Nanomechanical characterization of hair cuticle under high-temperature and chemical treatments using Atomic Force Microscopy approach

Context

The daily use of straighteners and shampoos has become a widespread practice. Characterization of such treatments effects on hair properties is essential to develop better cosmetic products and promotes biological and cosmetic science.
Hair fibre is made up of cuticle and cortex cells, which are mainly filled with keratin protein. The cortex takes up most hair fibre composition and the cuticle is the outermost region that protects the cortex.

The cuticle consists of flat overlapping cells (scales). Each cuticle cell is composed by various sub-lamellar layers with different cystine content that determines their mechanical properties. Indeed, cystine has the capacity to crosslink and a high cystine content corresponds to rich disulfide cross-link bonds, leading to high mechanical properties. The strength of these bonds might be strongly affected by water content in hair.

Given the importance of mechanical properties and structure of cuticle in hair health and appearance, it is important to understand these daily treatments effects on **hair mechanical characteristics.**

In this study, cuticule morphology and effective Young's modulus (stiffness characteristics) of hair surface were **measured in different conditions** (high-temperature and detergent) using force-volume and imaging modes of AFM.

The **correlation between treatments, nanomechanical properties and surface morphology** of hair is then discussed.

Why use **AFM ?**

Morphology of cellular structure of human hair has been traditionally investigated using electron microscopy. However, this technique provides limited capability to in situ study of hair mechanical properties in various environments. AFM overcomes these issues and can be used for such measurements and to obtain high-resolution imaging in ambient conditions, without requiring specific sample preparations and surface treatments.
In addition to the many advantages offered by AFM, BioMeca® has developped an analytical framework for analyzing AFM force curves to produce robust and internally quantitative nanomechanical quantification of hair cuticle. In this study, we propose two complementary approaches (i) mechanical and (ii) nanometric and structural, to study impact of chemical and high-temperature treatments.

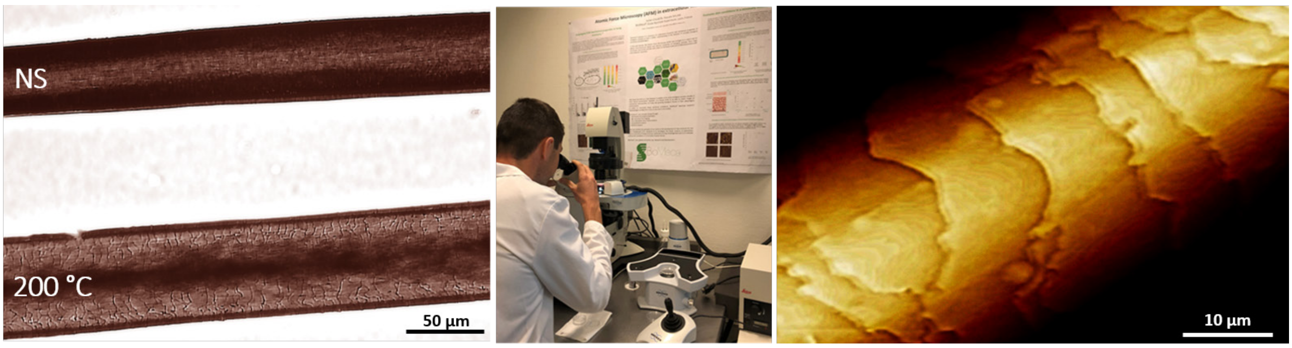

Figure 2 : On the left : *Optical image of a natural (up) and a high-temperature (down) submitted hair fibers taken by the AFM system used by BioMeca®.*
On the right : *AFM topographical image of hair fiber.*

Our AFM system is coupled with an epifluorescence microscope to overlay topographic AFM images, mechanical property maps and optical images.

