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An Advanced Micro Physiological System for Dermatological applications

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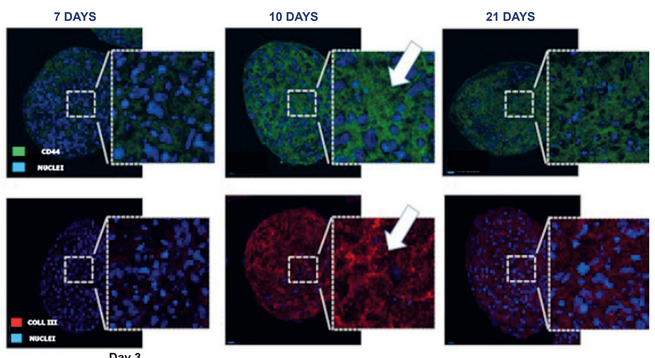
Introduction

A major limitation of *in vitro* 3D skin systems for dermatological research consists in the presence of an exogenous scaffold that doesn't allow to fully recapitulate both the physiological assembly of dermal extra cellular matrix (ECM) and the dynamic remodeling process that naturally occurs during aging, fibrosis or photoaging at dermis level. We developed an advanced Micro Physiological System (MPS) as scaffold-free spheroid generated with human primary fibroblasts at early passage that recapitulates human dermis in healthy conditions in terms of tissue architecture and *de novo* ECM assembly. The 3D geometrical guidance simulates the natural cell-cell and cell-matrix interactions in a physiological microenvironment allowing fibroblasts to develop the native tissue features. The ORA™ Micro-Physiological Dermis mirrors the physiological aging process during the time of culture, preserving donor's phenotype features (Fig.1, Rescigno et al. 2021). ORA™ Micro-Physiological 3D Dermis spheroids have been characterized by proteomics analysis (Fig.2, Aiello et al. 2022) and an intense metabolic activity has been underlined: early cytoskeletal modifications generate maximized cell-cell contacts and cell-matrix interactions leading to an efficient ECM self-assembly and remodeling by mechano-transduction processes.

Aim of the study

ORA™ Micro Physiological 3D Dermis System has been applied to address the UVA-induced ECM modifications occurring in photoaging process: UVA light (320-400 λ) deeply penetrates into the skin dermis inducing oxidative stress inflammation and modification of structural proteins that lose their antioxidant and pro-apoptotic properties resulting in a progressive and severe alterations of skin appearance (Meloni et 2010). A proteomic and transcriptomic integrated approach was applied for gaining a deeper understanding of the UVA effects on dermis.

1A



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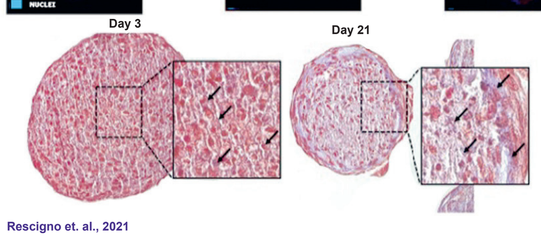


Fig.1 ORA™ Micro-Physiological Dermis (40 years old donor fibroblast). (**A**) Immunofluorescence for Hyaluronic Acid receptor CD44 (green) and COL III (red); nuclei were counterstained with DAPI (blue): a significant modification in their expression and spatial distribution is observed at day 10.
(**B**) The Masson Trichrome staining suggests that ECM organization evolves during the culture time reaching a mature collagens organization (black arrows) at 21 days.

