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3D scaffold free micro-dermis model: an innovative tool to explore dermal matrix remodeling

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INTRODUCTION

The hanging drop technology has allowed the development of scaffold free tissues to address pre-clinical testing in different industrial sectors. In the cosmetic sector the research applied to the dermal compartment is particularly interesting being the dermis the most important target of antiageing products.

AIM OF THE STUDY

Develop a new 3D scaffold free micro-dermis model starting from human primary dermal fibroblasts for cosmetic ingredient testing.

METHOD

InSphero plate technology has been used for microtissue formation. Micro-dermis models have been produced by using the hanging drop technology starting from a suspension (5000 cells/microtissue) of human primary dermal fibroblasts (Tebu-Bio). The results refer to cells from a 40 years old donor.

RESULTS

A multiple endpoint analysis approach has been adopted to characterize the established micro-dermis model in term of microtissue evolution in size (Figure 1), viability (Figure 2), ECM deposition and structure (Figure 3 and 4), ECM components and receptors localization (Figure 5) during 21 days.

MICRO-DERMIS SIZE AND VIABILITY EVOLUTION

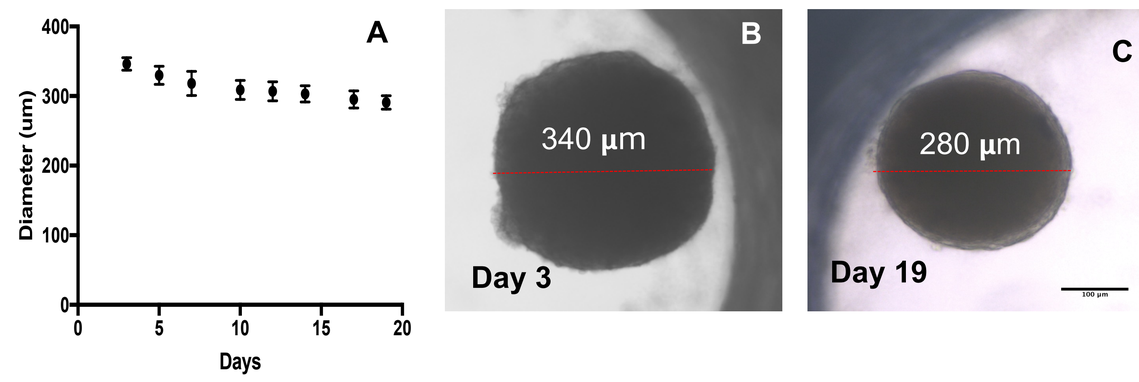

Figure 1. **Microtissue size** is highly reproducible and conserved over time demonstrating that the micro-dermis model can be kept in culture for at least 21 days while preserving its morphology (A). Micro-dermis images at day 3 (B) and at day 21 (C) suggest a slight reduction of microtissues size due to tensile and contraction forces according to ECM maturation and elastic and structural fibres deposition.

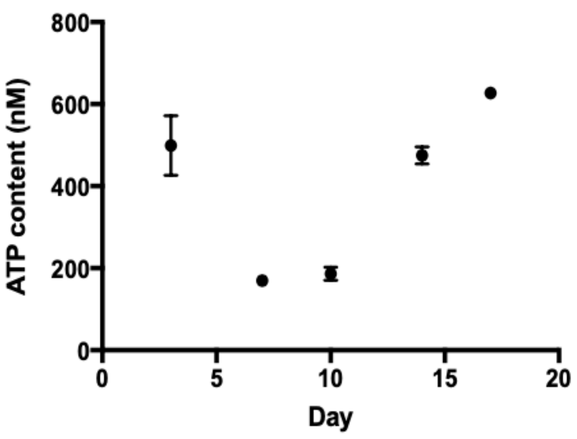

Figure 2. **Micro-dermis viability** by ATP quantification: in long-term culture (at 21 days) microtissues preserve their cellular viability showing an ATP content similar to microtissues at day 3.