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Results

High temperature effects on **hair fibers**

Temporary hair straightening by high temperature application is one of the daily practices used for hair styling but might damage hair. The work undertaken by BioMeca® here examines effects of such thermal treatments with a straightener iron on the cuticular structure of hair fibre.
For this purpose, natural hair fibres (without treatment nor straightening - NS) of the same thickness were collected from the temporal area of a volunteer (caucasian, light brown, wavy hair). Each fibre is straightened with a "classic" straightener (ceramic plate). In order to determine the influence of temperature, each fibre is straightened at different temperatures (***Figure 2A***). The first observation is deformation of hair fibre: the more fibre is submitted to high temperature, the wider it appears (optical images: ***Figure 2A and 2C***). This hair flattening is due to stress caused by heating plates.
Then, we studied cuticle's state of each hair fibre by AFM. The experiment consisted of acquisition of topographic images of surface and mechanical properties measurements of the cuticle along hair fibre. AFM images (***Figure 2B***) clearly show presence of debris at the surface of the fibre treated at high temperature, indicating cuticle damages.
Image analysis does not show significant differences on the height of the scales (***Figure 2D***). One of the essential information from this study is the increase of cuticle stiffness when hair is submitted to high temperatures (***Figure 2E***). A significant difference in stiffness increase is observed here from 130°C upwards.

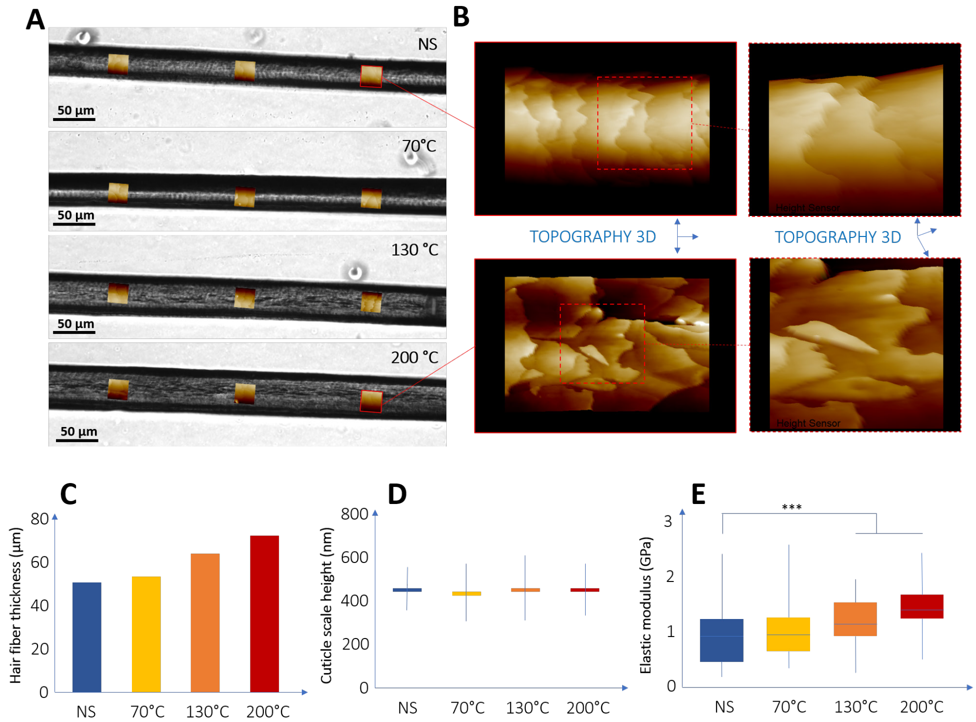

Figure 3 *: **A.** Optical and AFM images of hair fibres non-straightened (NS) or straightened with a flat iron (at 70, 130 and 200°C). **B.** 3D topographical images of cuticule from non-straightened hair (NS) and straightened at 200°C. Image analysis and mechanical measurements allow to obtain statistical quantification of hair fibre width (**C**), height of scales (**D**) and cuticle rigidity (**E**) in different conditions.*

These results indicate that high-temperature treatment **damages cuticle and can lead to hair embrittlement**. It has already been shown that **straightening induces removal of hydrophobic top-layer of hair cuticle** and a consequent **reduction of surface hydrophobicity**. Here, we show that high temperature treatment **also induces cuticle's stiffening.**

We can conclude that the significant rigidity's increase might **lead to the cuticle weakening by making it more breakable.**

Results

Chemical treatments effects on **hair mechanical properties**

Detergents are a common ingredient found in the composition of soaps and shampoos. Most of them are known to be strong tension-actives and can cause skin irritation. Frequent or even daily use of shampoo can thus have negative effects on hair health. In this exploratory study, we used AFM in order to visualize detergent's effect on surface morphology of hair fibre and mechanical properties of its cuticle.