2

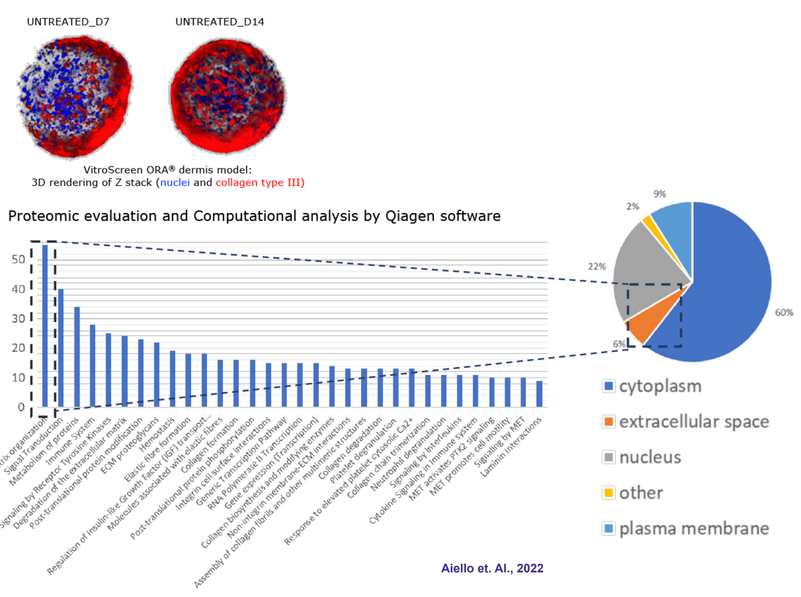


Fig. 2 Proteomic characterization in a time-course experiment on ORA™ spheroids (59 years old donor fibroblasts). The integrated approach of network analyses based on high-resolution mass spectrometry showed the physiological phenotype of spheroids and the ECM mirroring the native *in vivo* dermis features.

Experimental Design

ORA™ Micro-Physiological 3D Dermis spheroids were produced by using Akura plates (InSphero AG, Swiss) starting from a suspension (10^3 cells/spheroid) of human primary dermal 51 years old donor fibroblasts (Innoprot, Spain), formed by hanging drop method, followed by transfer in Akura TRAP plates and cultured for 3 days. Experimental design and read-out parameters are described in Fig.3 and Fig.4 respectively.

3

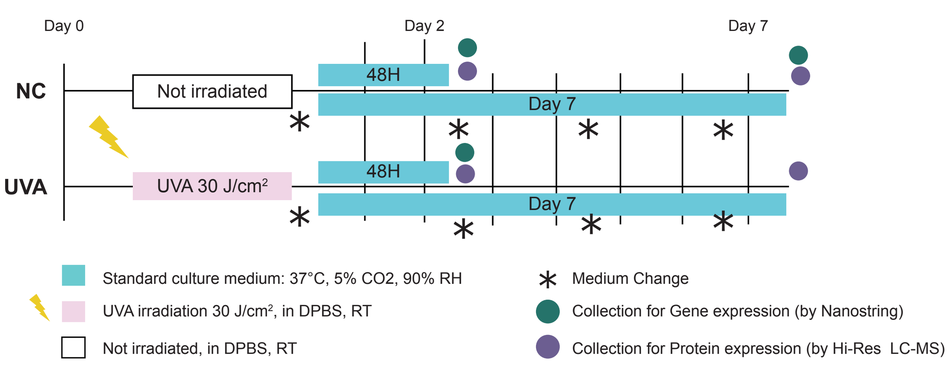


Fig. 3 Experimental Design. UVA series were transferred in DPBS and irradiated with UVA at 30 J/cm² (Oriel Solar Simulator equipped with Xenon Lamp and Filter WG 335 3 mm). Untreated and unexposed samples (NC) were transferred in DPBS 1X and placed at RT in the dark in the same conditions of the exposed series. After UVA exposure 30J/cm², fresh medium was added to all series for 48h and 7 days (D7) of recovery. All treated series were compared to NC and to untreated exposed samples (UVA).

