The K-probe® imaging system as a new tool to analyze human skin aging

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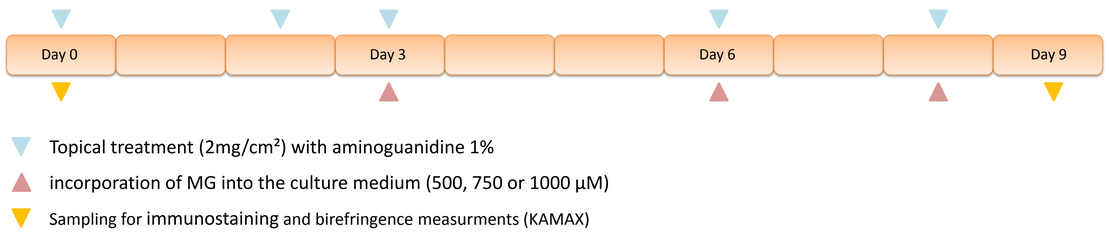
Skin aging
Ex vivo
Glycation
Collagen
Polarization
K-probe®

Glycation is a natural intrinsic process responsible for cross-linking between reducing sugars and proteins. This process, leading to an accumulation of residues called advanced glycated end products (AGEs) like carboxymethyl-lysine residues (CML) and pentosidine, contributes largely to the aging of the skin.
Classical microscopy approaches, including immunohistochemistry or immunofluorescence, are valid tools in order to detect AGEs in the cutaneous compartment. This study shows the correlation between the formation of AGEs and the alteration of the collagen bundles by measuring their birefringence. We used the Xpolar® polarimetric scanners (K-probe® imaging system) as alternative and complementary tool to visualize the glycation in the skin using an *ex vivo* model.

Material and methods

To this purpose, human skin explants from healthy donors have been treated daily, as following with methylglyoxal (MG), a compound known to generate AGEs in the skin, and/or aminoguanidine.



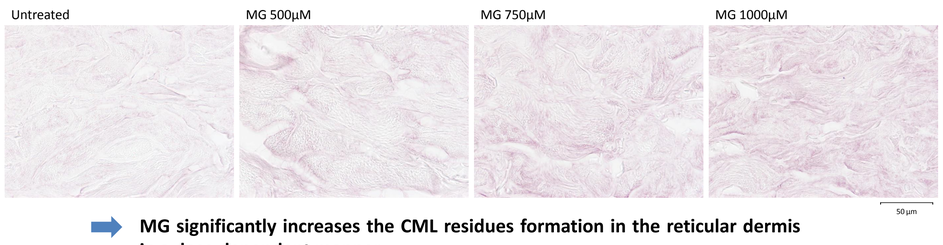
Firstly, we determined the presence of AGEs into the skin by immunohistochemistry using specific antibodies raised against CML and pentosidine, two main compounds generated by the glycation process. AGEs have been quantified by image analysis. Successively, we analyzed the same skin samples by the K-probe® imaging system.
Then, to test this new technology, we evaluated the activity of aminoguanidine, which is usually used as an anti-glycation reference in many study designs. *Ex vivo*, it is especially used at 1% (in CMC gel), applied topically and in combination with MG at 500 µM.

K-probe®, the first polarimetric scanner for histological slides, is an innovative apparatus using XPolar® contrasts which allows an accurate and complete sample analysis, with a sub-micrometric resolving power. The process uses a low optical power and non-contact imaging apparatus which does not cause any degradation on the samples.
The technique is based on the measurement of birefringence: when a birefringent material is irradiated with polarized light, the polarization of the transmitted light is modified. These modifications can be quantified through the measurement of a parameter, called "Kmax". Then, the "Weighted Mean K" parameter is obtained by the summation of all the "Kmax" of an image, divided by the sample area exhibiting birefringence.
This parameter is specific to each Xpolar® image, it notably reflects the density of the fibers on a given field of view. The birefringence induced by the sample is measured qualitatively and quantitatively and then displayed on the screen in the form of a colorimetric map.

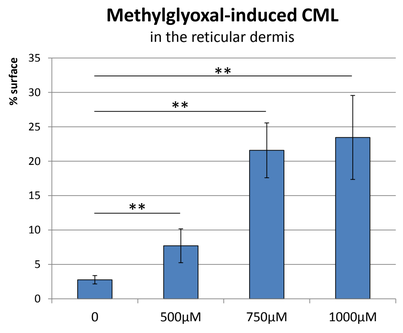
Results

Effect of methylglyoxal on the formation of AGEs and on the modification of the birefringence of collagen bundles.

