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Journal of Dermatology & Cosmetology

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Vitreoscilla filiformis extract promotes keratinocyte migration and stemness when exposed to photo-pollution

Abstract

Introduction: Vitreoscilla filiformis (Vf, ATCC 15551) is a non-pathogenic, filamentous Gram-negative bacterium originally isolated from sulfur-rich thermal waters of Molitg-les-Bains in the French Pyrenees, historically known for their skin-repair and wound-healing properties. The non-replicative probiotic fraction derived from this bacterium, Vitreoscilla filiformis extract (Vfe), contains essential nutrients beneficial for skin cells and the skin microbiome. Vfe interacts primarily with toll-like receptor 2 (TLR2) expressed on keratinocytes and other skin cells, promoting epidermal renewal, strengthening the skin barrier, and modulating immune and antioxidant responses. Since ultraviolet radiation combined with air pollution can induce oxidative stress, barrier disruption, and immune dysregulation in the skin, these assays investigated the regenerative and stemness-preserving potential of Vfe under photo-polluted conditions.

Materials and methods: Two in vitro assays were conducted. The first evaluated the regenerative potential of Vfe (0.3%) using a 3D reconstructed epidermal model composed of cultured keratinocytes exposed to UVA1 radiation (7.4 J/cm²) and benzo[a]pyrene (BaP, 5 nM). The second assay assessed keratinocyte stemness preservation using a colony-forming assay under similar photo-polluted conditions. Holoclones were quantified, and colony-forming efficiency (CFE) was calculated. Results were normalized to untreated controls to determine relative CFE (rCFE).

Results: Vfe (0.3%) significantly accelerated keratinocyte migration, increasing regeneration up to threefold in unexposed cells and 1.5-fold following UVA1/BaP exposure. At Day 20, Vfe improved reconstructed epidermal architecture by 2.7-fold compared with exposed untreated samples. Additionally, Vfe (0.00125%) preserved keratinocyte stemness, increasing holoclone formation by 28.8% after UVA1 exposure and by 50.8% under combined UVA1 and BaP stress.

Conclusion: Vfe promotes epidermal regeneration and preserves keratinocyte stemness under photo-polluted conditions, suggesting a protective role against environmental skin damage. Further studies are required to clarify the underlying molecular mechanisms.

Keywords: Vitreoscilla filiformis, probiotic, keratinocyte stem cells, regeneration, UVA1, benzo(a)pyrene

Volume 10 Issue 4 - 2026

Muriel Liboutet,[1] Elise Paucod,[2] Nathalie Deshayes,[3,4] Gabrielle Misset,[4] Hussein Fayyad-Kazan,[5] Walid Rachidi,[2] Audrey Gueniche[1]

[1]L'Oreal Research and Innovation, Luxury Division Dept, Chevilly-La-Rue, France
[2]University of Grenoble Alpes, CEA, Inserm, IRIG-UA13 BGE, Biomics, Grenoble, France
[3]L'Oreal Research and Innovation, Advanced Research Dept, Aulnay-sous-Bois, France
[4]Biotherm, Levallois-Perret, France
[5]Lebanese University, Faculty of Science, Laboratory of Cancer Biology and Molecular Immunology, Beirut, Lebanon

Correspondence: Muriel Liboutet, L'Oreal Research and Innovation, Luxury Division Dept, Chevilly-La-Rue, France

Received: October 07, 2026 | **Published:** November 23, 2026

Introduction

Vitreoscilla filiformis (Vf, ATCC 15551) is a gram-negative, non-pathogenic, non-photosynthetic, filamentous bacterium, first isolated from sodium sulfurized thermal water originating from Molitg-les-Bains in the French Oriental Pyrenean Mountains. This thermal water has been known since Roman times for its skin-repair and wound-healing properties.[1]