4

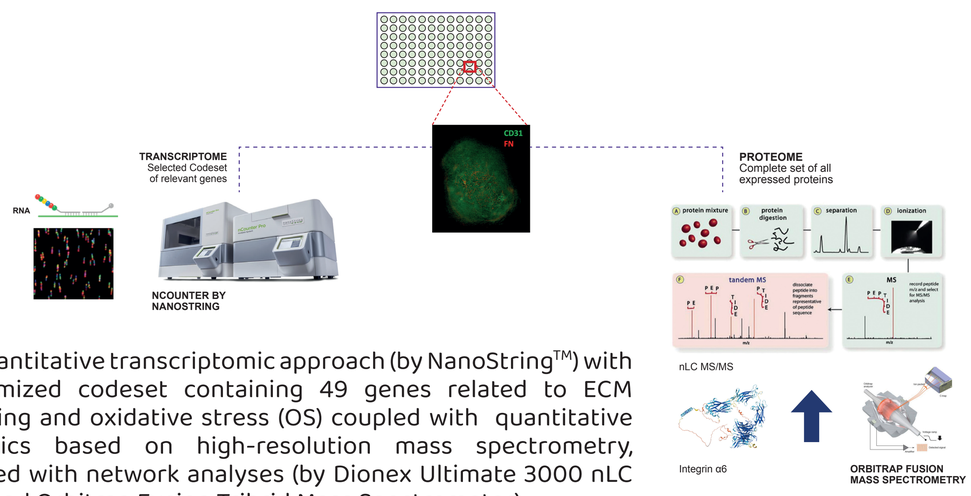


Fig. 4 Quantitative transcriptomic approach (by NanoString™) with a customized codeset containing 49 genes related to ECM remodeling and oxidative stress (OS) coupled with quantitative proteomics based on high-resolution mass spectrometry, integrated with network analyses (by Dionex Ultimate 3000 nLC system and Orbitrap Fusion Tribrid Mass Spectrometer).

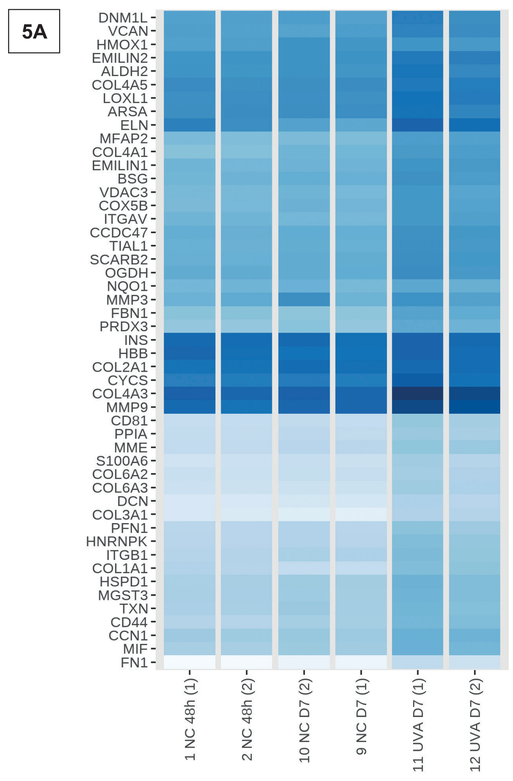
RESULTS

Transcriptomic by Nanostring analysis

The log2-transformed expression values analysis demonstrated a stable expression profile in homeostatic conditions (NC at 48H and D7) and a global decrease of log2-transformed expression values (Fig. 5A).

The genes associated with the ECM gene set are mostly downregulated in the UVA exposed series compared to the control (e.g. COL1A, ELN, COL3A1, FN1) (Fig. 5B) confirming the detrimental effect of UVA on ECM and global dermis architecture. The oxidative stress gene set shows a more varied response pattern, although two genes related to mechanism of response and protection resulted upregulated (Fig. 5C). In particular, HMOX1, involved in regulation of inflammation as antioxidant effector (Nisar et 2015) while NQO1, involved in quinone detoxification, as a superoxide scavenger (Rysava et 2020) resulted both up regulated after 7 days suggesting a stimulus to restore the dermis healthy physiology after the UVA stress.

