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Collagen XVIII: A key interfacial component of the skin architecture

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INTRODUCTION

Collagen XVIII belongs to the multiplexins, known as extracellular matrix proteins that contain multiple triple-helix domains (collagenous domains) interrupted by noncollagenous domains. Besides, as collagen XVIII is a basement membrane (BM) heparan sulfate proteoglycan, it holds the structural properties of both collagen and proteoglycan. This collagen is expressed ubiquitously in various BM structures throughout the body. In skin and compared to other collagen types, collagen XVIII displays the broadest repartition as it could be synthetized by keratinocytes, endothelial cells, epithelial cells of the sweat glands and hair follicles stem cells, and adipocytes (1). However, the complete physiological role of collagen XVIII is not fully understood, even if its localization and ultrastructural organization reveal that it is an important component of all BM molecular networks present in skin (2). Understanding its expression modulation with age is of great interest for cosmetic research and could provide new strategies to counteract the loss of tissue structure and cohesion seen during aging of epidermis, dermis, hypodermis, and scalp.

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METHODS AND RESULTS

SPECIFIC ANTIBODY DESIGN

To specifically target the collagen XVIII portion involved in tissue structure, and not its endostatin domain (Figure 1), we designed an affinity-purified rabbit antibody against its two collagenic domains: COL1 and COL 5. This antibody was used for collagen XVIII immunolocalization on skin biopsies of different ages and for the design of an enzyme-linked immunosorbent assay (ELISA) on keratinocytes allowing cosmetic ingredient screening.

COLLAGEN XVIII EXPRESSION DURING SKIN AGING

At first immuno-labelling of 12 skin samples coming from face lifting was performed to localize and quantify this protein in the skin structure (n = 6 for 8–15 years old and for 30–69 years old). Briefly, 7-µm cryosections were fixed using cold methanol/acetone during 10 min and unspecific sites were blocked using 10% normal goat serum diluted in phosphate-buffered saline (PBS) at room temperature. Then sections were incubated within the primary antibody and after two washes in PBS, the sections were incubated with Cy3-labelled anti-rabbit antibody and counterstained with Dapi. Sections were examined using a Zeiss LSM 700 confocal microscope and the acquisition was realized using the Z-stack mode of the Zen program. Quantification data are presented as mean values and standard error of the mean from at least three measures performed on biological triplicate samples for each skin samples (Figure 2).

As compared to young skin specimens, collagen XVIII staining was less intense and homogenous. In a 60 years old skin sample, heterogeneity of the *lamina densa* labeling was observed as well as zones where the *lamina densa* starts to duplicate and detach from the dermal epidermal. Quantification allowed to evidence a strong collagen XVIII decrease as skin ages (–43.5% for older group).

The age decrease of collagen XVIII was confirmed using quantitative reverse transcription polymerase chain reaction (qRT-PCR) study on keratinocytes extracted from 50 abdominal skin biopsies (Figure 2, right), which evidenced that the proteomic decrease occurs

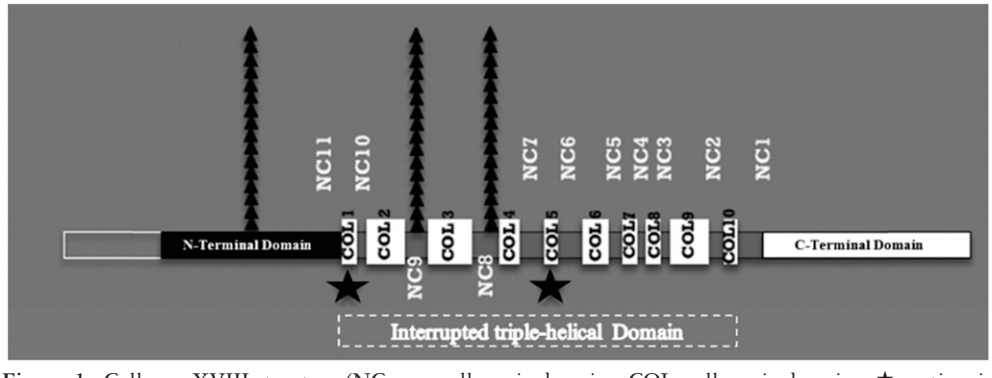

Figure 1. Collagen XVIII structure (NC: non collagenic domains, COL: collagenic domains, ★: antigenic area).