Vf extract (Vfe, Biotech Plankton™, Biotherm, France), a non-replicative probiotic fraction, is an original extract of *Vf*. It contains nutritional essential elements for both skin cells and skin microbiome.[1] Vfe is a natural, 100% biodegradable ingredient that has been produced in a unique sustainable process. Vfe has been produced on a large scale for over 20 years by an exclusive, green, bio-fermentation process to generate bacterial lysate. The well-defined, sterile culture medium contains autolytic yeast extract (2 g/L), soya papain peptone (2 g/L), glucose (3 g/L), Heller microelements (1 ml/L), and $CaCl_2$*2 H_2O (60 mg/L), at pH 7.2. The bioreactor medium is inoculated with *V. filiformis* (20 ml/L). After being harvested, Vfe is separated from the culture medium by centrifugation at 10,000 *g* for 10 min, and the biomass is stabilized by heat treatment at 121°C for 30 min.[1] Vfe primarily acts by binding to the toll-like receptor 2 (TLR2), which is expressed on the surface of all skin cells, including that of keratinocytes.[2] Vfe has a high skin bio-affinity and mimics activities that are shared with commensal skin microorganisms. It was highly efficacious *in vitro* and in clinical trials regarding epidermal renewal, reinforcement of the skin barrier, modulation of immune responses and stimulation of endogenous antioxidants.[1,3] Ultraviolet radiation interacts with air pollution, leading to the production of skin damage including oxidative stress, barrier function alteration and immuno-dysregulation.[4] To counter these constant environmental and physiological stresses, the skin relies on its capacity for self-renewal and tissue homeostasis. As recently reviewed by Hsu and Fuchs,[5] maintaining cutaneous integrity and optimizing tissue fitness depend on dynamic, highly coordinated crosstalk within stem cell niches.[5] In the epidermis, stem cells continually balance self-renewal, cell competition, and differentiation to maintain tissue architecture and reinforce both physical and immunological barriers. However, environmental stressors, such as photo-pollution, can compromise stem cell function, disrupt cellular competition, and impair the regenerative capacity of the tissue.

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In this context, the emerging concept of "skin fitness" shifts the paradigm from traditional corrective skincare toward proactive preservation of cellular resilience and stemness. Enhancing skin fitness involves fortifying the natural defense mechanisms of skin stem cells and maintaining their ability to repair and regenerate damaged tissue when exposed to environmental stress. Given its known capacity to interact with TLR2, stimulate endogenous antioxidants, and promote epidermal renewal,[1,3] *Vitreoscilla filiformis* extract (Vfe) represents a promising candidate to support skin fitness under adverse conditions.

We herewith report results from 2 *in vitro* assays evaluating the cutaneous regeneration/differentiation and keratinocyte stemness preservation by Vfe, especially under photo-polluted conditions.

Material and methods

A first assay by means of a 3D epidermal regeneration model (Episkin™, France) using cultured keratinocytes (normal human epidermal keratinocytes, NHEK) assessed the regeneration potential of Vfe 0.3 % added at Day 0, Day 13 and Day 17.[5] All conditions were performed in triplicate. Living dermal equivalents were ballasted with a metal ring. Keratinocytes were isolated and pre-amplified from skin samples derived from breast tissue of healthy donors undergoing aesthetic surgery and seeded on a living dermal equivalent around the ring (the centre of the ring remained empty). After immersion in culture media containing serum, samples were lifted to an air-liquid interface inducing epidermal differentiation and stratification. The ring was removed at Day 10. Keratinocytes were exposed to UVA1 radiation (7.4 J/cm²) at Day 0, Day 13 and Day 17 and continuously exposed to Benzo[a]pyrene (BaP) 5nM. The control samples did not receive Vfe supplementation. Keratinocyte migration kinetics via following the epithelised area up to Day 20 was assessed through collagen colouring using haematoxylin. Time quantification was made using HISTOLAB® software (Alphelys, France).

The 2nd assay confirmed the keratinocyte stemness preservation potential of Vfe 0.00125% under photo-polluted conditions by maintaining the survival of keratinocyte holoclones (under the experimental conditions, holoclones are colonies of at least 0.5mm in diameter, with regular shape). The colony-forming efficiency (CFE) was defined as the number of colonies formed per number of cells seeded. Data were normalized to the untreated control to express relative CFE (rCFE) changes.[6]

For this assay, 8 different cell lines were used. Four lines originating from mammary plasties are referred to by the abbreviation of HKPM for Human Keratinocyte Primary Mammal. The donors' ages ranged from 3 to 38 years, and the phototypes were mostly phototypes I to III, with one exception of phototype VI. Four lines were purchased (CTI Biotech, France) and originated from mammary plasties, abdominal plasties, and foreskin circumcision.

