EVALUATION OF THE IMPACT OF MITOCHONDRIAL FUNCTIONS ON SKIN. A CELL-TO-SKIN ANTI-AGING STRATEGY

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POSTER ID: 538

INTRODUCTION

Mitochondria are the power house of cells. Increasing evidence points the fact that an alteration in mitochondrial functions is a potential target of the aging process in skin. In this work, we focused on several models that can help evaluating the effect of active ingredients and cosmetic skin care products on the mitochondrial functions of skin cells. Work was first done on the cellular level (*in-vitro*) and then on the skin level (explants & Reconstructed Human Epidermis (RHE)). This provides a complete cell-to-skin anti-aging strategy of evaluation. The following points were studied:

CELLULAR LEVEL:

Mitochondrial fission and fusion: Mitochondria are highly dynamic cellular organelles, with the ability to undergo the highly coordinated processes of fission (division of a single organelle into two or more independent structures) and of fusion (the opposing reaction). These actions occur simultaneously and continuously in skin cells and the balance between them regulate the overall energy balance and metabolism. Although not fully understood, alteration in the fission/fusion process appears to be involved in several activities that are crucial to the health of skin cells.

Wound healing: As an end point of the effect of mitochondrial fission/fusion process, the wound healing capacities of skin cells were studied (scratch wound assay).

SKIN LEVEL:

Mitofusin 2 (MFN2): MFN2 is a mitochondrial membrane protein that participates in mitochondrial fusion and contributes to the maintenance of the mitochondrial network and regulates mitochondrial metabolism and intracellular signaling. Mourier & al (2015) showed that loss of MFN2 leads to impaired mitochondrial respiration and reduced ATP production. In this study, Mitofusin 2 of skin cells was targeted as a marker of mitochondrial state.

Epidermal thickness: In-house Reconstructed Human Epidermis (RHE) were used to measure the effect of mitochondrial function on the thickness of the epidermis.

METHODOLOGY

Mitochondrial fission and fusion: Confluent Normal human fibroblasts seeded in multi well plates were exposed for 1 hour to a model simulating environmental aggressions/oxidative stress/aging (300µM of a hydrogen peroxide solution (H_2O_2)). Cells were then washed and incubated overnight with an active ingredient. Cells were stained with the MitoTracker™ probe and the mitochondrial network was observed.

Wound healing: Human keratinocytes were seeded in a 6 well microplate till confluence and then the cell layers were "scared" with disposable pipette tips. Depleted culture medium containing or not the active test ingredient was added. The cells were then incubated for 3 days. Pictures were taken from day 0 to day 3.

Epidermal thickness: In-house RHE were treated or not with the tested product. Observation of epidermal thickness was done at the end of the maturation process.

Mitofusin 2 (MFN2): Human skin explants were irradiated (UVA+B) and then the tested product was applied onto the skin surface. After UV irradiation, the skin explants were replaced in new survival medium for 72 hours. Skin samples were then embedded in a freezing medium before cryosectioning. Sections were fixed and then stained by specific anti-MFN2 antibodies. The quantity of staining is proportional to the quantity of MFN2.

