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3D model for menopausal skin: advancing personalized and specialized active ingredients and solutions

Cécile NESPOLO[1], Loïc PENTECOUTEAU[1], Céline LAPERDRIX[1], Pierre-Yves MORVAN[1], Romuald VALLÉE[1].

[1]CODIF INTERNATIONAL, 61 Rue du Commandant l'Herminier, CS 51749 F-35417 Saint-Malo Cedex, France.

1. Introduction

Menopause is a major physiological transition, characterized by the cessation of ovarian function and a sharp decline in circulating key female hormones, particularly 17β-estradiol. Each year, more than 47 million women worldwide enter this life stage, and with rising life expectancy, the postmenopausal population is steadily increasing. By 2030, it is projected that over 1.2 billion women will be postmenopausal, highlighting menopause as a global concern for both public health and quality of life [1, 2]. The median age of onset is around 51 years and is preceded by a perimenopausal phase lasting two to eight years, during which hormonal fluctuations begin to manifest clinically and biologically [3, 4].

In addition to reproductive changes, menopause is associated with over 35 symptoms that can significantly affect physical and emotional well-being, including hot flashes, sleep disturbances, cognitive complaints, and musculoskeletal discomfort [1]. Among these, the impact on skin remains under-recognized despite its clinical and psychological relevance. As a hormone-responsive organ enriched in receptors for key female hormones (ERα, ERβ, and GPER), the skin undergoes accelerated aging following hormonal withdrawal [5, 6], which clinically presents as dryness, thinning, reduced elasticity, wrinkle formation, and sagging, features that affect not only skin function but also self-perception and social identity [7, 8].

Key female hormones, particularly 17β-estradiol, play a central role in skin homeostasis, regulating fibroblast activity, extracellular matrix remodeling, and antioxidant defenses [8]. After menopause, the decline in hormonal production from ovarian sources leads to reduced synthesis of collagen and elastin, increased matrix metalloproteinase (MMP) activity, diminished vascularization, and heightened oxidative stress. These changes impair skin structure and resilience, compromise barrier function and repair, and contribute to visible aging [9–14].

At the epidermal level, hormonal deficiency alters barrier function, increasing transepidermal water loss (TEWL), decreasing hydration, and heightening sensitivity [15, 16]. These effects

are partly due to changes in the lipid composition of the *stratum corneum*, including reduced ceramide levels and shorter lipid chain lengths [17]. Key female hormones also modulate the expression of proteins involved in epidermal differentiation and barrier integrity. A recent clinical study showed that hormone replacement therapy (HRT) enhances filaggrin expression and increases the thickness of K10-positive layers following irritant challenge, underscoring their role in maintaining epidermal homeostasis [18].

However, HRT is not suitable for all patients due to safety concerns and contraindications [19], which has prompted growing interest in topically applied, biologically informed skincare strategies. The development of such approaches requires physiologically relevant models capable of capturing the complexity of hormone-deficient skin. While traditional monolayer cultures offer molecular insights, they do not reproduce the architectural and functional features of native skin. Full-thickness 3D models better reflect skin physiology and are increasingly used to simulate conditions such as aging or hormonal imbalance [20, 21].

In response to this need, we developed and characterized a novel full-thickness *ex vivo* 3D model based on human skin explants cultured in a medium deprived of three key female hormones. This model mimics the hormonal context of menopause and enables the study of biological and functional alterations associated with hormone withdrawal. In this proof-of-concept study, we focused on two essential proteins, filaggrin and claudin-1, key markers of epidermal barrier integrity and homeostasis [22, 23], and explored the potential of 0.5% vitamin C as a topical intervention to restore their expression under hormone-deficient conditions.