Cells were cultured in a humidified incubator at 37 °C and 5% CO_2 in semi-defined Epilife® medium (Episkin, France) supplemented with 1% HKGS (Human Keratinocyte Growth Supplements), 1% penicillin–streptomycin, and 400 µM calcium. Cells were seeded at 500,000 cells in a T75 flask (75 cm²), corresponding to 6,667 cells/cm², in 10 mL of supplemented Epilife medium. At 60–80% confluence, cells were detached using 1.5 mL of 0.05% trypsin/EDTA per flask. Experiments were performed at passages 1 and 2, and exceptionally at passage 3.

At Day 0, keratinocytes were seeded at a clonal density of 40 cells/cm² in 6-well plates for the curative assay and at 1000 cells/cm² for the preventive assay. Treatments (± BaP), ± long UVA exposure, and medium replacement with medium ± VF were performed at Day 3 and Day 7. For the curative assay, cells were fixed and stained between Day 10 and Day 14. For the preventive assay, cells were trypsinised and reseeded at Day 8, fixed and stained between Day 18 and Day 22.

For the treatment with 5 nM BaP, the culture medium was removed from the wells and replaced with 1.5 mL (per well of a 6-well plate) of 5 nM BaP in Epilife medium, followed by incubation at 37 °C for 30 minutes. The BaP-containing medium was then removed and replaced with Epilife medium ± Vfe 0.00125%.

Multi-well plates to be irradiated with UVA were placed one at a time under a Waldmann UVA 700L lamp with a power of 19.2 mW/cm², covered with a filter allowing only long UVA wavelengths (340–400 nm) to pass through. The plate was irradiated at 1.85 J/cm². Non-irradiated plates were kept in the dark at room temperature for the same duration. Irradiation of cells co-exposed to BaP and long UVA was performed immediately after BaP treatment, with no waiting time.

Cells were observed daily using an inverted microscope (Olympus CKX41, Olympus, Japan), and colonies were photographed using a camera (TC210 – 630-2678, Cloudfront, France) in order to characterize the clones.

Presto Blue absorbance analysis was performed using the Ensight™ multimode microplate reader (PerkinElmer, USA). After cell fixation and staining, multi-well plates and flasks were photographed using the ChemiDo™c MP Imaging System (Bio-Rad Laboratories, USA), with a white tray, "Coomassie Blue Gel" setting, "Medium" zoom (W: 15.5 cm, L: 12.4 cm), and "Auto optimal" exposure time to obtain the best contrast for crystal violet staining.

Accurate cell counting was performed using the Cell Insight CX5 High-Content Screening (HCS) Platform (Thermo Fisher Scientific, USA) at 4× magnification.

Images acquired with the ChemiDo MP Imaging System were analyzed using ImageJ software, an open-source software for processing and analyzing scientific images, to count the number of colonies and to analyze colony size. Images acquired with the CellInsight™ CX5 High-Content Screening (HCS) Platform were analyzed using HCS™ Studio (Thermo Fischer Scientific, USA) software to determine the number of cells per well.

Results

Assay n° 1 showed that Vfe 0.3% significantly ($p<0.05$) accelerated the cell migration process up to 3-fold at Day 20 in unexposed cells, and up to 1.5-fold after exposure to UVA and BaP compared to untreated keratinocytes with UVA and BaP (Figure 1). At Day 20, Vfe 0.3% improved the skin architecture by 2.7-fold in UVA/BaP-exposed reconstructed skin, compared to untreated but exposed samples (Figure 2 and Figure 3).

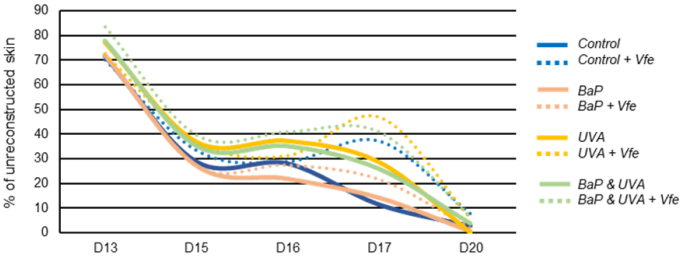


Figure 1 Keratinocyte migration kinetics up to 20 days with and without *Vitreoscilla filiformis extract 0.3%* supplementation.

Vitreoscilla filiformis extract 0.3% significantly ($p<0.05$) accelerates the migration speed process (up to 3 times at D17 in basal conditions, and up to 1.5 times in (photo)-polluted conditions.

