Studying blue light exposure impact on skin mechanical properties using atomic force microscopy (part 2)

Context

With the emergence of new technologies, we are increasingly exposed to artificial light from television, computers, tablets and smartphone screens. This is not without consequences for our health.

We currently know the risks for our eyes, however, the effects on the skin are still little known. Despite some positive aspects of low energy blue light, higher dose irradiation could have harmful effects on skin. Indeed, several studies have revealed that blue light might reduce collagen and elastin's synthesis, which are responsible for skin resistance and elasticity. We assumed that blue light could affect the mechanical properties of the skin and thus on skin health and in particular on skin ageing.

In a first study (experimental note #9), we've proved that blue light acts on the mechanical behaviour of fibroblasts and on collagen polymerisation. Here, we propose to use atomic force microscopy (AFM) to full skin mechanical properties. The main objective of this study is therefore to study whether daily exposure to blue light is also responsible for changes in the physical properties of full skin.

Figure 1 : The blue light box and the AFM system used at BioMeca. Stiffness tomography methodology developed by BioMeca.

Why use **AFM ?**

The skin is a complex organ composed of several superposed compartments. Due to this layered structure, it is difficult to accuratly measure the mechanical properties of its different compartments in a in vivo or ex vivo (full skin explant) context.

In addition to the many advantages offered by AFM, such as being able to measure full skin explants maintained in an air-medium interface, BioMeca has developped an analytical framework for analyzing AFM force curves to produce robust and internally quantitative nanomechanical quantification of thick samples in depth (stiffness tomography method). In this study, we propose two complementary approaches (i) macroscopic and mechanical in a first hand and (ii) nanometric and structural in other hand to study the impact of blue light exposure.

Our AFM system is coupled with an epifluorescence microscope (Figure 1) so that we are able to overlay topographic AFM images, mechanical property maps and optical images.

We propose to use AFM to measure mechanical properties of full skin explant (Figure 2). The utilization of our stiffness tomography approach allows 3D reconstruction of mechanical properties in depth and the quantification of elastic modulus of dermis and epidermis. This approach has been validated for this

This study aimed to characterize the effect of blue light irradiation on mechanical properties of **full skin explant**. We've demonstrated that daily exposure to blue light might impact the overall skin function, physical function of dermis and barrier function of epidermis. Here, we show that **blue light irradiation impacts full skin physical properties by increasing stiffness of dermis and epidermis**.

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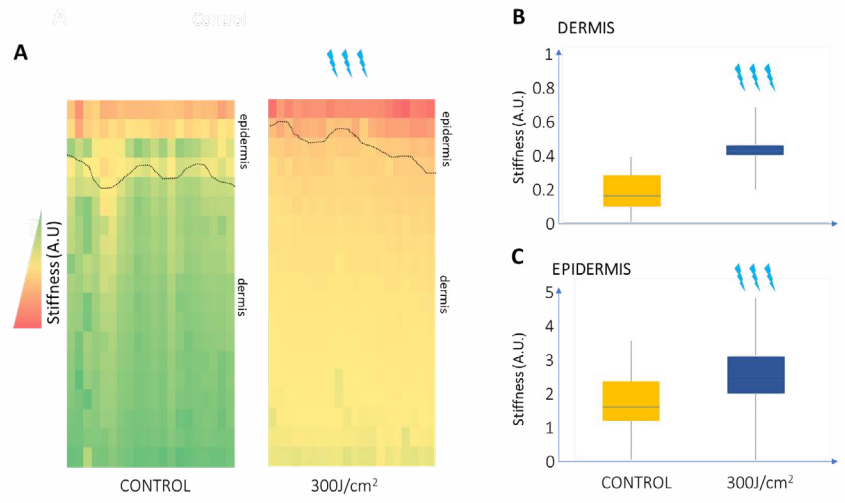

Figure 2 : A. *Stiffness tomography of a skin explant before (control) and after (300 J/cm²) blue light irradiation. For this study, the quantities of energy emitted chosen is 300 J/cm² corresponding to a daily exposure (11 hours/day) of 4 months.* ***B. and C.*** *Variation of stiffness before and after blue light irradiation of the dermis and epidermis compartments respectively.*

After this overview of the impact of blue light exposure on full skin's mechanical properties, we decided to investigate more precisely the different compartments to better understand what causes this stiffness increase. To do so, we worked on skin sections. This approach provides more local measurements of mechanical properties. The skin explants previously studied by the stiffness tomography method are then frozen and cut into 20 µm thick sections.

