2 EFFICACY STUDIES

2.1 *In vitro* studies

The *in vitro* tests were carried out with the pure synthetic molecule used to obtain **CLOTHOLINE®: Coumaroyl Methoxytryptamine (CmMT),** or on the molecule isolated from Bleuet used to obtain **CLOTHOLINE LV: Centaurea cyanus flower extract (Ccf extract)**. Products are diluted in the cell culture medium.

2.1.1 Activation of the expression of Klotho gene

- **Protocol**

We used qPCR microfluidic technology according to the protocol of Fluidigm®. Microfluidic technology comes from crossing the world of nanotechnology and gene analysis by q-PCR. System miniaturization has led to the development of a chip that currently allows analyzing 96 conditions *versus* 96 genes. The chip we designed integrates genes that cover the entire range of skin activity including the Klotho gene KL for skin longevity.
Normal Human Dermal Fibroblasts (NHDF) were seeded at the concentration of 10 000 cells by well in 96 wells plates. After adhesion during the night, CmMT was introduced at 3ppm during 24 hours. Then, the supernatant was eliminated and the cells collected in specific lysis solution for mRNA extraction. Lysates were transferred on plate in order to purify mRNA. Afterwards, a reverse transcription system was used. According to the Fluidigm protocol, specific stages for 96x96 chip preparation were starting. A preamplification step was carried out with the primers used in the chip. Pre-amplified cDNA/PCR mix and primers were deposited on the chip. The mix blending was undertaken by the IFC controller™ and then the chip was placed in the BioMark™ system in order to carry out real time PCR.

- **Results**

CLOTHOLINE® strongly increases Klotho gene expression in dermal fibroblasts. The stimulation reached 244% relative to the control (Figure 1).

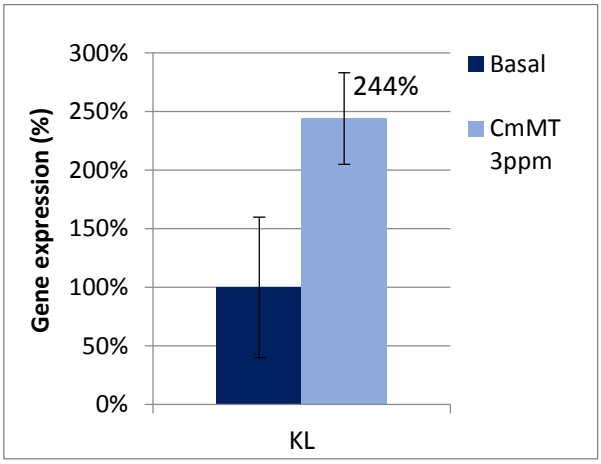

Figure 1: **CLOTHOLINE®'s active ingredient activates Klotho gene expression.** *qPCR (Fluidigm) on NHDF after 24h of CmMT treatment (3ppm).*