

2.1.3 Delay of the senescence

Lamin B1 is a nuclear protein which establishes a two-dimensional matrix in the internal nuclear membrane. It is involved in the nuclear stability, the structuration of the chromatin and the gene expression control. Its expression decreases during the cellular senescence caused by aging, thus Lamin B1 is an interesting new aging marker. However, its overexpression is harmful for cells. Indeed, the overexpression of Lamin B1 increases the rate of proliferation and delays the appearance of the cells senescence leading to the stop of the cellular cycle in G1/ S phases (Freund et al, 2012). This protein must be maintained at a constant level to ensure homeostasis of the metabolism.

❖ Protocol

NHDF were incubated until pre-senescence without treatment or with 5ppm or 10ppm of CLOTHOLINE® active ingredient during 56 days.

Then young NHDF freshly prepared and the pre-senescent NHDF pretreated or not with CLOTHOLINE® were seeded in 96 wells plate during 24 hours. The cells were washed and fixed with formalin. After permeabilization and blocking, cells were incubated with anti-Lamin B1 antibody and revealed with secondary antibody linked to Alex 488. Finally cells nuclei were marked with DAPI. Lamin B1 was observed and quantified by automated fluorescence microscopy (Arrayscan™ (Cellomics)) (Figure 3A).

❖ Results

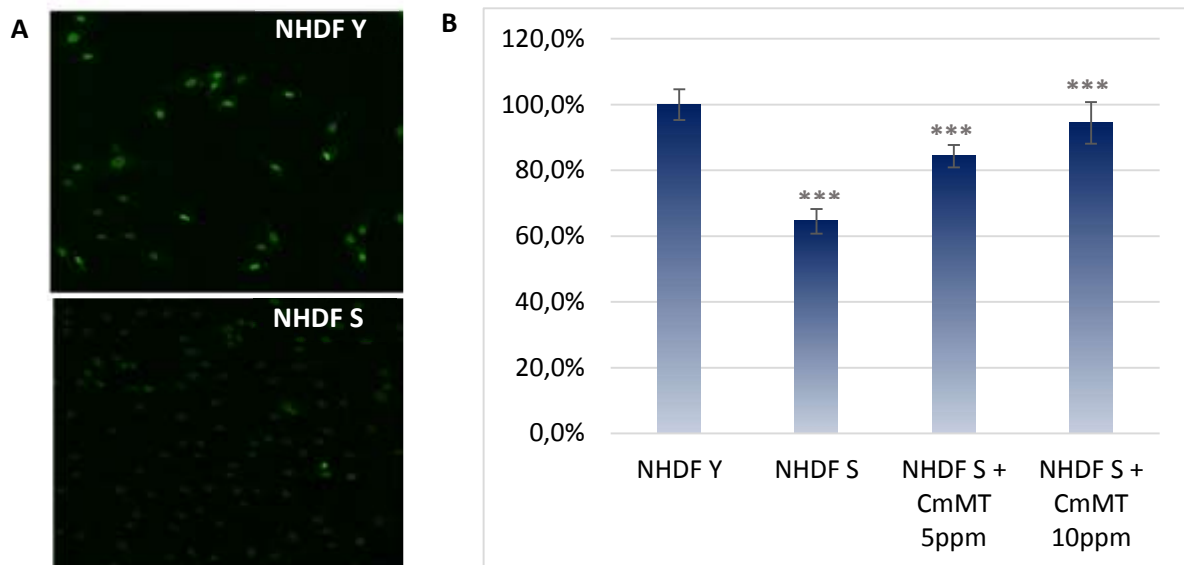


Figure 3: **CLOTHOLINE®'s active ingredient maintains Lamin B1 expression in pre-senescent fibroblasts.** Pictures of Lamin B1 labelled by immunofluorescence in young cells (NHDF Y) and pre-senescent cells (NHDF S). Lamin B1 is labelled in green (A). Diagram of Lamin B1 expression in young NHDF (Y), pre-senescent NHDF (S) and treated NHDF during 56 days (S+) quantified by automatic fluorescence microscopy (B).

CLOTHOLINE® is able to delay the cell senescence. Untreated senescent NHDF show a Lamin B1 expression rate largely lower than young NHDF while senescent NHDF treated with CmMT show a maintained rate of Lamin B1 which approaches the one of young NHDF (Figure 3B). Moreover, CmMT doesn't lead to Lamin B1 overexpression that would have been harmful for the cells. Thus, CLOTHOLINE® contributes to the dermal homeostasis and subsequently to extension of skin youthfulness.