BioMeca

§ experimental note#9

Studying blue light exposure impact on dermis mechanical properties using atomic force microscopy

Context

Light is necessary for life, but prolonged exposure to artificial light is a matter of increasing health concern. Humans are exposed to increased amounts of light in the blue spectrum produced by light-emitting diodes (LEDs) found in screens, smartphone, which can interfere with biological process. The LED technologies are relatively new; therefore, the long-term effects of exposure to blue light across the lifespan are not understood. Here we focus the impact of blue light exposure on skin health and in particular on skin ageing.

The aim of this study is to understand and to determine if blue light acts on the mechanical properties of skin by studying the physical behavior of fibroblasts, cells responsible for collagen and elastin synthesis and dermis sustaining capacity. Therefore, impaired dermal fibroblast function has significant impact on the properties of skin connective tissue. Fibroblast activity and dermis organization represent preferred targets for the development of cosmetic products. However, a combined study of collagen organization and physical activity of cells is complex to implement. Here, we propose to use atomic force microscopy (AFM) to visualize and quantify the mechanical activity of fibroblasts and collagen network organization before and after blue light exposure.

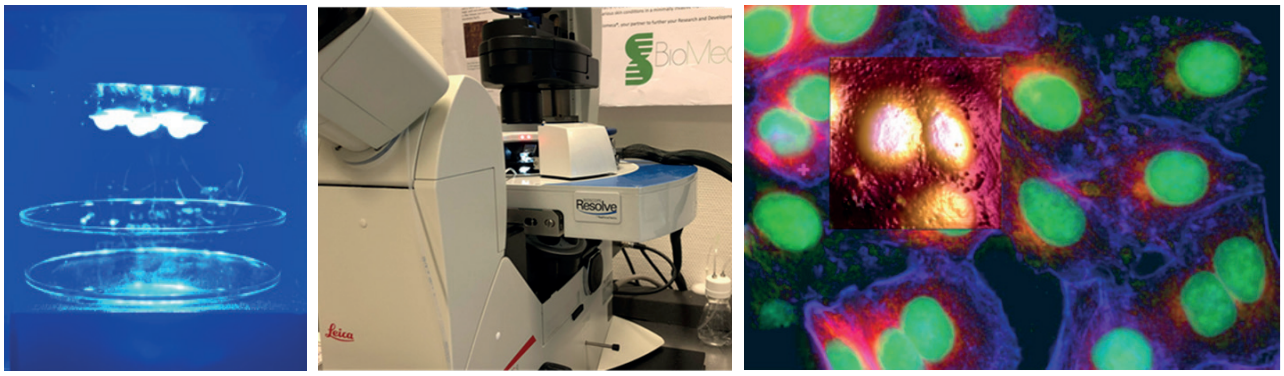

Figure 1 : The blue light box and the AFM system used at BioMeca, correlative mapping can be achieve thanks to a coupling with fluorescence microscopy.

Why use **AFM ?**

Due to the complex structure of dermis, investigation of the fibroblast activity in physiological conditions is extremely difficult and challenging. Indirect readouts of the magnitude of fibroblast contraction force have been already studied by collagen gel assay. These measurements, however, are performed on a macroscopic scale and thus lack spatial senstitivity toward local heterogeneity of cell-generated tensile force. AFM appears to be a unique tool to direclty adress local mechanical properties changes generated by cell1 as well as high-resolution imaging of collagen network:

- AFM experiments can be performed in the culturing medium to ensure cells grow in their normal physiological conditions, temperature can be controlled and drugs or ingredients can be added in situ during experiment,
- AFM can achieve high resolution imaging (at the nanometer scale) which is perfectly suitable for the imaging of the cell-matrix interactions and collagen structure,
- AFM can track mechanical properties changes in real time.

Our AFM system (Bioscope Resolve – Bruker) is coupled with an epifluorescence microscope (DMi8 – Leica) (Figure 1) so that we are able to overlay topographic AFM images, mechanical property maps and fluorescent signal.

BioMeca has developped a set of experimental conditions (blue light box, Figure 1) and an analytical framework for analyzing AFM force curves to produce robust and internally quantitative topographic and nanomechanical maps. In this study, we propose two complementary approaches (structural and mechanical) to study the impact of blue light exposure.

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We propose to use AFM to image and to map the mechanical properties of cell and cell-matrix interactions (Figure 2) and to image collagen structure and organization (Figure 3) . Those data can be obtained as well on cells grown on collagen lattice as on more complex in vitro models (reconstructed skin models, explant cryosection, etc.).

An example of a study done by BioMeca is presented here. This study aimed to characterize the effect of blue light irradiation on mechanical properties of fibroblasts and collagen and in particular to demonstrate that daily exposure to blue light (Figure 5) may impact the physical-sustaining function of dermis.