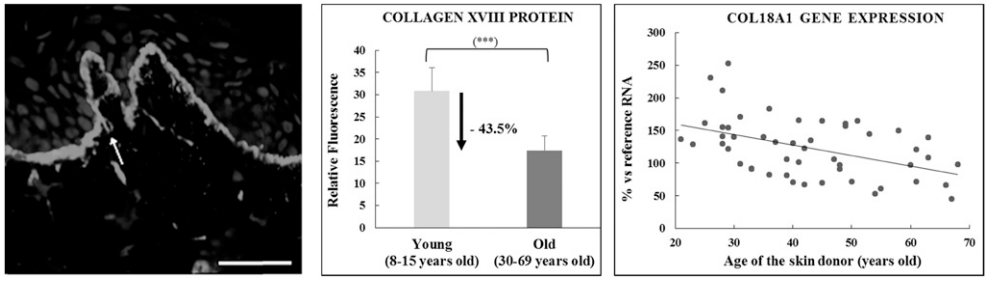

Figure 2. Collagen XVIII localization in skin section and decrease with aging. Left: Mature representative collagen XVIII localization at the dermal epidermal junction X25. Middle: Collagen XVIII protein level in facial skin biopsies (n = 6 per age group, Student's *t* test, ***$p < 0.001$. Right: Col18A1 gene expression in keratinocytes from abdominal skin biopsies (n = 50 from 21 to 68 years old).

mainly through COL18A1 gene regulation. Gene expression was also reduced after UVB irradiation, highlighting the implication of this target in photoaging (data not shown).

NEW ELISA DEVELOPMENT TO SELECT ACTIVE INGREDIENT PROMOTING COL XVIII SYNTHESIS

In order to restore the collagen XVIII age decrease, a screening test was developed to identify the best ingredient able to induce collagen XVIII synthesis in adult keratinocytes. For this purpose, a new ELISA test was designed on keratinocytes extracted from a 36 years old skin specimen using the specific affinity-purified rabbit antibody previously described. After culture and ELISA protocol optimizations, several vegetal extracts were evaluated and *Khaya senegalensis* bark extract was identified as one of the most efficient (Figure 3).

NEW NONCONTACT DEVICE DESIGN FOR MECHANICAL PROPERTIES EVALUATION IN VITRO ON RECONSTRUCTED SKINS TREATED BY *K. SENEGALENSIS* BARK EXTRACT

In order to evaluate the mechanical behavior, a specific device was designed for the purpose of this evaluation. It allows the assessment without any disturbance and preconditioning of the stressed area. This new specific noncontact device is based on a controlled air flow system and a high laser measurement of displacement (Figure 4). A specific software

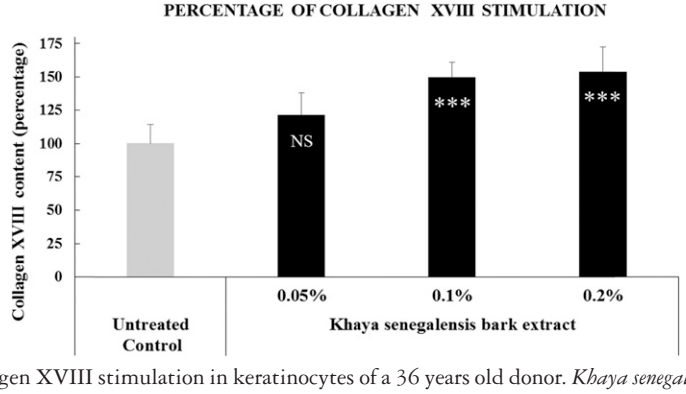

Figure 3. Collagen XVIII stimulation in keratinocytes of a 36 years old donor. *Khaya senegalensis* bark extract demonstrated significant collagen XVIII stimulation by 0.1%. Data presented as mean values and standard error of the mean from at least seven measures. Statistical significance was assessed running a multiple comparisons versus untreated control, Dunnett's method, ***$p < 0.001$, NS: not significant.

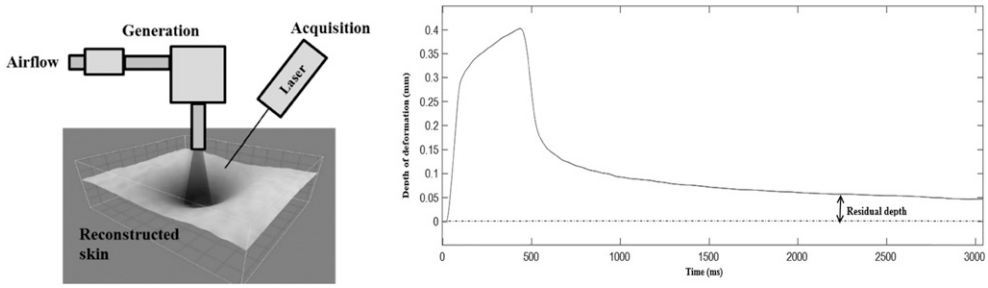

Figure 4. Non-contact device used for mechanical evaluation and skin deformation curve obtained.

developed under LabView™ language (National Instruments, Austin, TX) allows it to control the whole system.

