mtDNAQuant: a new molecular test for quantification of mitochondrial DNA as a read out of stress and the antioxidant effect of vitamin C

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INTRODUCTION

Molecular-based assays are useful tools to monitor cell or tissue response to a variety of target compounds, drugs, cell-signaling molecules, as well as toxic or stressful molecules. Irritating, corrosive, phototoxic, oxidative and antioxidant properties of molecules are evaluated in the field of cosmetics.

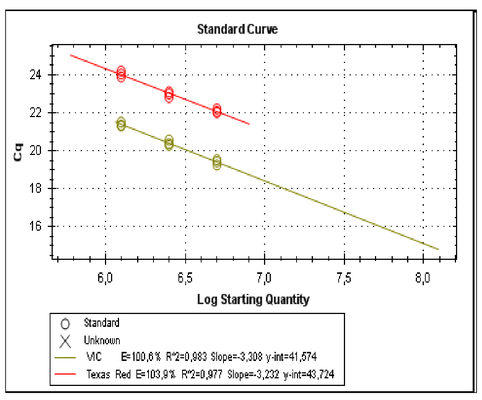
The method presented here is based on the quantification of both mitochondrial DNA (mtDNA), and genomic DNA (gDNA) by duplex quantitative PCR. The calculation of a mtDNAQuant index corresponding to the ratio mtDNA/gDNA evaluates mtDNA copy number per cell, and indirectly mitochondrial biogenesis. Cellular assays of toxicity such as hepatocytes exposed to alcohol, epidermis exposed to detergent have initially allowed us to develop this molecular tool.

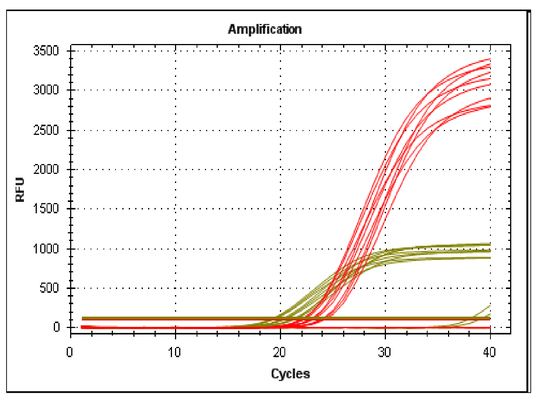
In the present study, mtDNAQuant was evaluated on Sterlab reconstructed human epidermis (RHE) and human keratinocytes in culture.

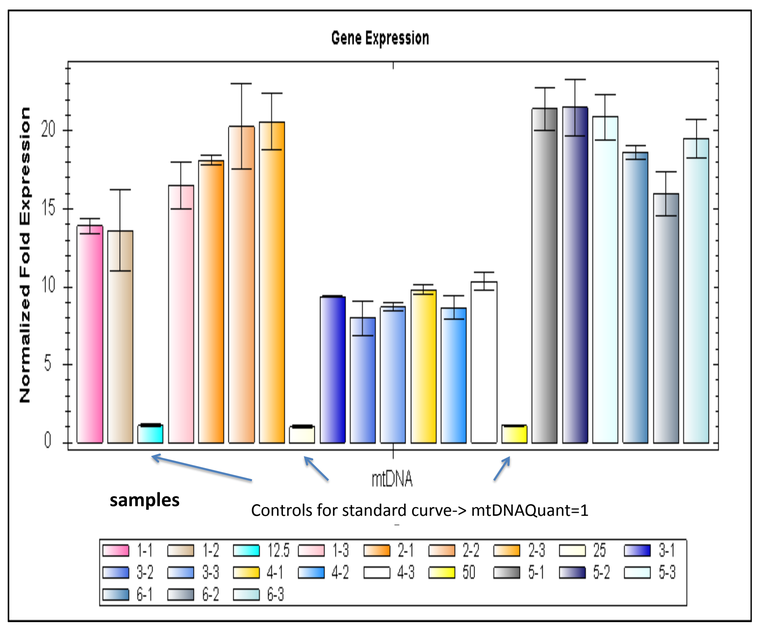
MATERIALS and METHODS

mtQuant index is the ratio of mitochondrial DNA (VIC) and genomic DNA (Red) quantified by duplex qPCR

mtDNAQuant standard curve → **Example of mtDNAQuant index**



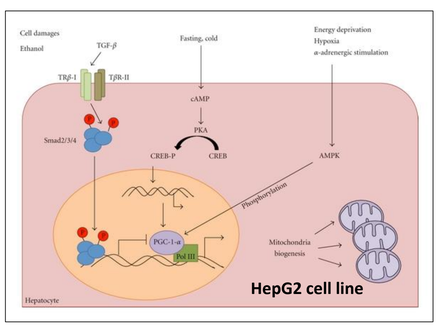


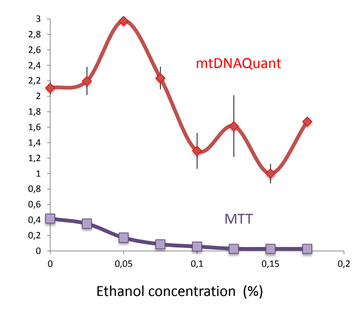


☑ **mtDNAQuant is evaluated in 1h20 minutes**

☑ **The control DNA, prepared from whole blood, dehydrated and encapsulated, is stable for at least 5 years.**