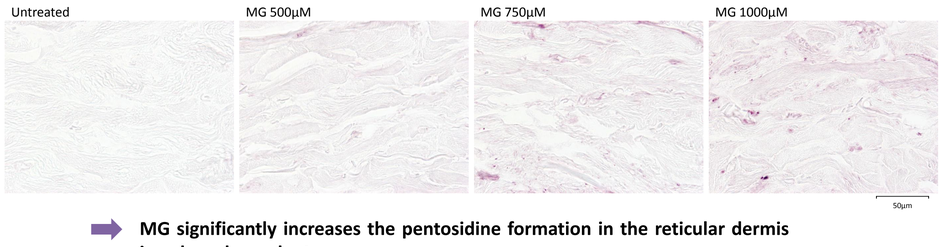
CML immunostaning in the reticular dermis



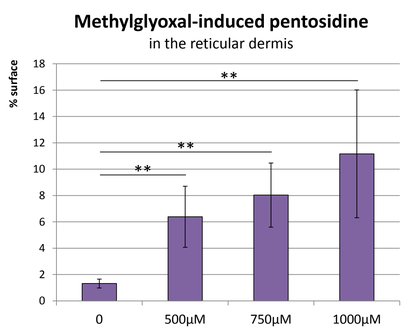
MG significantly increases the CML residues formation in the reticular dermis in a dose dependent manner



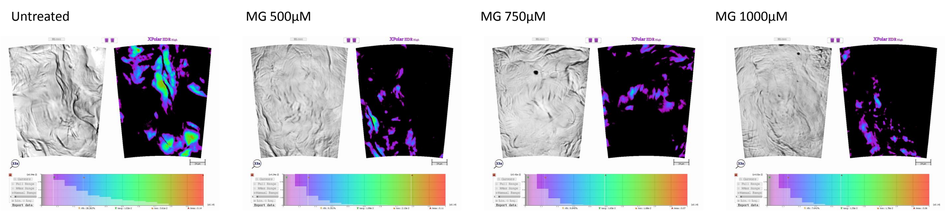
Pentosidine immunostaning in the reticular dermis



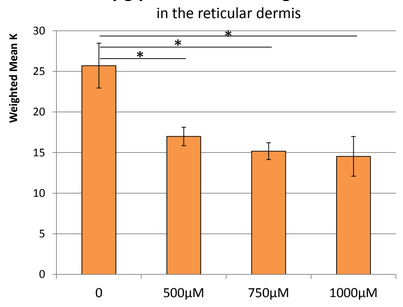
MG significantly increases the pentosidine formation in the reticular dermis in a dose dependent manner



Weighted MeanK measurements in the reticular dermis

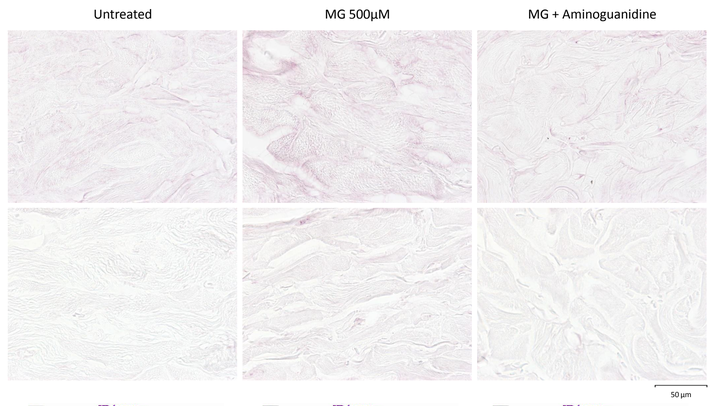


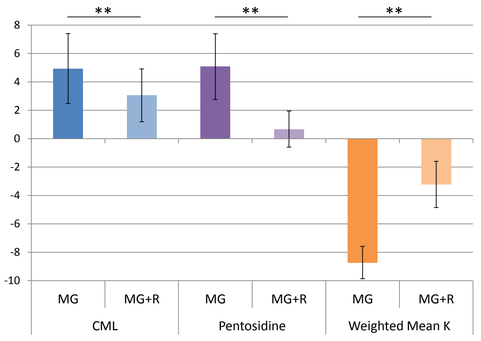
MG significantly decreases the weighted Mean K in a dose dependent manner.



* Significant with $p<0.05$, ** significant with $p<0.01$

Protective effect of aminoguanidine against MG-induced AGEs and MG-modification of collagen bundles birefringence.





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%**.

These results show the ability of the K-probe® measurement to highlight the activity of glycation products.

Conclusions

As widely described, MG-treated *ex vivo* skin samples show a significant increase of carboxymethyl-lysine and pentosidine in the dermis.
On the same samples, birefringence of collagen bundles measured using K-probe® shows a decrease of the "weighted Mean K".
The decrease of this factor is probably due to chemical and structural modifications induced by methylglyoxal on collagen fibers leading to a modification of its optical proprieties.
Taken together these data show that the K-probe® imaging system is an alternative and valid method to study the process of skin aging and that could help in the development of active ingredients and end-products with anti-aging activity.