EARLY MODULATION OF *DE NOVO* COLLAGEN SYNTHESIS

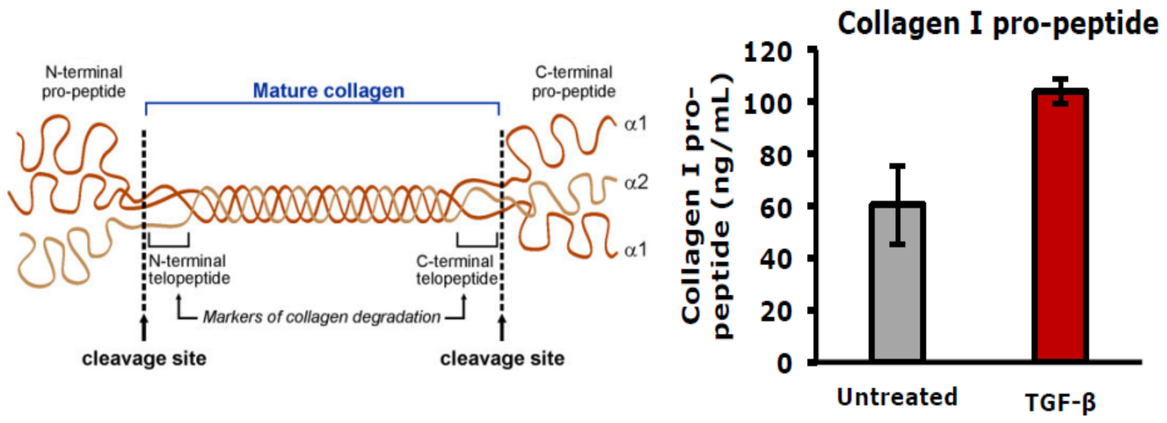

Figure 3. During **collagen maturation** and prior to ECM assembly, the pro-peptide located at N-terminal and C-terminal of collagen chains are cleaved and C-terminal pro-peptide released (scheme on the left). The collagen I pro-peptide was quantified in the culture media after 48h treatment with TGF-**β**1 (10 ng/mL), an ECM soluble modulator, to monitor ECM matrix deposition and remodeling. The results (on the right) suggest that treatment with TGF-**β**1 stimulated *de novo* collagen synthesis within micro-dermis.

DERMIS ECM DEPOSITION AND STRUCTURE

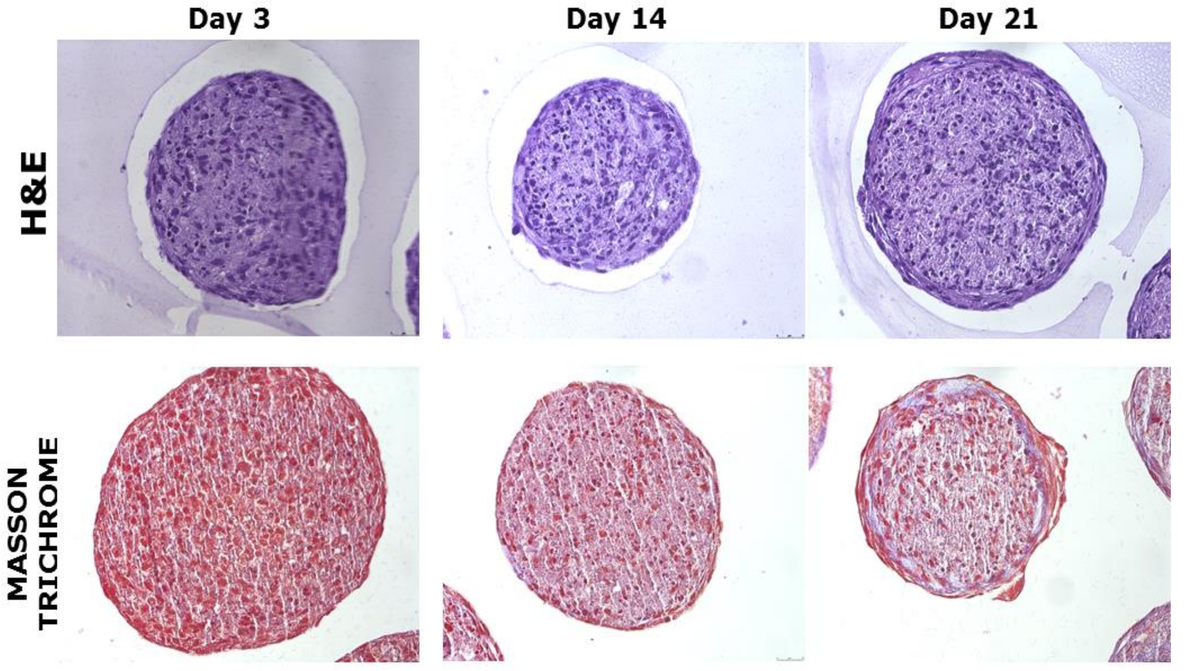

Figure 4. H&E staining shows the evolution of microtissue morphology from day 3 to day 21. ECM deposition monitored by Masson's trichrome staining (ECM in blue) suggests progressive ECM deposition in the microtissue by the fibroblasts, mimicking ECM production *in vivo*: cells are perfectly embedded in the newly formed ECM and necrosis is not observed. The human dermal fibroblasts forming the microtissues appear differently organized during long-term culture recapitulating with time ECM structure and complex organization. The results show a physiological and progressive dynamic maturation of the model: during the culture time collagen deposition and structural features of ECM evolve and change, indicating a "maturation" phase of the 3D construct triggered spontaneously by dermal fibroblasts before reaching the complete final architecture of dermal reference tissue.