2. Materials and Methods

2.1. Explant origin

Human full-thickness skin explants (Ø12 mm) were obtained from abdominoplasty procedures performed on female donors aged 31, 45, and 49 years (CTI Biotech or Biopredic International). All procedures involving human biological material were conducted in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

2.2. Culture conditions and treatment protocol

Upon reception, the explants were incubated overnight at +37 °C under 5% CO_2 in skin medium (Ref: CTIGM.ExVivo.060, CTI Biotech) for acclimatization. The following day, they received a topical application of 50 µL of either 0.5% vitamin C (Ref: A0278, Sigma-Aldrich, in 0.9% NaCl) or NaCl 0.9% alone (control). Explants were maintained in either a menopausal medium (deprived of three key female hormones) or a non-menopausal medium (supplemented with hormones). Treatments and culture media were renewed every 2 to 3 days. At day 7, explants were washed, fixed in 3.7% formaldehyde, and embedded in paraffin. Immunohistochemistry was performed to assess the expression of proteins involved in epidermal differentiation and barrier function. Each experimental condition was performed in five biological replicates (n = 5).

2.3. Histological and Immunohistochemical Analyses

A 4 µm paraffin section from each explant was first stained with hematoxylin to confirm tissue integrity. Immunohistochemical staining was then performed to assess the expression of two markers: filaggrin, involved in epidermal differentiation and natural moisturizing factor production, and claudin-1, a tight junction protein essential for barrier function [22, 23]. Primary antibodies used were anti-filaggrin (Santa Cruz, Clone AKH1) and anti-claudin-1 (Abcam, ab15098), following standard protocols. Detection was performed using specific fluorescent secondary antibodies (Roche). Images were acquired using the NDPview2 software and quantitatively analyzed with NIS-Elements software (Nikon Instruments Inc.). Results were expressed as stain intensity values for a defined area of interest.

2.4. Data normalization and calculation of protection percentage

All fluorescence data are expressed as mean intensity values, calculated across 15 to 18 image acquisitions per donor ($n = 5$ explants for 31 and 45-year-old donors, n=6 explants for 49-year-old donor). The percentage variation between conditions was computed using the following formula:

- To assess the effect of menopausal conditions:

% Variation = [(Menopausal value – Non-menopausal value) / Non-menopausal value] × 100

- To evaluate the effect of vitamin C treatment under menopausal conditions:

% Variation = [(Vitamin C treated value – Untreated menopausal value) / Untreated menopausal value] × 100

Negative values indicate a reduction in fluorescence intensity relative to the reference condition, while positive values indicate an increase.

2.5. Statistical Analysis

Raw data were compiled and analyzed using Microsoft® Excel. Statistical comparisons between groups were performed using a two-tailed Student's t-test. Outliers were identified and excluded using the Grubbs' test. Differences were considered statistically significant at $*p < 0.05$. Higher significance levels were indicated as follows: $**p < 0.01$ and $***p < 0.001$. Trends toward significance were denoted with the symbol # ($p < 0.1$).

3. Results

3.1. Menopausal conditions significantly downregulate filaggrin expression in human skin explants

To investigate the impact of key female hormone deprivation on epidermal barrier markers, the expression of filaggrin and claudin-1 was evaluated by immunofluorescence after 7 days of culture in a hormonally deprived ("menopausal") medium, in comparison to a control medium supplemented with female hormones.

As illustrated in Figure 1, hormonal deprivation led to a marked reduction in filaggrin expression. In explants from the 31-year-old donor, filaggrin fluorescence intensity decreased by 45% under menopausal conditions compared to the non-menopausal control ($p < 0.001$). Representative images further confirm the visible reduction in filaggrin signal across the *stratum granulosum* (SG).

Similar trends were observed in explants from the 45-year-old donor, which exhibited a more pronounced decrease (–85%), while the 49-year-old donor showed a non-significant reduction of 34%.

