25TH IFSCC CONFERENCE
COSMETHIC SCIENCE AND CONSCIENCE
MILAN 2019
30 SEPTEMBER 1-2 OCTOBER

N° 12

A new way to investigate the collagen glycation process through its optical properties

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Glycation is a natural intrinsic process leading to an accumulation of residues called advanced glycated end products (AGEs) like carboxymethyl-lysine residues (CML) and pentosidine. This process contributes largely to the aging of the skin.
Classical microscopy approaches, including immunohisto-chemistry, are valid tools to detect AGEs formation in collagen network. Nevertheless, they lack any information about the structural changes of collagen fibers that can affect their function.

To this purpose, AGEs in human skin explants were induced by methylglyoxal (MG). Successively, the presence of CML and pentosidine was determined by immunochemistry.

Finally, polarimetric measurements and atomic force microscopy (AFM) were performed on the same samples to demonstrate the correlation between the formation of AGEs and the alteration of collagen bundles and fibers.

Ex vivo-reproduced Glycation

Glycation was inducted by MG in *ex vivo* human skin explants. In parallel some explants were co-treated with MG and aminoguanidine (antiglycation reference product) .

Histology

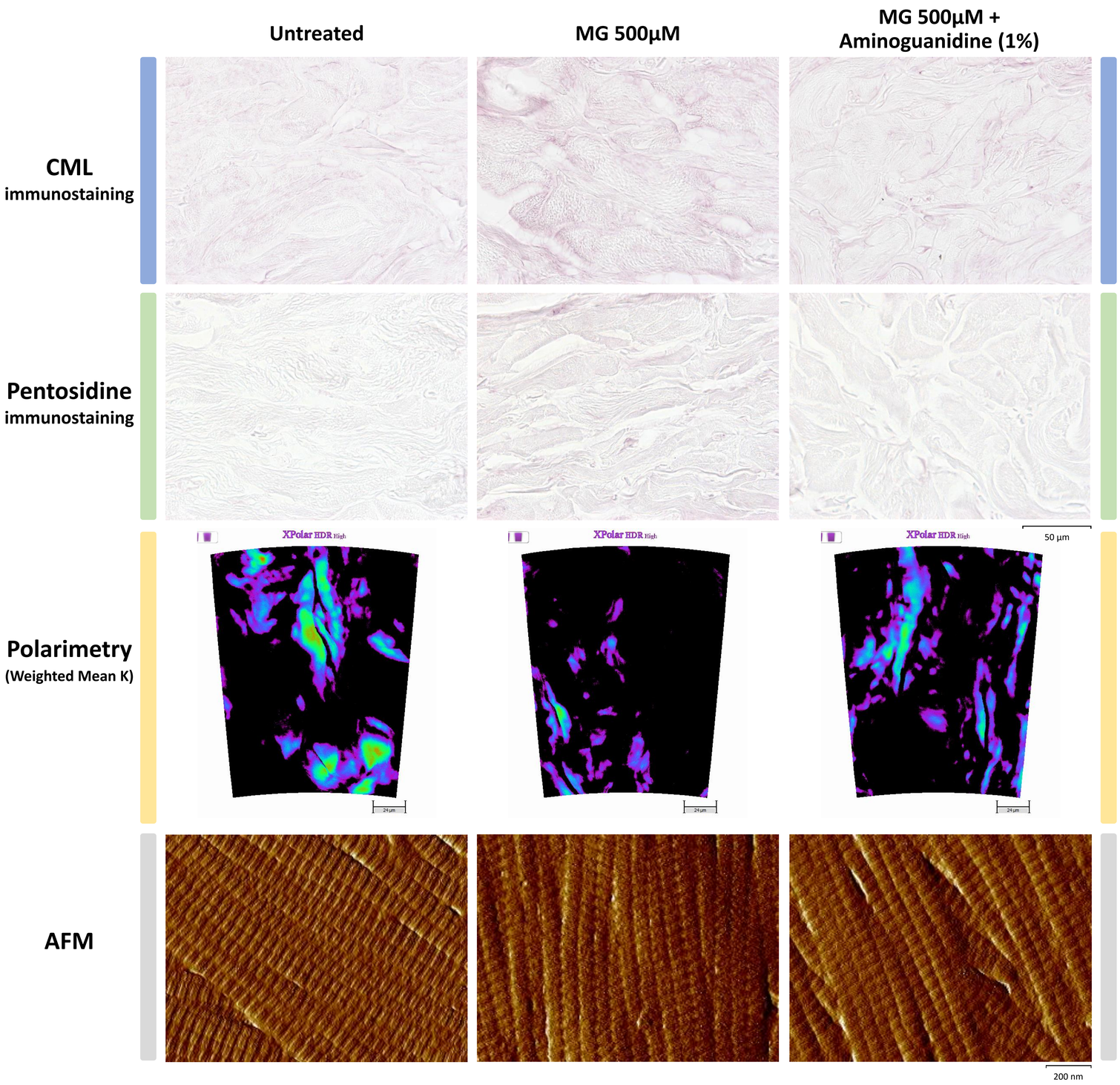
CML and pentosidine were detected on skin sections by immuno-histochemistry in dermal collagen network using specific antibodies.