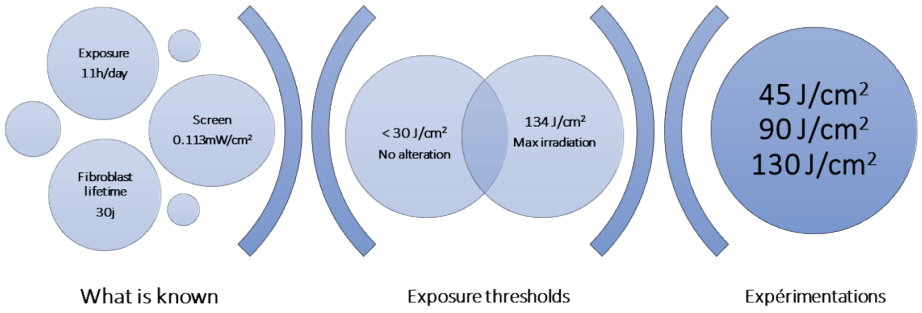

***Figure 2** : In skin, a fibroblast has a maximum lifespan of 30 days. In addition, the average of screen time per person is 11h/day. The maximum irradiation energy to be supplied to the cells has therefore been calculated as a function of these parameters. For this project, the quantities of energy emitted chosen are 45 J/cm², 90 J/cm² and 130J/cm².*

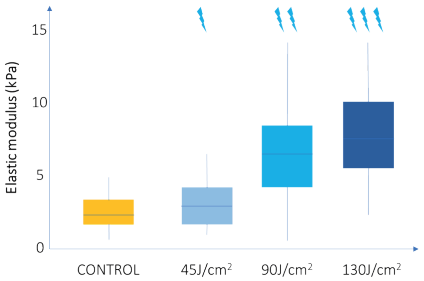

***Figure 3** : Elastic modulus of fibroblasts exposed or not to different dosage of blue light..*

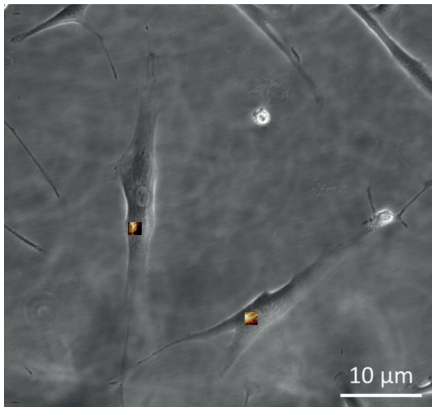

***Figure 4** : Optical and AFM images of human primary fibroblasts.*

Various quantitative readouts* will be then extracted from those data and images as listed below :

- Cell morphometry (area, perimeter, circularity, aspect ratio, etc.)
- Cell mechanical properties (stiffness, elastic modulus, traction force)
- Morphometric parameters of collagen network (fiber density, fiber thickness, fiber orientation)
- Morphometric parameters of collagen fibers (thickness, period measurement, etc.)
- Network mechanical properties (tension , strain stiffening)

AFM data can also be supplemented with fluorescence data : cytoskeleton activity (actin, tubulin staining) , cell activity trackers (lysotracker, mitotrackers ...) ....

**Data are provided as a detailed report with graphics (box plots ...) supported by statistical tests and excel files with numerical data.*

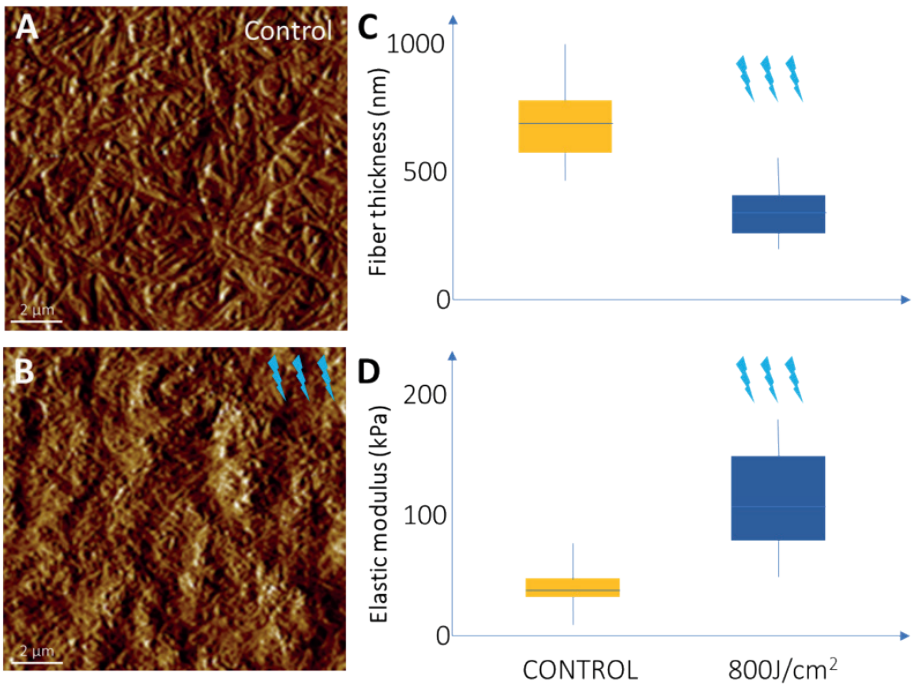

Figure 5 *: **A.** AFM topographical image of collagen I polymerized during 18h. **B.** AFM topographical image of collagen I polymerized during 18h under blue light (800J/cm2). **C.** Analysis of collagen I fibers tickness on both conditions. **D.** Elastic modulus of collagen I lattice in both conditions.*
Blue light irradiation has a significant effect on collagen network architecture in term of structure and mechanical properties. Collagen fibers are thinners when they are exposed to blue light during polymerisation process. Moreover the stiffness of the collagen lattice increase as function of blue light exposure.

This study demonstrates how daily exposure to blue light may accelerate aging. Here, we show that both fibroblasts traction capacity and collagen polymerisation process are impacted by blue light exposure. This method is ideal to study a quantitative effect of a protective product against digital pollution .

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