Citation: Liboutet M, Paucod E, Deshayes N, et al. Vitreoscilla filiformis extract promotes keratinocyte migration and stemness when exposed to photo-pollution. *J Dermat Cosmetol.* 2026;10(4):91–94. DOI: 10.15406/jdc.2026.10.00329

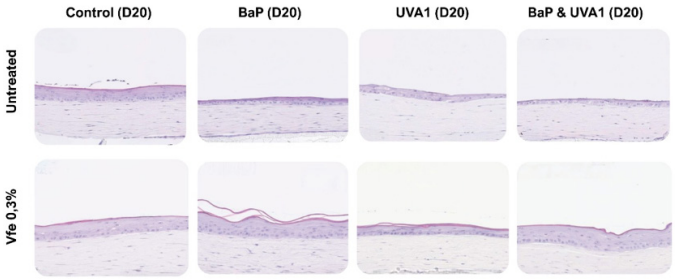


Figure 2 Epidermal thickness effect of *Vitreoscilla filiformis* extract 0.3% at Day 20.

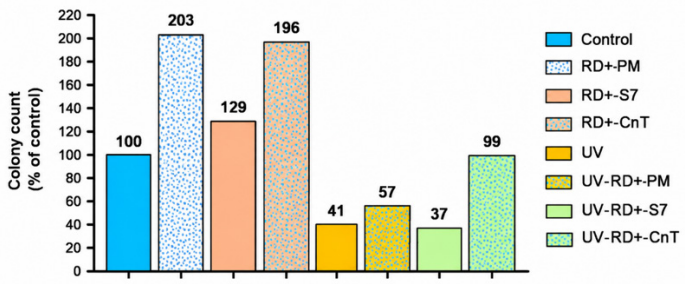


Figure 3 Reconstructed epidermal thickness after 20 days with *Vitreoscilla filiformis* supplementation.

Vitreoscilla filiformis extract 0.3% increases the epidermal thickness in wounded zones up to 2.7 times after 20 days under (photo-) polluted conditions.

In assay n° 2, BaP alone induced a low but noticeable level of cell mortality. The effects of long UVA exposure were lethal at 7.4 J/cm² and caused about 50% cell mortality at 1.85 J/cm². Finally, co-exposure to BaP and long UVA further increased cell mortality.

The effect of Vfe on skin regeneration, and therefore on the ability of keratinocytes to form colonies of the largest possible size, was demonstrated. Depending on inter-individual variability and the response of each cell line, VF produced a curative effect on the number and/or size of colonies after exposure to BaP alone, long UVA alone, or their co-exposure.

Vfe 0.00125% protected clones from UVA1 exposure (rCFE: +28.8% holoclones vs. untreated samples) and from combined UVA1 and BaP exposure (rCFE: +50.8% holoclones vs. untreated samples); (Figure 4 and Figure 5).

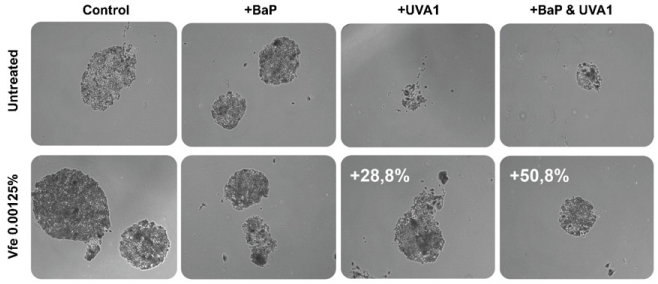


Figure 4 *Vitreoscilla filiformis* 0.00125% extract promotes keratinocyte stemness under photo-polluted conditions.

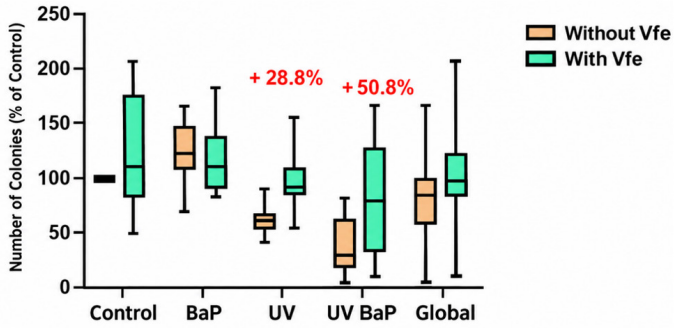


Figure 5 *Vitreoscilla filiformis* 0.00125% significantly ($p<0.05$) protects from UVA and BaP pollution.

