Evaluation Of The Air Pollution Effects On Human Skin Using An Original 3-D Model

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INTRODUCTION
Every day the skin is more or less exposed to air pollution which has a direct effect on its health. Scientific studies have highlighted the negative effects of pollution on the skin moisture. The skin loses its suppleness and radiance. It has been shown that cigarette smoke decreases the hydration of the skin (Morita A., 2007). Pollution also stimulates the production of pro-inflammatory molecules, including TNF-alpha, IL-6 and IL-8 (Ushio H et al., 1999). But nothing was previously described on the effect on atmospheric gas pollution on skin hydration.

OBJECTIVE
Evaluate the effect of pollution on skin hydration and inflammation, using an original ex-vivo model of human skin explants exposed to car exhaust gas, and secondly, to measure the protective and moisturizing effect of an original hydrolyzed marine polysaccharide isolated from a red alga (Rhodophyceae) and named MHF.

MATERIAL AND METHODS
- **Product**：“Marine Hydrolyzed Fucellaran (MHF)” is a sulfated kappa-carrageenan composed of galactose and anhydroglactose units, extracted from the red algae Fucellaria lumbricalis and modified by a controlled depolymerisation by an original method using supercritical CO2 (CODIF’s innovation).
- **Assay systems** : Human skin explants of 12mm diameter (obtained by cosmetic surgery).
  - **Pre-treatment protocol**: topical application of 20 mg of MHF, diluted at 0.5% during 30 minutes or at 1.5% during 60 minutes before each gas application: once at 10% for immediate effect (protocol 1), or several times for cumulative effect at 0.5%, D3, D4, D5 and D6 (protocol 2), and at D0, D2, D3, D6, D8 and D9 (protocol 3).
  - **Treatments with gas**: diesel car without particulate filter collected in a specific bag and applied on skin for 5 hours for immediate effect or 30 minutes for cumulative effect.
- **Analytical evaluations**: hydration by corneometry, Inflammation by IL-8 dosage, COX-2 by quantitative RT-PCR and MIF by immunoassay.
- **Image analysis**: Optical microscope with a camera.

RESULTS

Effect Of Pollution On Skin Hydration
The skin treatment by car exhaust gas (1 x 5 hours) significantly decreased by 11% the hydration rate of exposed skin (Figure 1). The hydration rate is thus sensitive to pollution contact, and this decrease may be linked to the inflammation state.

Effect Of Pollution On SPRRs Proteins
Pollution decreased SPRRs proteins SPRR1 and SPRR2 proteins were clearly detected in the upper part of the epidermis (Figure 5A). Gas decreased SPRR1 by 31% and SPRR2 by 27% in comparison with untreated skin (Figure 5B/5D, respectively).

Protective Effect Of MHF On SPRRs Proteins
MHF stopped skin protein by increasing SPRR1 expression by 38% and SPRR2 by 79% in comparison with untreated skin exposed to gas for 3 days (Figure 5F/5G). The ingredient limited the reduction of SPRR1 and SPRR2 in stressed skin. The protection of both SPRRs could explain, at least partially, the protection against pollution induced-dehydration.

Effect Of Pollution On Skin Inflammation
A treatment of skin explant by one contact with car exhaust fumes for 5 hours increased by 60% the COX-2 expression (Figure 6). The skin treatment with gas increased by 36% the IL-8 secretion (Figure 7). The increase of COX-2 and IL-8 expression is a good indicator of an inflammation state induced by atmospheric air pollution.

Protective Effect Of MHF On Skin Inflammation
The cumulative application of gas (5 x 30 minutes from Day 0 to Day 6) increased the number of COX-2 positive cells in the epidermis (Figure 8B). The pre-treatment by MHF before each gas application protected skin by decreasing COX-2 positive cells (Figure 8C). Thus the marine ingredient reduced the pollution induced inflammation.

CONCLUSION
Pollution induced a clear reduction of hydration rate in ex vivo skin explants. It also reduced the SPRRs expression in the epidermis, and increased of COX-2 positive cells in the epidermis. MHF protects the skin against dehydration induced by exposure to car exhaust gas. Importantly, its ability to protect was dependent to the number of contacts with gas. MHF prevented the loss of SPRRs expression in the epidermis. MHF also decreased the number of COX-2 positive cells. The ex vivo model is interesting to measure the side effects of car exhaust gas, and to select protective ingredients that might be intended for people with sensitive skin.