5A



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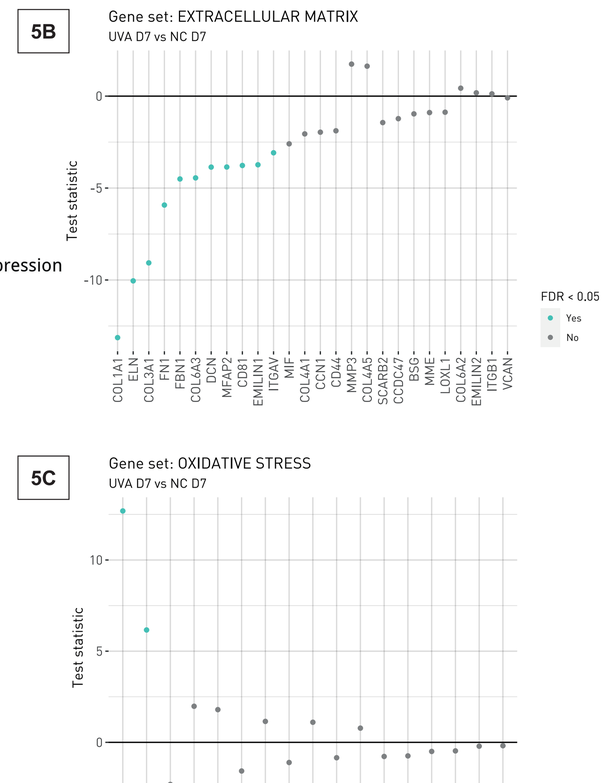

5C

Comparison	Downregulated genes	Upregulated genes
Day 7 UVA vs NC	COL1A1, ELN, COL3A1, FN1, FBN1, COL6A3, MFAP2, DCN, EMILIN1, CD81, ITGAV	HMOX1, NQO1

Fig. 5 Transcriptomic analysis by Nanostring™ on a customized codeset based on ECM and oxidative stress response genes. (**A**) Observed expression levels as a heatmap that displays the log2-transformed expression values. Differential Gene Expression (DGE) at Day 7 in irradiated samples versus NC for ECM genes (**B**) and oxidative stress response related genes (**C**).

Proteomic by high-resolution Mass Spectrometry

1. Nrf2-mediated oxidative stress response at day 7, enhanced in UVA vs control and decreased P_UVA vs UVA. The UVA exposure modules the activation of Nrf2, responsible to the inducible expression of a group of detoxication enzymes (e.g. GSTM1, GSTM3) via MAPK pathway, and the increase HMOX1 as antioxidant enzyme (Fig.6A).
2. Epithelial Adherents Junction Signaling at day 7, enhanced in UVA vs control and decreased P_UVA Data showed a negative modulation of epithelial adherents Junction Signaling induced by UVA radiation after 48h (not shown) and 7 days (D7) suggesting a negative impact on ECM structure and tissue integrity. To confirm the oxidative stress damage RAS-like proto-oncogene A and the proteins belonging to the RAS oncogene family (RAB7A and RAB5C) were found overexpressed after both UVA exposures. RAS-mediated proliferative overdrive may induce replicative stress and activation of DNA damage responses (Fig. 6B).

Additional analysis indicated that at 7 days a depletion of glycolytic enzymes, including glyceraldehyde 3-phosphate dehydrogenase (GAPDH), pyruvate kinase M2 (PKM) and phosphofructokinase (PFKL) was induced by UVA to promote a switch from Glycolysis to oxidative phosphorylation or oxidative stress likely correlated to ROS production.