Reconstructed skin (Mimeskin™; BASF Beauty Care Solutions, Lyon, France) was produced from human-cell-cultured keratinocytes and fibroblasts isolated from human skin (30- and 18-year-old women's breast cells, respectively). Briefly dermal reconstruction was performed during 28 days with a daily medium change and epidermal reconstruction was performed including a 3 days immersion step and a 14 days air–liquid interface step. *Khaya senegalensis* bark extract at 0.05% was introduced at each cell medium replacement during the reconstructed skin growth.

For each skin sample, residual depth, corresponding to the residual deformation at the end of measuring cycle, was calculated (Figure 4). The smaller the value of residual depth is, the more elastic the skin is. *Khaya senegalensis* bark extract at 0.05% significantly decreased the residual depth parameter, demonstrating an improvement of the skin elasticity (Figure 5).

CLINICAL EVALUATION OF *K. SENEGALENSIS* BARK EXTRACT

The study was a double-blind, placebo-controlled, and randomized study with 25 female volunteers (Fitzpatrick skin type I, II, or III; ages 53–65) with self-perceived loss of skin elasticity, crow's feet wrinkle grade between 3 and 4, and visible pores as graded by a clinical scientist.

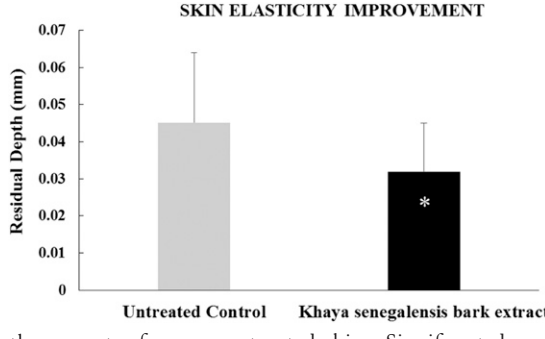

Figure 5. Residual depth parameter from reconstructed skins. Significant decrease of the residual depth observed for the skins treated with *K. senegalensis* bark extract 0.05%. Data are presented as mean values and standard error of the mean from 13 measures. Statistical significance was assessed running Mann–Whitney test, $*p < 0.05$.

Each of the placebo and *K. senegalensis* bark extract (1%) products was applied to a half-face in a randomized manner and twice a day for 56 days.

The efficacy of *K. senegalensis* bark extract was evaluated for certain skin parameters associated with aging, e.g., skin elasticity on temple with cutometer, cheek curvature (surface heterogeneity), and skin wrinkles (data not shown) using fringe projection analysis (AEVA technique).

Cutometer results: Skin elasticity. Measurements were taken using the Cutometer® SEM 575 (Courage & Khazaka) and immediate elasticity (R7) was calculated as explained in Figure 6. The closer the value of R7 is to 1 (100%), the more elastic the skin is.

After only 28 days of treatment, a 9.6% improvement of the immediate elasticity (R7) over placebo was observed (Figure 7). This improvement was confirmed after 56 days of treatment (11.6% improvement). By stimulating collagen XVIII synthesis in all skin layers, *K. senegalensis* bark extract dramatically improved skin elasticity.

Fringe projection results: Skin cheek curvature. Curvature is a new parameter to identify any feature that has a defined curvature without taking into consideration of the depth. This parameter could be related to the visibility of the skin feature. The more detected density of the curvature suggested more visibility of the skin feature. An increase of this parameter is linked to an increase of skin surface heterogeneity (pores and microrelief). Curvature density was detected and analyzed by the Optocat software after image acquisition with the AEVA 3D-HE 3D Imaging System.

After 28 and 56 days of treatment by *K. senegalensis* bark extract, cheek curvature (surface heterogeneity) showed improvement by 22.8% and 38.2%, respectively, compared to the placebo (Figure 8). By stimulating collagen XVIII, *K. senegalensis* bark extract induced a visible pore reshaping effect (pictures not shown), leading to a significant improvement in skin surface heterogeneity (curvature).

CONCLUSION

Our results show a decrease of the total amount of collagen XVIII during skin chrono- and photoaging. We demonstrated that collagen XVIII is a relevant structural target for

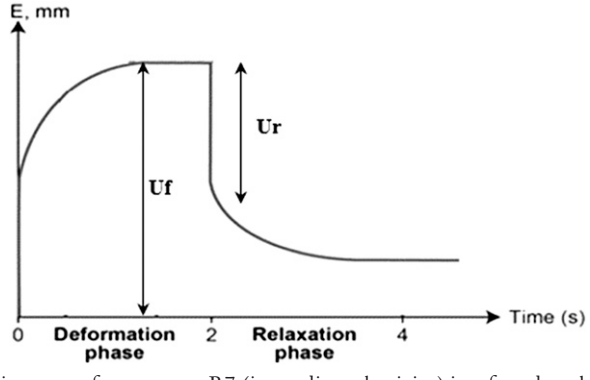

Figure 6. Stress–strain curve of cutometer. R7 (immediate elasticity) is referred as the biological (net) elasticity. It is the portion of elasticity compared to the final distension. It is represented by the ratio of "the immediate retraction" to "final distension," i.e., Ur/Uf.