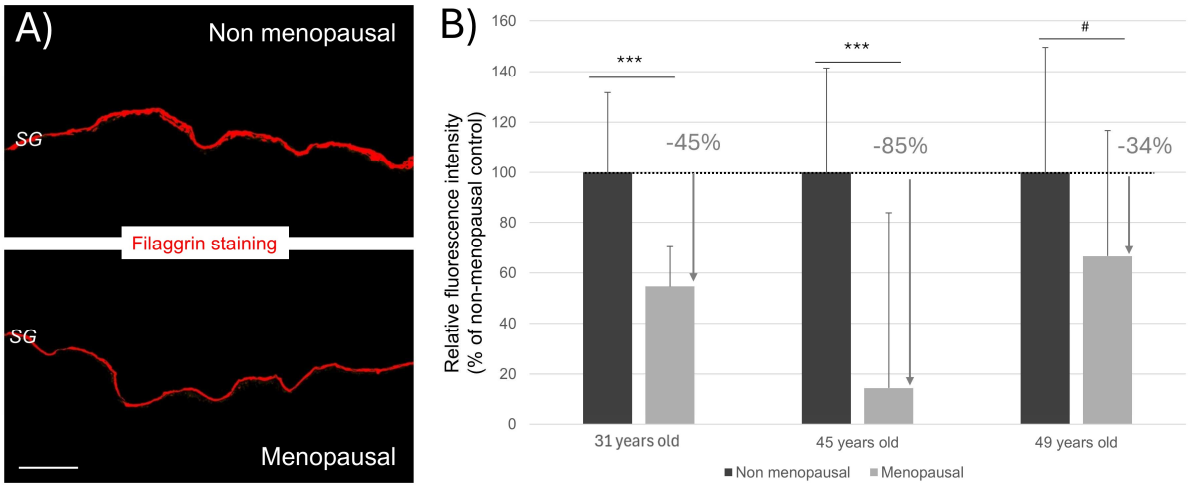

Figure 1. Donor-dependent decrease in filaggrin expression under menopausal conditions.
(A) Representative immunofluorescence images showing filaggrin staining (red) in human skin explants from a 31-year-old donor after 7 days of culture under non-menopausal (top) or menopausal (bottom) conditions. The signal is localized primarily in the *stratum granulosum* (SG). A visible reduction in fluorescence intensity is observed in the hormone-deprived condition. Scale bar = 100 µm.
(B) Quantification of filaggrin fluorescence intensity expressed as a percentage of the non-menopausal control (set to 100%) for each donor. A strong decrease is observed in the 31-year-old (-45%, n = 5) and 45-year-old (-85%, n = 5) donors (***$p < 0.001$), whereas the 49-year-old donor exhibited a non-significant reduction (-34%, $^{\#}p < 0.1$, n = 6). Values are expressed as mean ± SEM (n = 5 or 6 explants per condition, depending on the donor).

3.2. Claudin-1 expression is moderately reduced under menopausal conditions

The impact of hormonal deprivation on tight junction integrity was further evaluated through claudin-1 immunostaining. As shown in Figure 2, culture under menopausal conditions led to a donor-dependent decrease in claudin-1 expression.
In explants from the 45-year-old donor, a significant reduction of -46% was observed ($p < 0.01$). For the 31-year-old donor, claudin-1 intensity decreased by 24%, though this variation did not reach statistical significance. The 49-year-old donor exhibited a non-significant reduction of 14% compared to the hormone-supplemented control.
This moderate yet consistent decrease in claudin-1 expression suggests that tight junction remodeling is also affected by hormonal deficiency, albeit with less variability than filaggrin. These findings further validate the model's ability to reproduce barrier impairment features relevant to menopausal skin physiology.