Discussion

The present assays highlight the protective and regenerative effects of Vfe on keratinocytes under both basal and stress-induced conditions. In assay n°1, Vfe at 0.3% significantly accelerated cell migration, demonstrating a 3-fold increase in unexposed cells and a 1.5-fold increase in cells subjected to UVA and BaP exposure compared to untreated controls. This indicates that Vfe can enhance keratinocyte motility and potentially expedite wound healing, even under conditions of environmental stress. Correspondingly, reconstructed skin treated with Vfe 0.3% showed a 2.7-fold improvement in skin architecture at Day 20 after UVA/BaP exposure, further supporting its role in maintaining tissue integrity under genotoxic and oxidative stress.

Assay n°2 provided complementary insights into cytotoxic challenges and the protective capacity of Vfe. BaP alone induced modest cell mortality, whereas long UVA exposure demonstrated dose-dependent lethality, with 7.4 J/cm² causing complete cell death and 1.85 J/cm² resulting in approximately 50% mortality. Notably, the combination of BaP and UVA synergistically increased cell death, confirming that co-exposure exacerbates keratinocyte damage. These results underscore the importance of interventions capable of mitigating combined chemical and phototoxic stress.

The effect of Vfe on the regenerative potential of keratinocytes was evident in colony-forming efficiency assays. Treatment with Vfe enabled the formation of larger and more numerous colonies, reflecting enhanced clonogenic capacity. Importantly, these protective effects were observed across varying levels of stress and were subject to inter-individual variability, suggesting that Vfe exerts a consistent curative influence while accommodating intrinsic differences among cell lines. Vfe (0.00125%) significantly protected keratinocyte clones from UVA1 exposure (+28.8% holoclones) and from combined UVA1/BaP exposure (+50.8% holoclones) relative to untreated controls. These findings demonstrate that even minimal concentrations of Vfe can substantially preserve the regenerative potential of keratinocytes under conditions of oxidative and genotoxic stress.

Present work showed that Vfe at 1% accelerated *in vitro* keratinocyte migration (monolayers seeded on collagen I) compared with the control, demonstrating improved re-epithelialization efficacy (297 ± 84 migratory cells vs. 201 ± 28 for the control). In addition, using a *novel in vitro* 3D epidermal regeneration model that evaluates keratinocyte migration and mimics phase II of wound healing, Vfe significantly enhanced wound healing at day 16 (Vfe 0.3%) and day 17 (Vfe 0.1% and 0.3%).[5] Other research already showed that Vfe has anti-inflammatory, immunoregulation properties in indications such

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as AD and seborrheic dermatitis and enhances antioxidant defenses by stimulating mitochondrial manganese superoxide dismutase 2 (MnSOD2) activity at both the mRNA and protein levels.[3,7–10]

Conclusion

Overall, results from the herewith presented *in vitro* assays using a 3D reconstructed skin model under photo-polluted conditions emphasize the significant regenerative potential of Vfe. This effect may be partially due to its preservative potential on keratinocyte stemness. Further investigation is needed to elucidate the molecular mechanisms involved. By supporting epidermal stemness, as reflected by the preserved survival and colony-forming efficiency of keratinocyte holoclones, and by promoting re-epithelialization under photo-polluted stress, Vfe contributes to maintaining skin fitness. In line with current paradigms on skin homeostasis,[5] protecting the epidermal progenitor pool against environmental stresses appears essential to sustain tissue regeneration and preserve barrier integrity. Overall, these findings suggest that Vfe may represent a relevant strategy to support stem cell potential and enhance cutaneous resilience in photo-polluted environments. Further molecular studies are warranted to clarify the specific pathways involved in this stemness-preserving effect.

Acknowledgments

The authors acknowledge the writing support of Karl Patrick Göritz, SMWS, France.

Authors' contributions

EP performed the experiments. WR supervised the project. HFK performed the statistical analysis. All authors edited, read, and approved the manuscript.

Conflict of interest

All authors except Elise Paucod, Walid Rachidi and Hussein Fayyad-Kazan are employees of L'Oréal Group. Elise Paucod, Walid Rachidi and Hussein Fayyad-Kazan have no conflict of interest to disclose.

Funding

This research work was supported by Biotherm, France.

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