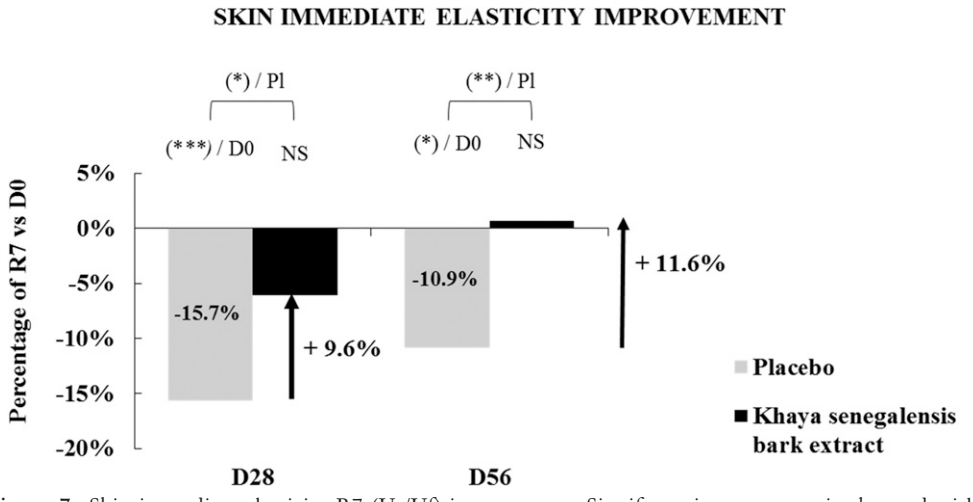

Figure 7. Skin immediate elasticity R7 (Ur/Uf) improvement. Significant improvement is observed with *Khaya senegelensis* bark extract treatment versus placebo. Percentage versus baseline on 25 volunteers (53–65 years old). Statistical significance assessed running Student's *t* test or Wilcoxon test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

skin improvement to fight skin aging. This discovery and newly developed methods and equipment offer new opportunities for clinical and cosmetic applications such as strengthening several skin structural interfaces. We demonstrated that *K. senegalensis* bark extract is an interesting active ingredient to boost collagen XVIII synthesis, thus leading to cosmetic applications targeting improvements of skin surface heterogeneity (pores and microrelief), wrinkles (data not showed) and skin elasticity. These findings allow us to claim the discovery of the "skin matrix lifter."

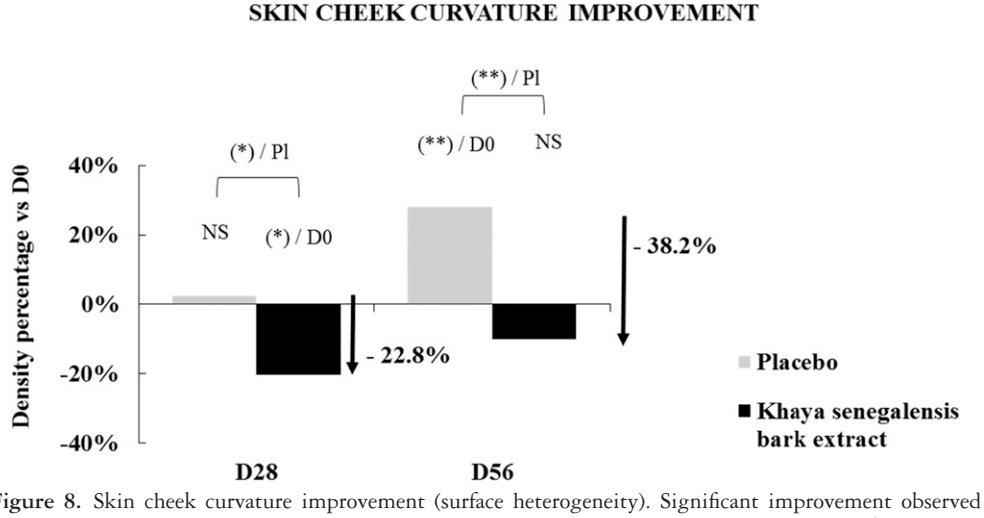

Figure 8. Skin cheek curvature improvement (surface heterogeneity). Significant improvement observed with *Khaya senegelensis* bark extract treatment versus placebo. Percentage versus baseline on 24 volunteers (53–65 years old). Statistical significance assessed running Student's *t* test or Wilcoxon test versus baseline, and Student's *t* test or Mann–Whitney test versus placebo test, $*p < 0.05$, $**p < 0.01$.

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