RESULTS

Mitochondrial fission and fusion

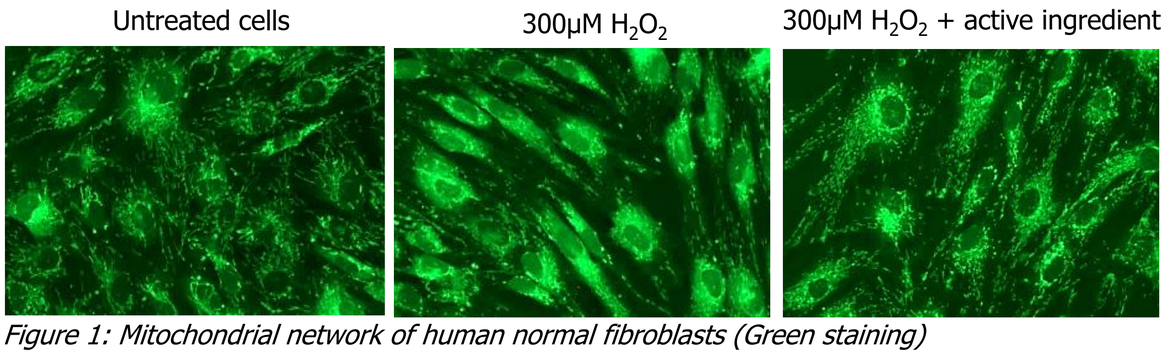

Figure 1: Mitochondrial network of human normal fibroblasts (Green staining)

H_2O_2 seems to damage the mitochondrial network (less mitochondria in fusion state and more in fission state).

The treatment of fibroblasts with the active ingredient seems to counterbalance the effect of H_2O_2 on the mitochondrial network; the initial state of the mitochondrial network was observed: more mitochondria in fusion state than fission.

Epidermal thickness

Figure 3: Effect of the tested product on the evolution of RHE maturation

Tested product, containing active ingredients that protect against mitochondrial disruption and improving wound healing helps the development of skin differentiation and contributes to skin homeostasis.

Wound healing

Figure 2: Wound healing effect of an active ingredient

Treatment with the active ingredient improves keratinocyte wound healing

Mitofusin 2 (MFN2)

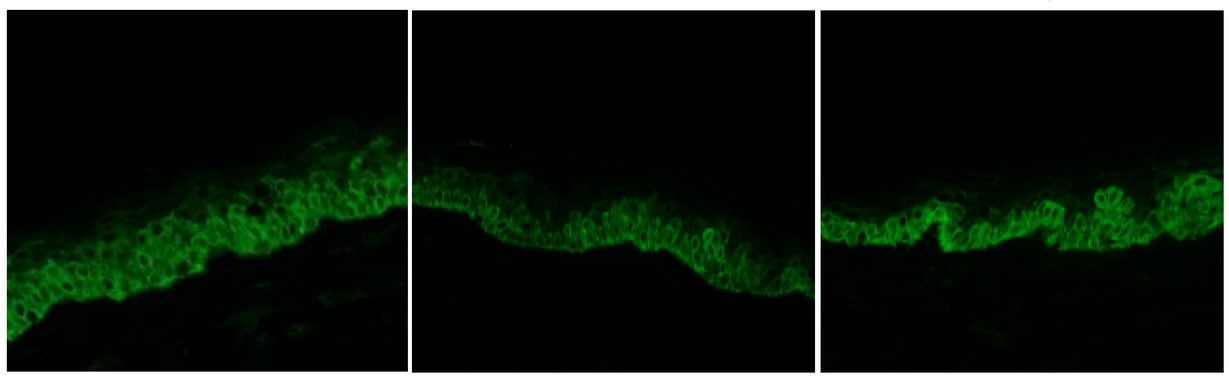

Figure 4: Visualization of MFN2 protein on skin explants (Green staining)

MFN2 staining is localized on the first layers of the epidermis (metabolically active cells).

Experimental UVA/B method used to simulate environmental/oxidative stress decreases the MFN2 content of epidermal skin cells. This reflects an alteration in the mitochondrial state.

The treatment of the skin explants with the tested product seems to preserve from this decrease and restores the mitochondrial state.

CONCLUSION

The described methods seem suitable to evaluate active ingredients and skin care products. The fission/fusion process was successfully detected and the effect of an oxidative stress (age modeling) was observed. The effect of an impaired fission/fusion process was highlighted by a decrease in wound healing in skin cells. On the skin level, the MFN2 protein seems to be affected by an oxidative stress (age modeling). This probably contributes to the accumulation of damaged mitochondria during skin aging leading to impaired mitochondrial respiration and reduced energy production.

The tested active ingredients and the final formulated skin care product showed interesting and significant protection against aging.

32nd IFSCC Congress
London 19–22 September 2022