6A NRF2-Mediated Oxidative stress Response

6B Epithelial Adherens Junction Signaling

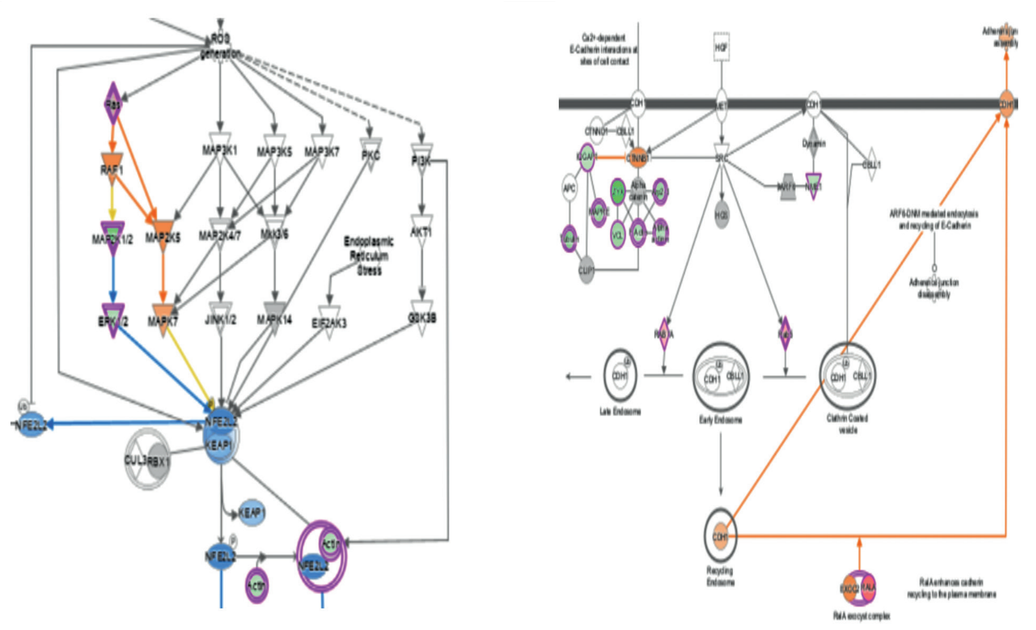

Fig. 6. Network analysis of proteomic data from High resolution mass spectrometry at day 7. (**A**) Nrf2-mediated oxidative stress response and Epithelial Adherens Junction Signaling (**B**) Orange color indicates an upregulation of the pathway, blue color indicates down regulation and the grey color a no regulation.

CONCLUSIONS

ORA™ Micro Physiological 3D Dermis Spheroids responses to UVA has been investigated in this research study based on an "omics" approach combining transcriptional profiling and high-resolution mass spectrometry followed by network analyses. In an experimental window of 7 days, we have been able to address the main pathways of UVA mediated photoaging process: within the several modulated pathways, most are involved in mitochondrial functionality, such as oxidative phosphorylation, TCA cycle, extracellular matrix reorganization and apoptosis.

- **Mitochondrial activity**. The modulation of multiple pathways such as Oxidative Phosphorylation, Glycolysis I and the Nrf2-mediated Oxidative Stress Response, highlights the role of mitochondrial dysfunctions in response to UVA.
- **Dermal architecture.** UVA induced the suppression of adhesion molecules, the degradation of ECM structural proteins (e.g. collagens, elastin) and the activation of matrix degradation enzymes (MMPs).

ORA™Micro Physiological 3D Dermis System has been shown to be responsive to biological relevant and relative high UVA doses (30 J/cm²): it has been possible in a relatively short experimental window to mirror the cascade of complex events following UVA exposure and to observe the recovery from the damage as it physiologically occurs *in vivo*. These features suggest a good predictive power allowing an interesting application to investigate the mechanism of active ingredients and drugs in protecting dermis against early and delayed UVA damages (Aiello, 2023). For all these reasons, ORA™ Micro Physiological 3D Dermis System represents a new and advanced tool to gain new mechanistic insights into physiological processes where ECM is mainly involved (i.e. aging process, photoaging, disease's pathogenesis and ECM fibrosis, communication with vascular compartments data not shown) and in general, in dermatological research. In particular, the plasticity of tissue and the innovative large scale production technique offers the possibility to generate miniaturized and customized dermis systems differing for architecture and metabolic activity according to the specific donor's profile (age, body area, gender, type of damage, etc.), paving the way to new and interesting perspectives in personalized care and medicine approaches. A major limitation of *in vitro* 3D skin systems for dermatological research consists in the presence of an exogenous scaffold that doesn't allow to fully recapitulate both the physiological assembly of dermal extra cellular matrix (ECM) and the dynamic remodeling process that naturally occurs during aging, fibrosis or photoaging at dermis level. We developed an advanced Micro Physiological System (MPS) as scaffold-free spheroid generated with human primary fibroblasts at early passage that recapitulates human dermis in healthy conditions in terms of tissue architecture and *de novo* ECM assembly. The 3D geometrical guidance simulates the natural cell-cell and cell-matrix interactions in a physiological microenvironment allowing fibroblasts to develop the native tissue features.

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The authors declare no conflict of interest