An example of an analysis of the dermis performed on these explants is presented Figure 3. We can observe that blue light irradiation of a full explant increases papillary dermis mechanical properties by increasing its stiffness (Figure 3C). Blue light irradiation acts also by modifying collagen structure by decreasing fibers thickness (Figure 3D) and by reticulate collagen fibers (Figure 3B).

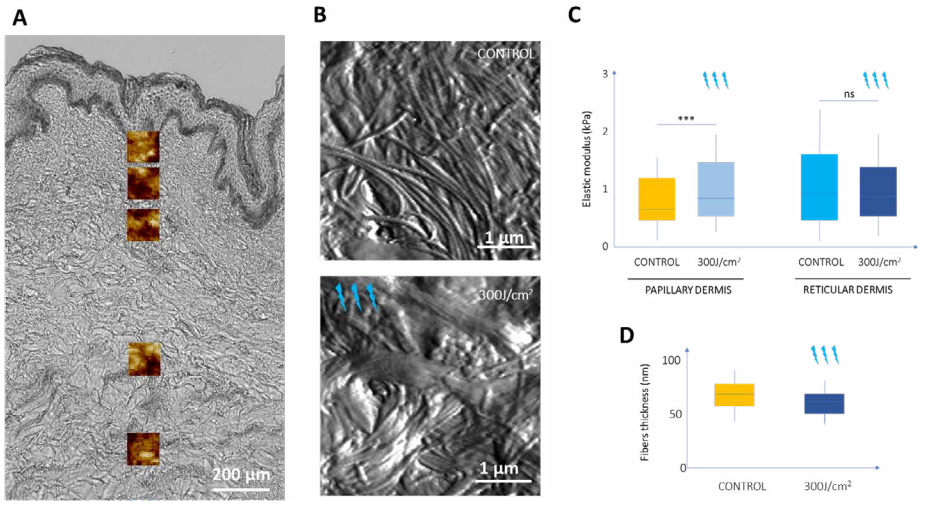

Figure 3 *: **A.** Optical image of a skin section and stiffness maps of the measured areas (papillary and reticular dermis).* ***B.*** *AFM images of the collagen network of a control explant (CONTROL) and an explant irradiated with blue light (300 J/cm²).* ***C.*** *Variation in the rigidity of the papillary and reticular dermis of explants irradiated or with blue light.* ***D.*** *Thickness of the collagen fibers of the control (CONTROL) and of the irradiated skin (300 J/cm²).*

Various quantitative readouts* are extracted from these data and images as listed below :

- Stiffness tomography reconstruction of full skin
- Full skin mechanical properties (ex-vivo epidermis and dermis properties)
- Morphometric parameters of collagen network and fibers (fiber density, fiber thickness, fiber orientation)
- Collagen network and elastic fibers mechanical properties (tension, elasticity, stiffness)

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

Here, we've showed that **full skin mechanical properties are impacted by blue light exposure**. The overall studies performed by BioMeca® have demonstrated how daily exposure to blue light may **accelerate aging by impacting** : (i) collagen's polymerization, (ii) mechanical properties of collagen network, (iii) activity of fibroblasts.

All these changes lead to the stiffening and loss of elasticity of the dermis compartment.

During the same experiment, we've also been able to demonstrate that **epidermis is impacted by blue light exposure**, which might impact skin barrier function. In conclusion, these methods are relevant to reveal **2 protective effects against blue light exposure's impact** :

- against digital pollution itself
- against "loss-of-barrier-function".

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