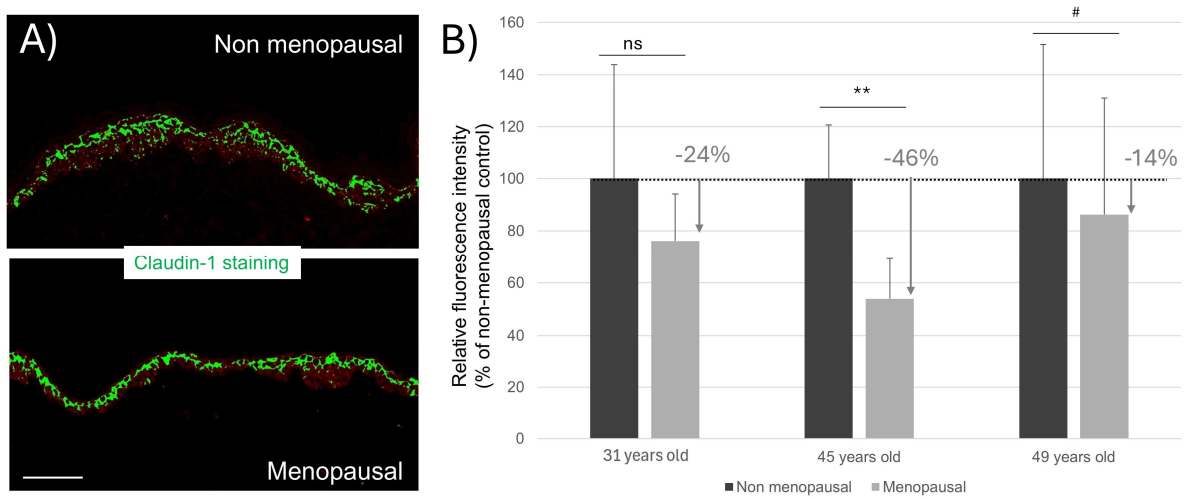

Figure 2. Claudin-1 expression is moderately decreased under menopausal conditions.

(A) Representative immunofluorescence images showing claudin-1 staining (green) in skin explants from a 45-year-old donor cultured for 7 days in non-menopausal or menopausal medium. Claudin-1 localizes to the upper epidermal layers, outlining tight junctions. Scale bar = 100 µm.

(B) Quantification of claudin-1 fluorescence intensity expressed as a percentage of the non-menopausal control for each donor (set to 100%). Results show a significant decrease in the 45-year-old donor (-46%, $^{**}p < 0.01$, n = 5) and non-significant (ns) decreases in the 31-year-old (-24%, n = 5) and 49-year-old (-14%, $^{\#}p < 0.1$, n = 6) donors. Data represent mean ± SEM ($n = 5$ or 6 explants per condition, depending on the donor).

3.3. Vitamin C shows promising effects on filaggrin expression under menopausal conditions

To explore whether topical intervention could mitigate the barrier impairments induced by hormonal deficiency, the effect of 0.5% vitamin C was evaluated on filaggrin expression in skin explants cultured under menopausal conditions. As shown in Figure 3**,** vitamin C treatment resulted in a donor-dependent increase in filaggrin fluorescence intensity compared to untreated menopausal skin.

In the 45-year-old donor, vitamin C application led to a 23% increase in filaggrin signal relative to the untreated menopausal condition. Similarly, a 14% increase was observed in explants from the 49-year-old donor. However, these improvements did not reach statistical significance ($p > 0.05$), likely due to inter-sample variability. Representative images confirm a visually enhanced filaggrin signal in the vitamin C-treated samples, suggesting a potential barrier-supporting effect under hormone-deprived conditions.

These findings support the utility of the model in evaluating active ingredients with potential to restore epidermal barrier function in postmenopausal skin, while also emphasizing the importance of donor variability in treatment responsiveness.

