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EXPLORATION OF BIOLOGICAL AND MECHANICAL CHARACTERISTICS OF A RECONSTRUCTED DERMAL MODEL

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INTRODUCTION

The 1970s saw the advent of cell culture methods, and the development of reconstructed dermal models, with the Bell's model. Nowadays, full reconstructed skin models are highly developed. They reflect a large part of the biomechanical properties of the skin that are mainly explained by the contractile power of fibroblasts (Bell et al, 1979).

We interested in Bell's model with fibroblasts conditioned by Transforming Growth Factor (TGFβ1, 5 ng/ml). TGFβ1 is known to stimulate cellular function and to improve contractile power of fibroblasts as it is implicated in healing process. Resistance and elasticity seem also to be improved but nothing was measured and described in literature.

We decided to characterize mechanical properties of Bell's model studying its behavior towards compression and decompression forces, representative of resistance and elasticity. Technics like indentation allowed us to measure the model hardness. Then, these physical results were conforted with microscopic measurements and observations using AFM technic. It highlighted cohesion forces between fibroblasts and the surrounding matrix, inside the dermal model. Finally, the study was completed by fibroblasts distribution observation within the matrix by two-photon microscopy.

RESULTS

Contraction of equivalent dermis

Studying the equivalent dermis diameter standardized to the cell density, we confirmed the results that show a faster contraction for the fibroblasts treated by TGFβ1 compared to the untreated ones. Differences were observed from the first hours after their achievement up to around 4 days, after which the effect is smoothed. Indeed, cells gradually differentiate into myofibroblasts which are able to express more collagens, fibronectin and growth factors. They organize the matrix around them and thus lead to the contraction of the equivalent dermis. Therefore, fibroblasts are essential for the contraction of models but the collagen also give plasticity and directly influence the network elasticity. However, beyond the 4 days observed, differences are less consequent. This confers biomechanical properties to the model that is undescribed until now.

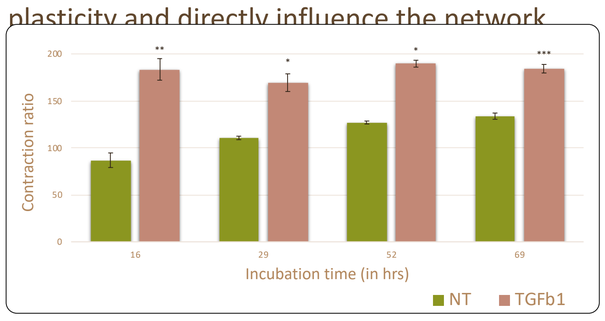
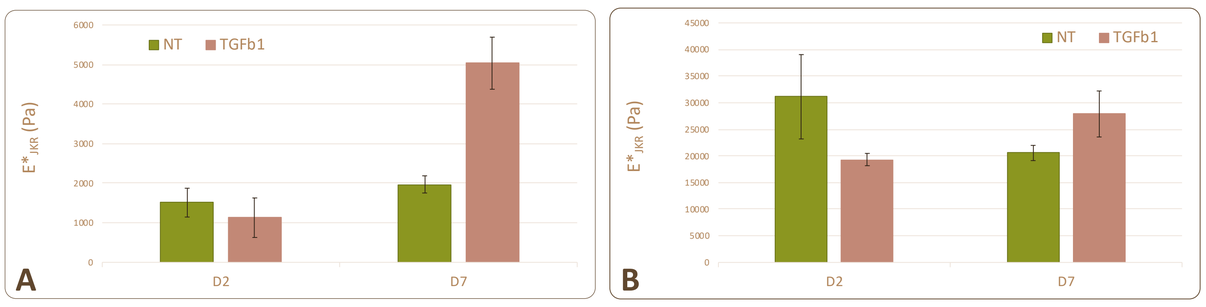


Figure 1: Contraction of the equivalent dermis size versus the cells density.
Treatment of fibroblast cultures were realized in flasks, with or without 5 ng/ml of TGFβ1 during 1 week. To prepare equivalent dermis, cells were combined with type 1 collagen in controlled pH, into 60mm Petri dishes.

Indentation and compression measurements

Considering the Bell's model heterogeneity, we investigated the middle of the model and its peripheral zone for the indentation measurements. For the middle zone, the global Young's modulus showed a very significant increase for the TGFβ1 treated condition, after 7 days (Figure 2A). This reflects a better rigidity of the model. At D2, no difference was observed. These results were also obtained at D7 for the collagen lattice border while we measured a decrease of the Young's modulus at D2 for TGFβ1 treated fibroblasts.



