

## INTRODUCTION

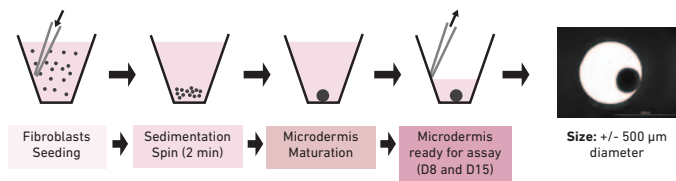
Spheroids as microtissues are a powerful alternative to standard 2D cell culture for *in vitro* studies. 3D scaffold-free spheroids are formed within a few days from a cell suspension that aggregates in Ultra Low Affinity plates (ULA plates). The advantages of spheroids exclusively composed of fibroblasts rely on the physiological production of the extracellular matrix thanks to the aggregative capability of fibroblasts to

self-assemble in a round tridimensional structure. This microdermis presents a complex tissue organization that closely mimics the architecture and composition of the human dermis *in vivo*. The aim of this study was to characterize structural and biomechanical properties of spheroids composed of normal human fibroblasts at 8 and 15 days of culture.

## MATERIALS & METHODS

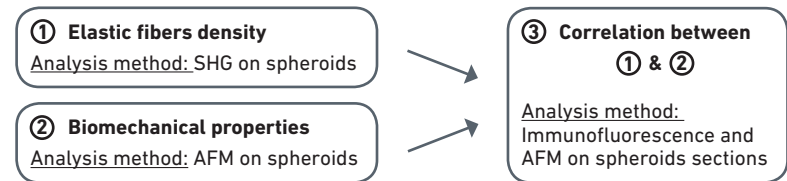
### 3D Microdermis formation

GravityTRAP ULA plate (Microtissue Culture and Assay Platform, PerkinElmer)



### Biomechanical characterization

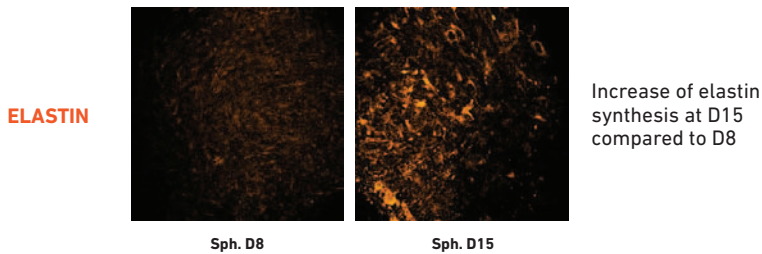
AFM and SHG analyzes at D8 and D15



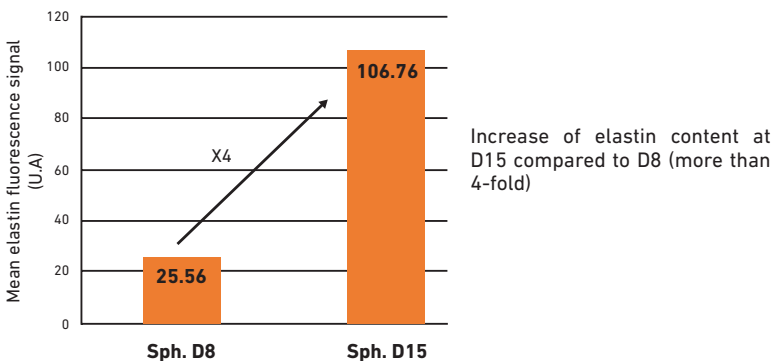
## RESULTS

### ① Elastic fibers density within the microdermis

Second Harmonic Generation microscopy on spheroids at D8 and D15



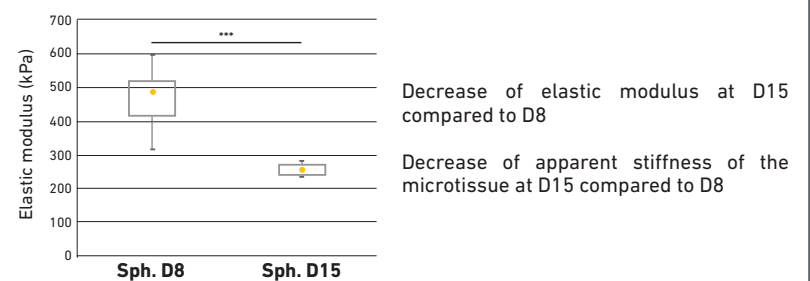
**Figure 1:** Z projection images of elastin deposits within fibroblast spheroids after 8 days and 15 days of culture. Image realized by Second Harmonic Generation Microscopy (Objective X40).



**Figure 2:** Analyzes of the SHG images. Mean elastin fluorescence signal quantification (grey level) within fibroblast spheroids after 8 days and 15 days of culture.

### ② Biomechanical properties of the microdermis

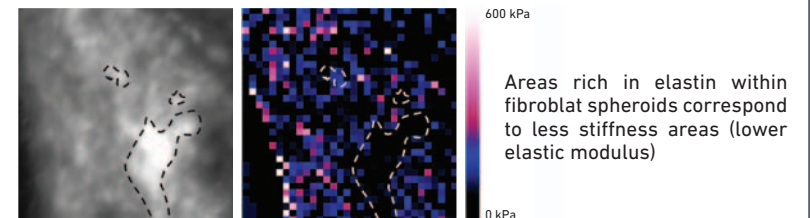
Atomic Force Microscopy on spheroids at D8 and D15



**Figure 3:** Distribution of elastic modulus extracted from fibroblast spheroids at 8 days and 15 days of culture. Student test: \*\*\* p-value < 0,0005.

### ③ Correlation between elastic modulus maps and elastin deposits density

Immunofluorescence and AFM on spheroids sections at D15



**Figure 4:** Immunofluorescence imaging of elastin within fibroblast spheroids at D15. Each image is associated with its mechanical properties map (elastic modulus is represented in kPa). Areas rich in elastin are identified by dots and correspond to soft areas.

## CONCLUSION

These results highlight the elastic properties of the microdermis at D15, which is mechanically softer than a spheroid at D8, and this thanks to the presence of elastic fibers. Therefore, a spheroid at D15 is a biomimetic model in which mechanical and structural elastic properties are related to each other. This correlative study outlines spheroids as a reliable microdermis model to test active ingredient efficiency on elastin synthesis and biomechanical properties of the microtissue.

**Take-home message**