CD 44 and COLLAGEN III IF

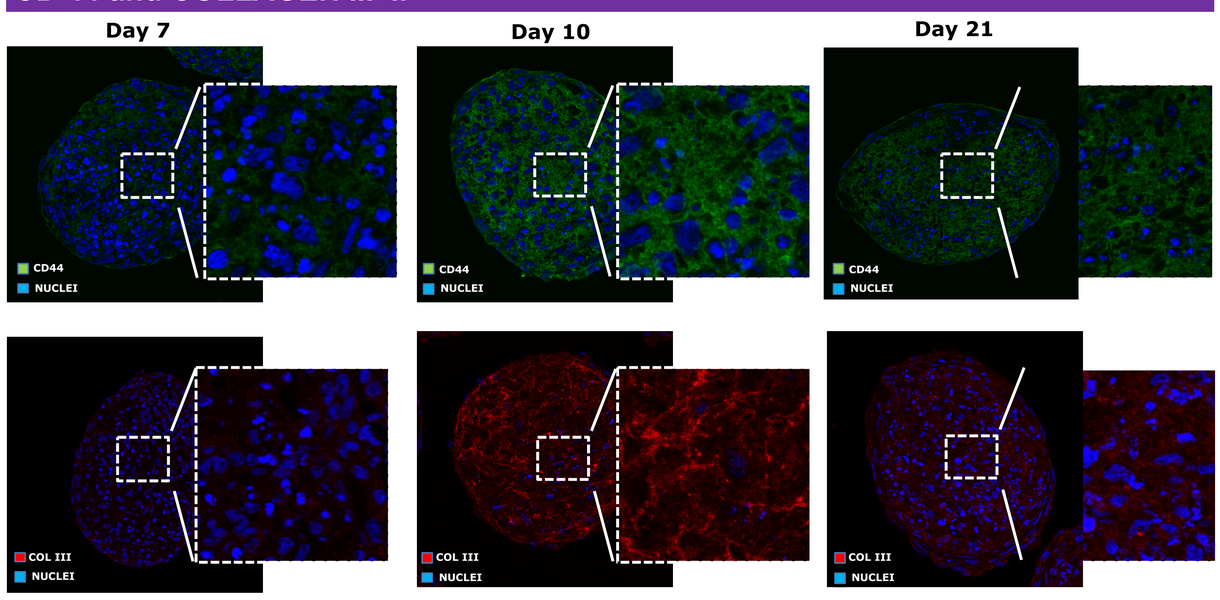

Figure 5. The functional molecules, such as **CD44** which acts as receptor of hyaluronic acid, and structural molecules, such as **Collagen III** (COL III), are identified in the micro-dermis by immunostaining at day 7, 10 and 21, during the microtissue formation. CD44 green signal; COL III red signal; nuclei counterstained with DAPI (blue signal).
The results suggest that micro-dermis main features reach a stable maturation after 21 days of culture. The dynamic expression of CD44 and COL III during the development of 3D micro-dermis gives an increasing completeness to the tissue: CD44 and COL III enriches the *de-novo* ECM in GAGs expression and collagen fibers, essential components for ensuring support and biomechanical resistance to the dermis. Thanks to these features, the resulting 3D dermal construct represents a novel tissue platform that better mimics the native structure and architecture of the human dermal compartment.

Conclusions

The results show that the micro-dermis model evolves in size, viability, morphology and functionality indicating ECM maturation, remodeling and improve tissue complexity.
The novel scaffold free micro-dermis model resulted viable up to 28 days allowing to set experimental designs for long-term and repeated exposures.

The established micro-dermis model represents a promising and relevant tool for:

- **medium/high throughput ingredient screening**: the spheroids robustness allows for the application of treatment doses closer to *in vivo* ranges.
- identification of **ingredient mechanism of action** with particular focus on ECM protein deposition
- assessment of ECM production and remodeling in both **homeostasis** and **stress conditions**
- developing microtissues from donor with different ages/race towards the development of **personalized products**
- developing **advanced models** by co-culturing defined cell types to assess product efficacy on a more complex biological system (e.g. an advance co-culture model with endothelial cells has been developed and is currently in use to assess the effects of antioxidant molecules and botanical ingredients on microcirculation)