Polarimetry

Optical properties of collagen bundles, were measured with K-Probe scanner. It displays color map and delivers the Weighted Mean K coefficient (phase delay weighted by fibers density)

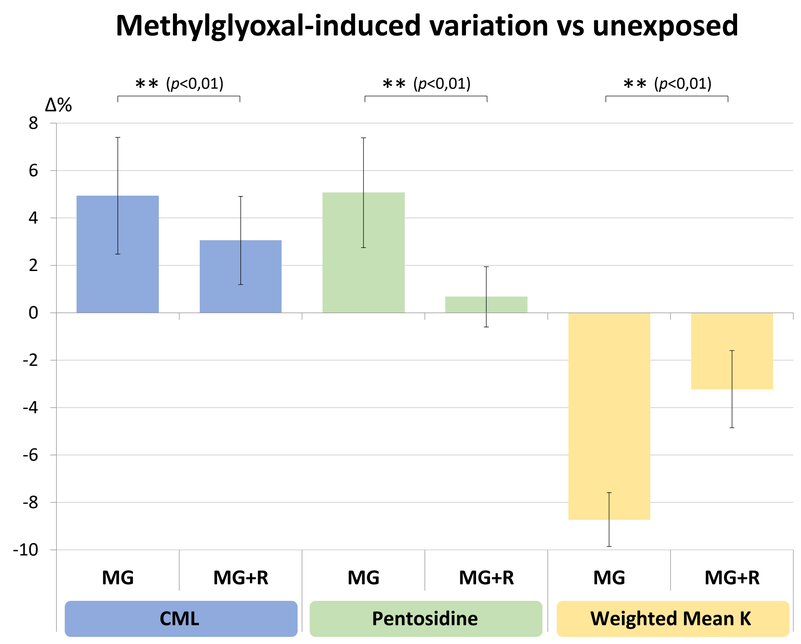
AFM

Collagen fibers were observed by AFM at very high spatial resolution (nano-metric level). The AFM images show the striated structure of collagen microfibrils.



As widely described, MG-treated *ex vivo* human skin explants show a significant increase of CML and pentosidine in the dermis.

On the same samples, birefringence of collagen bundles is also altered reflecting some modification of their physical properties.



Aminoguanidine (R) reduces MG-induced CML and pentosidine by 38%** and 87%** respectively.

In addition, it reduces the loss of collagen bundles birefringence by 63%**.

AFM shows that periodic striation of collagen fibrils is not altered by MG. But AGEs seems to form a “dusty” deposit on collagen fibrils.

Aminoguanidine protects collagen fibrils structure.

Conclusions

The AGEs formation on collagen fibers modifies their external structure and optical properties.
This multiscale work shows that polarimetric measurement is a complementary tool able to highlight the collagen alterations induced by glycation and is able to evaluate the effect of active ingredients and end-products with antiglycation feature.
This approach offers an alternative and valid method to study the process of skin aging and that could help in the development of dermo-cosmetics with anti-ageing activity.