For this study, untreated hair fibres were taken from a volunteer (caucasian, light brown, straight hair) in occipital area. The hair fibres were treated with different concentrations of detergent (1% and 10%) and then rinsed with clear water. Surface topology images (***Figure 3A***) and roughness analysis (***Figure 3C***) show:

- the removal of debris from hair surface as soon as 1% solution was used and
- a significant smoothing of cuticle surface with 10% solution treatment.

In addition to its morphological changes, we observe an important and significant decrease in elastic modulus of hair fibre treated with detergent (***Figure 3B***). The results of this study show that the use of a high dose of detergent induces a weakening of hair fibre by:

- eliminating the protective fatty layer of the cuticle and
- reducing effectiveness of the rigid protective barrier (A-layer, exo and endo-cuticle).

Various quantitative readouts* :

- Hair fibres morphometry characterisation (thickness, length)
- High-resolution cuticle surface images
- Morphometric parameters of the cuticle (scale's height, roughness, debris quantification)
- Mechanical properties mapping
- Elastic modulus quantification of hair cuticle

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

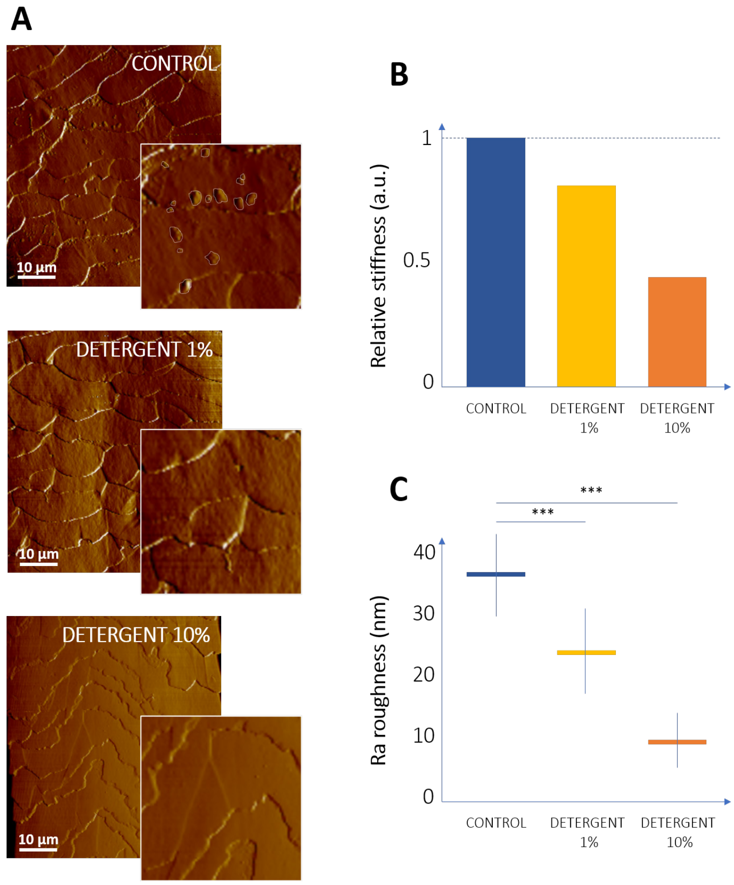

Figure 4 : ***A.*** *Topographical AFM images of hair cuticle treated or not with detergent at different concentrations.* ***B.*** *Effect of detergent on cuticle stiffness. Relative stiffness corresponds to the ratio between mean elastic modulus of control hair fibre and mean elastic modulus of treated fibre.* ***C.*** *Roughness (Ra) quantification as a good indicator of cuticle surface condition.*

These results show that hair treatments such as high-temperatures or frequent use of detergents **affect both structure and mechanical properties of the cuticle**. These two treatments act in two ways, (i) **high temperature induces stiffening of the cuticle** and (ii) **application of detergent softens it**. In both cases, **the cuticle becomes brittle and the cortex is exposed to external aggressions.**

The analysis of mechanical properties associated with surface morphology study is a **powerful quantitative method to determine state of the hair and is ideal to study effect of hair protective products.**