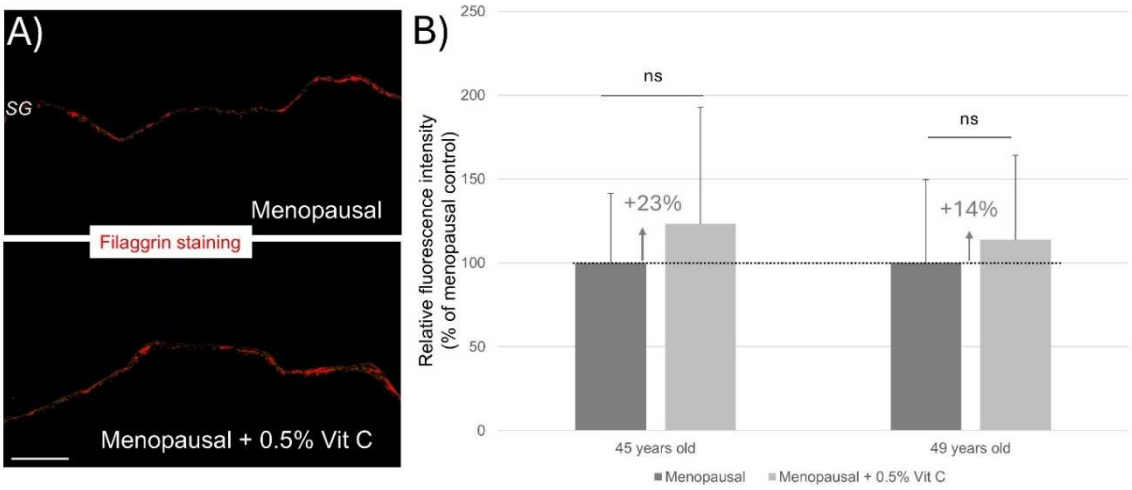

Figure 3: Impact of 0.5% vitamin C on filaggrin expression in menopausal skin explants.
(A) Representative immunofluorescence images of filaggrin staining (red) in skin explants from a 45-year-old donor cultured for 7 days under menopausal conditions, either untreated (top) or treated with 0.5% vitamin C (bottom). Signal localization is observed in the *stratum granulosum* (SG). Scale bar = 100 µm.
(B) Quantification of filaggrin fluorescence intensity, expressed as a percentage of the untreated menopausal control (set to 100%). Increases of +23% (45-year-old, n = 5) and +14% (49-year-old, n = 6) were observed, although not statistically significant (ns). Values are expressed as mean ± SEM (*n* = 5 or 6 explants per condition, depending on the donor).

3.4. Vitamin C treatment tends to restore Claudin-1 expression under menopausal conditions

To explore the potential of vitamin C to restore tight junction integrity in hormone-deficient skin, claudin-1 expression was evaluated in menopausal explants treated with 0.5% vitamin C for 7 days. As shown in Figure 4, a +43% increase in claudin-1 fluorescence intensity was observed in explants from the 45-year-old donor compared to the untreated menopausal condition.
Although this improvement did not reach statistical significance (p=0.0817), the trend suggests a partial restoration of tight junction protein levels. Representative immunofluorescence images confirm a visually enhanced Claudin-1 signal following vitamin C treatment.
These results complement the filaggrin data and further support the use of this *ex vivo* model to screen active ingredients for their ability to modulate epidermal barrier proteins in menopausal skin.

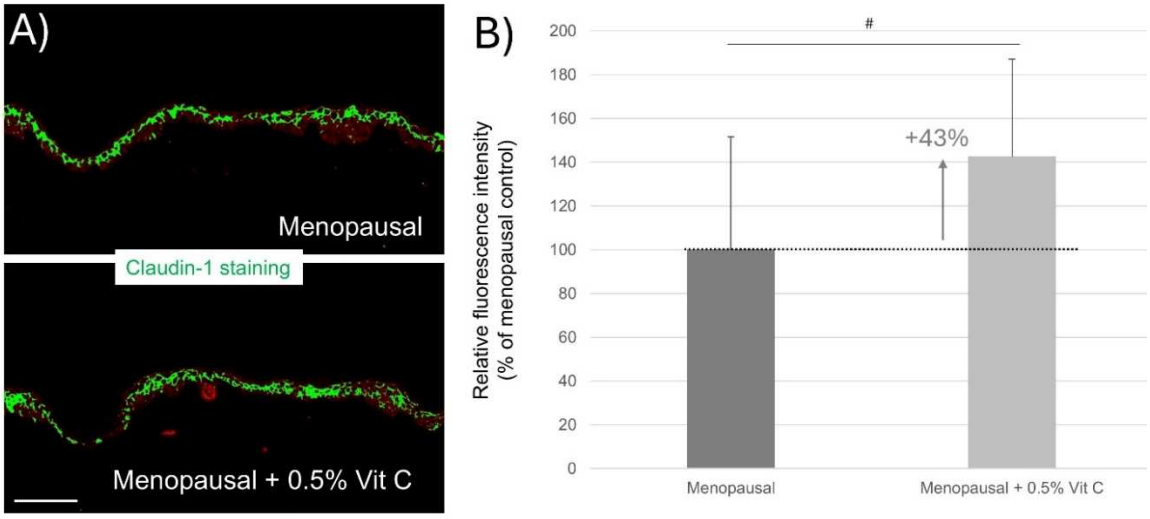

Figure 4. Vitamin C treatment tends to increase Claudin-1 expression in menopausal skin explants.