Mitochondria biogenesis allows tissue adaption under stress. Mitochondria biogenesis has been recently recognized as a central pathway in the adaptation of stress conditions in the liver, such as fasting, energy deprivation, hypoxia, or alcohol consumption, before mitochondria decrease if stress is prolonged or induces cell death.



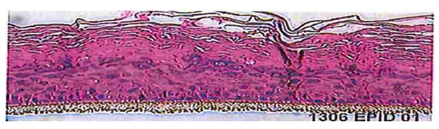


RESULTS and DISCUSSIONS

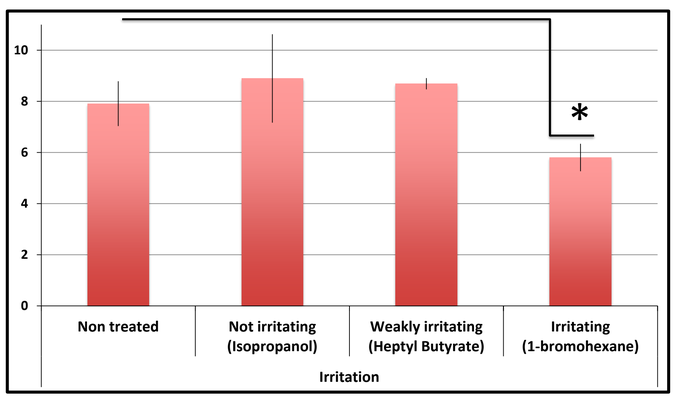
mtDNAQuant index of Sterlab reconstructed epidermis: Irritation & Phototoxicity tests

Reconstructed human epidermis (RHE) model

The Sterlab RHE model is a three dimensional, multilayers culture of normal human keratinocyte cells from a foreskin tissue, histologically similar to the cell layers of the human skin

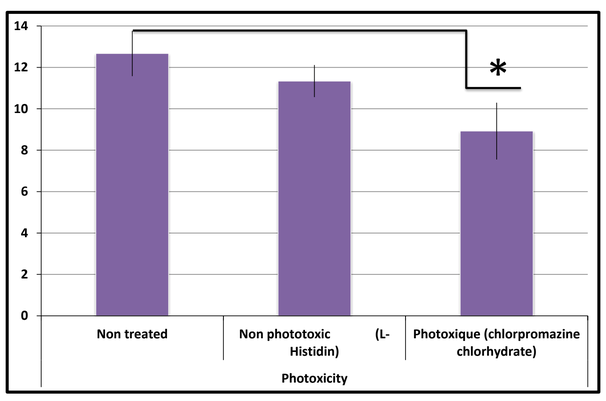


mtDNAQuant decreases with irritating drug



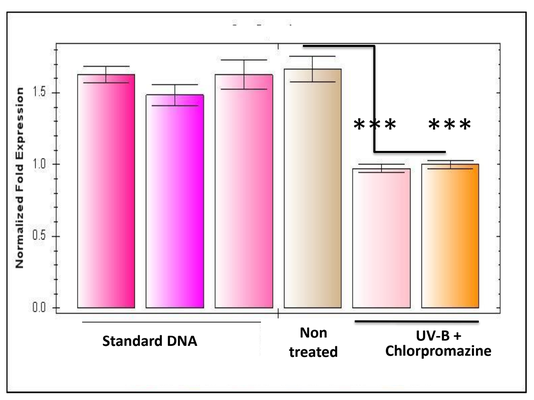
In an irritation test, mtDNA decreased by 30 % when reconstructed epidermis were exposed to a strongly irritating drug (1-bromohexane) compared to non treated reconstructed epidermis or exposed to a weakly irritating drug (Heptyl Butyrate).

mtDNAQuant decreases with UV-A+ photosensitizer



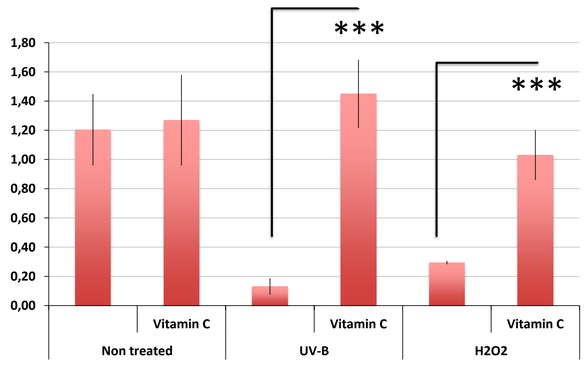
Reconstructed epidermis exposed to UVB (0,3J/cm²) had a mtDNAQuant index of 42 % compared to non-treated epidermis. When exposed to UVA (6J/cm²) and a phototoxic drug (Chlorpromazine chlorhydrate), mtDNAQuant decreased by 20%.