Figures 2: Young's modulus estimation according to the measuring zone, in the middle (A) and at the periphery (B) of the equivalent dermis.
Compression was performed by a spherical indenter (radius = 1.56/2). It moves in charge / discharge cycle with a control of the contact force (imposed effort F_n = 0.3mN, strain rate V = 50µm/s) and a measurement of the resulting displacement. The contact stiffness value allows a direct estimation of the Young's modulus (IVTV platform, Ecole Centrale de Lyon).

The measuring device used for the study of compression allows to apply a compressive force with a specific and regulated speed and to record the relaxation to get back to the initial condition. According to the results (Figure 3), equivalent dermis made with TGFβ1 pretreated fibroblasts applied greater forces compared to the untreated condition, but the differences were significant until particularly long times, such as D9, when there was no difference macroscopically. Thanks to the compression study, we were able to differentiate mechanical behavior of treated and untreated cells within equivalent dermis model even during a longer kinetics. To complete our mechanical knowledges, we wonder how these different forces are distributed inside the model. For that, we needed combination between mechanic and optic methods.

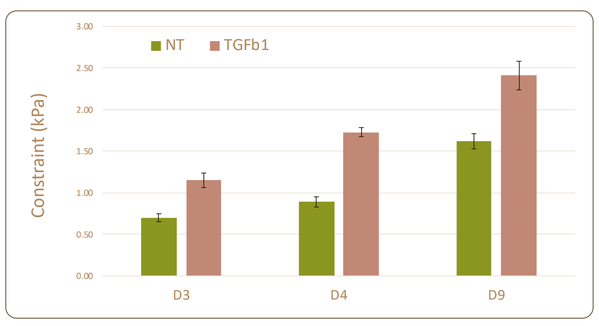
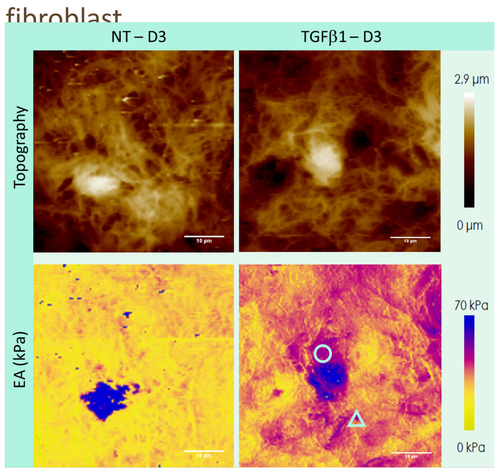
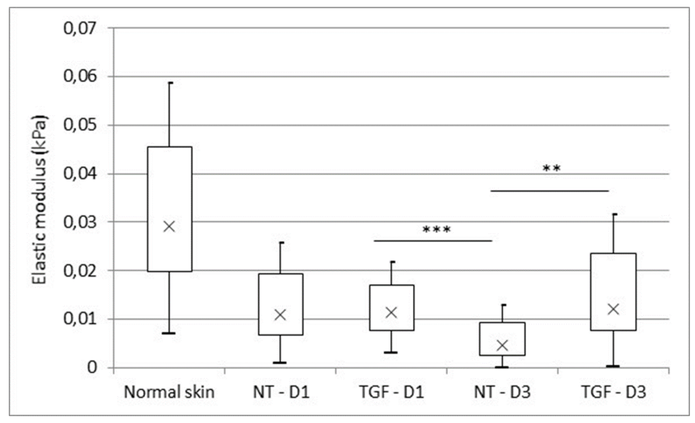


Figure 3: Compression measurement.
The compression measurements were performed with the Instron E10000 traction / torsion, equipped with a 50N sensor. Equivalent dermis were directly tested in the Petri dishes with the culture medium. They were subjected to a strain rate of 10 µm/s before a discharge performed at the same speed (IVTV platform).

Characterization by Atomic Force Microscopy

Applying this methodology, we accessed variations of elastic modulus linked to the topography (Figure 4). Thanks to DAPI nuclei colorations, we could focuse on cellular environment areas (2.5mm²). In the untreated models, the matrix seemed to be more regular compared to TGFβ1. Unfortunately, D1 models do not show any differences. As expected regarding the TGFβ1 properties on fibroblasts, there was an improvement of both fibers elasticity (Δ) and stiffness close to the nuclei area (O) after 3 days. The average elastic modulus of cellular environment area increased significantly. This trend to an increase of the local tension produced by the fibroblast.