(A) Representative immunofluorescence images showing claudin-1 staining (green) in skin explants from a 45-year-old donor cultured under menopausal conditions without (top) or with 0.5% vitamin C (bottom) for 7 days. Claudin-1 localizes predominantly in the upper epidermal layers. Scale bar = 100 µm. **(B)** Quantification of claudin-1 fluorescence intensity expressed as a percentage of the menopausal control (set to 100%). A +43% increase was observed following vitamin C treatment ($^{\#}p<0.1$, not statistically significant). Data are expressed as mean ± SEM (n = 5 explants).

4. Discussion

The decline in key female hormone levels during menopause leads to well-documented structural and functional impairments in the skin, including dryness, loss of elasticity, and barrier dysfunction [8]. These changes accelerate cutaneous aging and negatively affect quality of life [7]. As a hormone-sensitive organ enriched in receptors for key female hormones, the skin is highly responsive to systemic hormonal fluctuations, especially 17β-estradiol [5, 6]. In this context, physiologically relevant models are essential to replicate the specific biological alterations in postmenopausal skin. Our study aimed to both investigate the molecular impact of hormonal deprivation and validate an *ex vivo* 3D skin model adapted for screening active ingredients designed for menopausal skin.

This model successfully reproduced two key features of barrier impairment, significant downregulation of filaggrin and a consistent decrease in claudin-1. These proteins play essential roles in epidermal differentiation, hydration, and tight junction integrity [22, 24]. Their reduced expression mirrors patterns observed in inflammatory conditions like eczema and psoriasis, which are more frequent or severe in postmenopausal women [25], suggesting a link between the hypo-hormonal state and barrier-related vulnerabilities.

In addition, hormonal deprivation may promote low-grade inflammation by weakening barrier integrity, facilitating irritant or microbial penetration, and triggering immune responses. A recent study by Kiss *et al.* (2024) reported altered immune cell dynamics in postmenopausal women, including reduced Langerhans cell mobilization, in the absence of hormone replacement

therapy (HRT) [18]. In contrast, women receiving HRT showed higher filaggrin levels and thicker K10-positive layers, supporting the role of hormonal status in maintaining skin resilience. While HRT can partially restore cutaneous homeostasis, its use is often limited by contraindications and safety concerns [19]. This has stimulated interest in topically applied, non-hormonal solutions to target the needs of menopausal skin [26]. We used our model to test 0.5% vitamin C, an antioxidant known for its effects on redox balance, extracellular matrix maintenance, and inflammation control [27–30]. Although the increases in filaggrin and claudin-1 expression did not reach statistical significance, the positive trends suggest a potential barrier-supporting role under hormone-deficient conditions.

Importantly, donor variability was a key factor, reflecting differences in baseline hormonal context and tissue responsiveness. This highlights the need to consider menopausal status when interpreting explant study results. Our model, by standardizing hormone deprivation, offers a reproducible tool to better reflect the postmenopausal environment and to evaluate active ingredients under consistent biological conditions.

Such a platform contributes meaningfully to the development of personalized skincare strategies, particularly in light of the growing demand for effective, hormone-free interventions that address the specific challenges of aging female skin.

5. Conclusion

This study highlights the relevance of a full-thickness *ex vivo* 3D human skin model cultured without three key female hormones as a physiologically meaningful platform to investigate menopausal skin alterations. By reproducing hallmark features of barrier dysfunction, specifically the downregulation of filaggrin and claudin-1, this model enables mechanistic exploration and supports the evaluation of non-hormonal active ingredients. Although vitamin C did not significantly restore these markers in this preliminary assay, the observed trends and donor variability underscore the importance of using well-characterized, hormone-relevant models. In a context of increasing demand for personalized, hormone-free skincare, such models are essential for identifying safe and effective dermocosmetic solutions.

Further studies should broaden the molecular scope of analysis by including markers of inflammation, oxidative stress, and senescence, and explore combined strategies with antioxidants, barrier-supporting compounds, or senotherapeutics to better address the needs of postmenopausal skin.

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Conflict of interest statement

The authors report no conflict of interest.

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