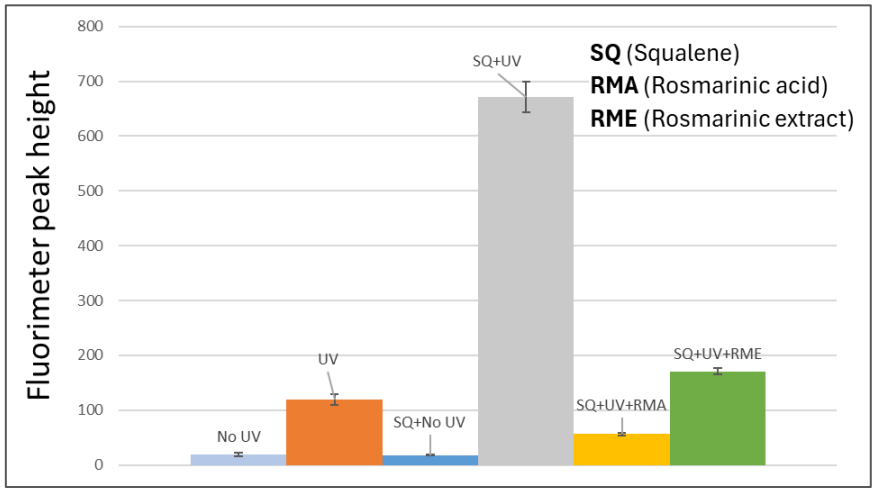


Figure 5. Rosemary and rosmarinic acid reduced the amount of oxidative damage done to the skin/scalp.

4. Conclusion

A method was developed to measure sebum-induced oxidation on a three-dimensional (3D) skin model. This model is more relevant for studying scalp conditions compared to tests conducted without sebum since the scalp naturally has high levels of sebum present, and the data demonstrated that the presence of sebum leads to increased damage to the stratum corneum. When rosemary extract or its active compound, rosmarinic acid, was introduced to the system at low concentrations, significantly less damage occurred to the cells. These findings suggest that these antioxidants can effectively protect the hair and scalp from the harmful effects of oxidized sebum.

5. References

[1] RM Trueb, Oxidative stress and its impact on skin, scalp and hair, *Int J Cosmet Sci*. 2021, 43, S9-S21

[2] RM Trueb, JP Henry, MG Davis, JR Schwartz, Scalp Condition Impacts Hair Growth and Retention via Oxidative Stress, *Int J Trichology*. 2018, 10, 262-270

[3] B Capitanio, V Lora, M Ludovici, JL Sinagra, M Ottaviani, A Mastrofrancesco, M Ardigo, E Camera, Modulation of Sebum Oxidation and Interleukin-1a Levels Associates with Clinical Improvements of Mild Comedonal Acne, *J Eur Acad Dermatol Venereol*. 2014, 28, 1792-1797

[4] JM Marsh, L Li, S Knowles, KCS Locker, K Pearson, R Bacon, K Kozak, T Laughlin, JR Schwartz, Scalp condition improvement with botanical extracts possessing chemical and physical antioxidant activity, *Int J Cosmet Sci.* 2025, 47, 297-304