mtDNAQuant decreases with UV-B+ photosensitizer



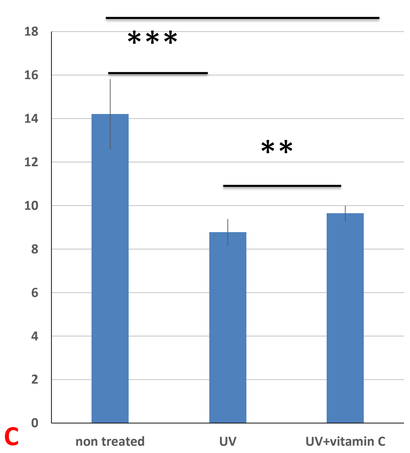
mtDNAQuant index on keratynocytes: oxidative stress and Vitamin C protective effect

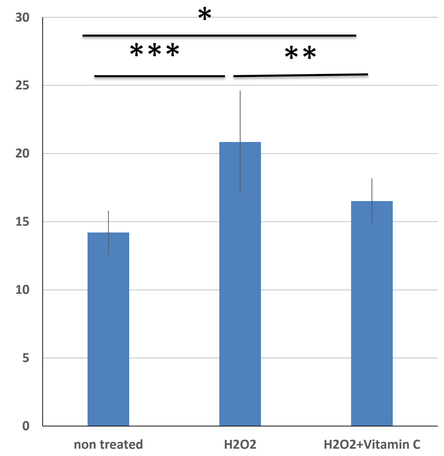
Superoxide dismutase (SOD) as a known read out of protective effect of vitamin C



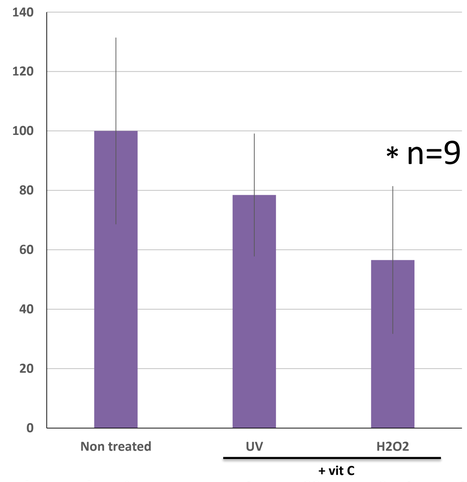
☑ **UV-B and H_2O_2 induce SOD decrease, which is restored by Vitamin C**

mtDNAQuant (n=6 to 9)





Net effect of vitamin C on mtDNAQuant (%)



☑ **Vitamin C decreases mtDNAQuant, partially restores it to the non treated level, a reflection of its protective effect on H_2O_2 induced mitochondrial biogenesis**

Two models of oxidative stress were developed on keratinocytes in culture either by exposing cells to UVB (0,15J/cm²) or to hydrogen peroxide (H_2O_2). The protective effect of vitamin C was evaluated in these models using mtDNAQuant and superoxide dismutase (SOD) quantification. SOD was strongly reduced by both UV (10 fold) and H_2O_2 (4 fold). Vitamin C was able to induce a modest decrease of mtDNAQuant after UV exposure. H_2O_2 lead to a 48% increase of mtDNAQuant, reflecting mitochondrial biogenesis due to stress, and vitamin C induced a significant decrease (44%) of mtDNAQuant following incubation of H_2O_2. Vitamin C was able to protect keratinocytes mitochondrial toxicity induced by H_2O_2, as measured by mtDNAQuant.

CONCLUSIONS

- **mtDNAQuant index is therefore a new indicator to follow toxicity and antioxidant properties of compounds at the level of mitochondria.**
- **mtDNAQuant test can potentially be adapted to any cell, tissue or paradigm aimed at following mitochondrial response.**

References: 1-Shokolenkko et al., 2009. Oxidative stress induces degradation of mitochondrial DNA. *NucleicAcid Research*, **37** (2539-2548).

2-OECD, 2013. *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method. *OECD Guideline for the Testing of Chemicals,* N°**439**.

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4-Kandarova, H.et. al., 2005. ECVAM feasibility study: Can the pre-validated in vitro skin model phototoxicity assay be upgraded to quantify phototoxic potency of topical phototoxins?. 5th World Congress, from http://www.mattek.com/pages/abstracts/372