STRUCTURAL AND BIOMECHANICAL PROPERTIES OF A NOVEL 3D MICRODERMIS MODEL: THE SPHEROID

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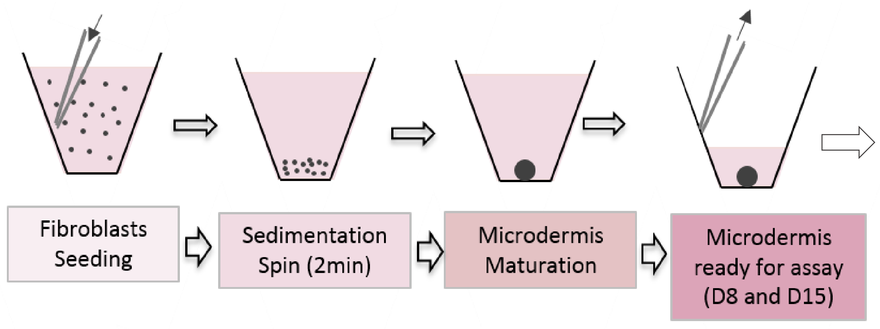
INTRODUCTION

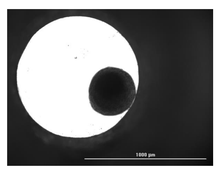
Spheroids as microtissues are a powerful alternative to standard 2D cell culture for *in vitro* studies. 3D scaffold-free spheroids are formed within a few days from a cell suspension using hanging drop technology. The advantages of spheroids exclusively composed of fibroblasts rely on the physiological 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 (Microtissue Culture and Assay Plateform, PerkinElmer)





Size: +/- 500 µm diameter

Biomechanical characterization

AFM and SHG analyzes at D8 and D15

① **Elastic fibers density**

Analysis method: SHG on spheroids

② **Biomechanical properties**

Analysis method: AFM on spheroids

③ **Correlation between ① & ②**

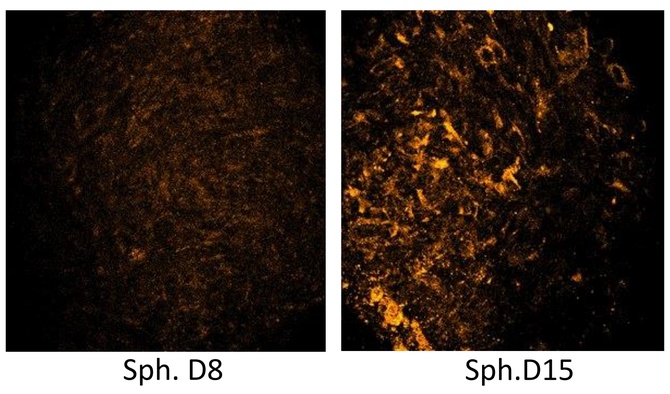
Analysis method: Immunofluorescence and AFM on spheroids sections

RESULTS

① Elastic fibers density within the microdermis

Second Harmonic Generation microscopy on spheroids at D8 and D15

ELASTIN



Sph. D8 Sph.D15

Increase of elastin synthesis at D15 compared to D8

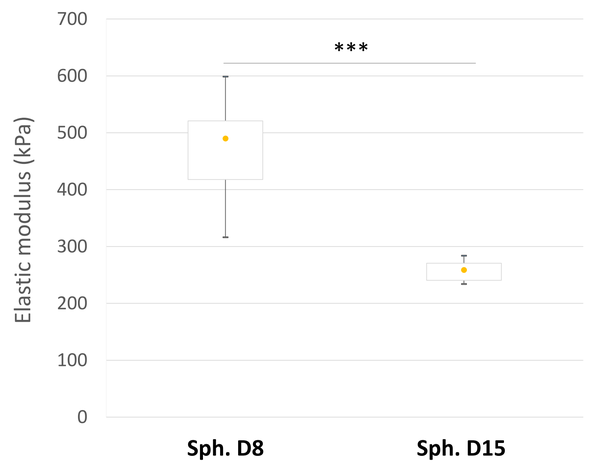
Figure 2: Z projection images of elastin deposits within fibroblasts spheroids after 8 days and 15 days of culture. Image realized by Second Harmonic Generation Microscopy (Objective X40).

Increase of elastin content at D15 compared to D8 (more than 4-fold)

Figure 3: 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



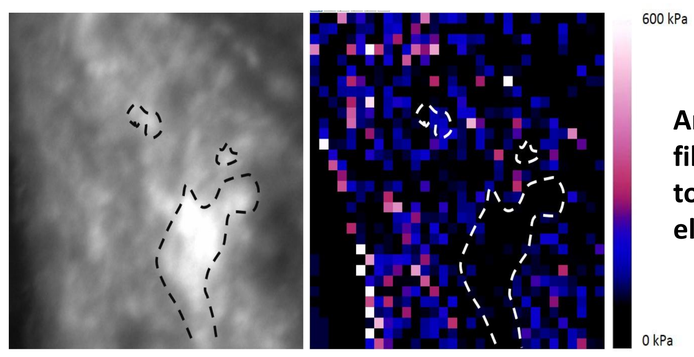
Decrease of elastic modulus at D15 compared to D8

Decrease of apparent stiffness of the microtissue at D15 compared to D8

Figure 1: 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



Areas rich in elastin within fibroblat spheroids correspond to less stiffness areas (lower elastic modulus)

Figure 4: Immunofluorescence imaging of elastin within fibroblasts 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

① **Microdermis at D15 is rich in elastic fibers**

② **Microdermis at D15 is mechanically softer than at D8**

③ **Areas rich in elastin in microdermis at D15 are mechanically softer**