Figures 4 and 5: Visualization and analysis of the elastic modulus.

AFM indentation experiments were carried out with a Bioscope Resolve (Bruker Nano Surface, Santa Barbara, CA, USA), mounted on an epiflurescence microscope (DMi600, Leica) with a ×20 objective. All quantitative measurements were performed using a special 0.8-nm-diameter spherical probe (SD-Sphere-NCH; Nanosensors). The spring constant of the cantilever was measured using the thermal tuning method (Hutter and Bechhoefer, 1993; Levy and Maaloum, 2009) and was 0.2N/m. All experiments were made in PBS 1x. Each AFM experiment consisted in acquiring a fluorescence image and a matrix of force curves (20 × 20 curves, with a 2.5-µm step). Measurement consisted of the analysis of 400 force curves extracted from 50 × 50 µm matrices with indentation points spaced 2.5 µm apart. The applied force was 10 nN. The quantitative data of the elastic modulus were extracted from each curve by applying the Hertz model for an indentation ranging from 0 to 1 µm. To record high resolution images, we used standard pyramidal tip (ScanAsyst Air, Bruker,k= 0.4 N/m) with PeakForceQNM AFM mode. The probe is oscillated at a low frequency (0.5 kHz). The maximum force during imaging was 50 nN. For each sample, the topology images of 1 µm2 (128 × 128 pixels) were collected in the dermis on dried section, for D1 and D3. Nuclei were labelled with DAPI stain to allow cells localization.

On figure 5, the graph shows significant enhancement of elastic modulus due to TGFβ1 conditions at D3 and the absence of variation from the control conditions for the early stage (D1). Interestingly the control elastic modulus value seemed to decrease while the TGFβ1 made it higher, with a trend to reach the normal skin data. That have to be confirmed.

Imaging reconstruction of dermal models

Finally, we decided to focus on two-photon excitation fluorescence microscopy (TPEF). Images of collagen matrix and fibroblasts were detected to depths up to 700 µm. In the non-treated model, fibroblasts showed a spindle shape with few interactions with the matrix. After treatment with TGFβ1, fibroblasts showed more numerous cytoplasmic projections. Each cell was surrounded by a large amount of organized collagen. We demonstrated the relevance of two-photon imaging to characterize cells morphology, collagen organization and interactions between them. Stacks of images with submicron resolution are able to produce high-resolution 3D imaging. A 3D representation of the matrix allowed a better understanding of the formation and the evolution of the model (Figure 6).

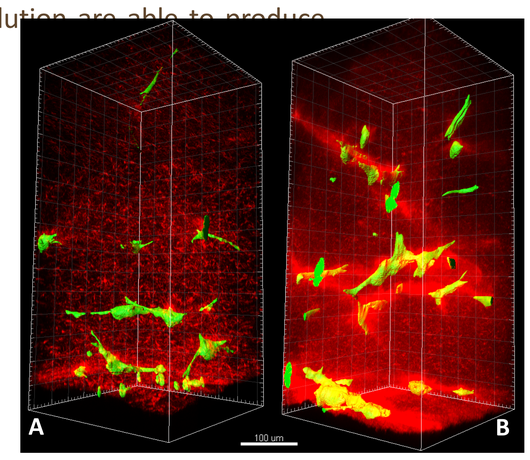


Figure 6: Macroscopic images of reconstructed dermal models.
Collagen matrices were fixed in 2% formaldehyde in PBS 1x for two hours. Cell morphology was visualized with a membrane labelling tracer (DiA). Images were produce using an upright two-photon microscope using a 25x water immersion objective lens (NA 0.9). Laser intensity was set at 100 mW and wavelength was fixed at 810 nm. Photo-multiplier tubes (PMT) were set at 800 V (PMT SHG) and 900 V (PMT TPEF). Two-photon investigations were carried out in the middle area of these models, treated (A) or not (B) with TGFβ1. Images included both SHG (collagen – red) and Two Photon Excitation Fluorescence (fibroblasts – green). Soft Imaris 364 x 364 x 700 µm³.

CONCLUSION

The evolutions in advanced technics using physical methods and the association between mechanics and optics ones allowed to characterize the Bell's equivalent dermis. The obtained results showed that there are significant differences between non-treated and TGFβ1 treatment in model behavior. Its mechanical properties make it an interesting study tool to assess cosmetic active ingredients efficacy.

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