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Care Creations™

Postbiolift™

Postbiotic technology for healthy aging

by Beauty Creations
another Care Creations™ product group Inspired by Life

Global Except US

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SUMMARY FILE

Postbiolift™	BC10152
Origin - Description	Powder made of *Lactobacillus crispatus* metabolites (postbiotic) for healthy aging
Regulatory data	
INCI	Maltodextrin (and) Lactobacillus Ferment
China	Each component is listed in Inventory of Existing Cosmetic Ingredient in China (IECIC 2021)
CAS#	9050-36-6, 2768389-50-8
Appearance	White to beige powder
Preservative	None
Natural labels	None
Naturalness content (ISO 16128)	100% from natural origin
Cosmetic use	
Properties	Helps to activate the synthesis of elastic fibers components: elastin, emilin-1, MFAP4, fibulin-5 and fibrillin-1 Helps to decrease tyrosinase activity Contibutes to decrease the melanin synthesis Helps to decrease the frequency of cell contraction
Applications	Anti-aging and photoaging face care
Formulation data	
Concentration of use	1%
Solubility	Soluble in water, insoluble in oils and fat
Incorporation method	Postbiolift is incorporated during the final process at a temperature below 30°C or at room temperature for cold processing
Optimal pH	3-8

BIOTIC INGREDIENT FOR HEALTHY AGING

The anti-aging cosmetic market is thriving, driven by emerging consumer demands and new technologies. At the same time, consumer antiaging demand has shifted toward functionality, healthy aging and natural safe ingredients.

Postbiolift is one of the two newly developed biotic ingredients (Probiolift and Postbiolift) to support graceful and healthy skin aging. Unlike other biotics existing on the market, the newly developed actives are the first to use a bacterium naturally present on the skin: *Lactobacillus crispatus*, a bacterium which has been found to decrease with age.

Consumer aspirations

Nutrition and internal balance have long served the evolving food and beverage industry well; the beauty sector can learn from the trends seen in that marketplace. This result in an increasing awareness of the health and skin benefits provided to consumers by biotic ingredients. Biotic ingredient is the broad term in which we categorize products infused with specific ingredients, like pre-, pro-, and postbiotics. Products with a biotic claim are associated with health, wellness and eco-friendliness. New beauty rituals involving biotic skincare products are taking over the personal care market with different claims including anti-aging. As a result biotic ingredients are booming on the market, most of them being substrate used by skin microbiome for its growth and metabolism called prebiotic or postbiotic which are soluble factors (metabolites) produced by the probiotic fermentation. The majority of available postbiotic on the market are derived from microorganisms used in nutrition and are not isolated from skin microbiota which is made of numerous commensal beneficial bacteria.

Regarding anti-aging claims, the demand is now orientated towards ingredients that enable the skin to age in good health starting from one's thirties, by targeting the impairment of biological processes of the skin (https://www.gcimagazine.com).

Postbiotics in anti-aging regimen

Skin aging manifestations occur from the age of 30 and evolve more or less rapidly depending on our genetic background and the influences of the external environment thanks to our lifestyle. Healthy aging is a continuous process of optimizing opportunities to maintain and improve skin health and beauty. Aged skin is associated with some signs like lack of elasticity, hyperpigmentation spots and wrinkles; some of them being more difficult to attenuate when well established; hence the necessity to tackle them during early aging.

To respond to consumer demand for efficacy and naturalness in anti-aging solutions, some postbiotics or fermented ingredient are commercially available. BASF Beauty Care Solutions have also developed a postbiotic technology; unlike those existing on the market, Postbiolift is the first postbiotic derived from bacteria naturally present on the skin which decreases with aging.

AGE-RELATED CHANGES IN FACIAL SKIN

Aging is an ineluctable process that affects human beings. Skin aging can be formally conceptualized into intrinsic and extrinsic aging, the latter not being easily disentangled from the former. Intrinsic aging is determined by each person's individual genetic clock, while extrinsic aging is engendered by external environment factors such as air pollution, smoking, poor nutrition, and sun exposure (Zhang *et al*, 2018). Recent articles have described worsening of some skin aging signs like wrinkles due to some habits such as wearing masks as a barrier measure against the covid pandemic (Park *et al*, 2021).

As the skin ages, it doesn't stay as plump and smooth as it once was. Fine lines, pigmented spots, sagging and wrinkles are an inevitable consequence of aging, (Liu *et al*, 2019; Ahmed *et al*, 2020). From around the age of 25 the first signs of aging start to appear on the surface of the skin. Fine lines appear first, then over time coarse wrinkles, loss of elasticity and volume become noticeable. UV rays are a significant accelerator of skin aging. Thus, facial signs related to skin wrinkles/texture and pigmentary spots were found significantly more accentuated among sun exposed women in a test in Japan and showed an early onset (20–30 years) (Flament *et al*, 2019).

The importance of aging lies in the enormous consumer demand for agents or treatments that can prevent or reverse its signs (Longo, 2016). In light of this, it is mandatory to detect early skin aging signs when the process can be readily reversed or, at least, minimized. We need an understanding of biological disturbances which occur at epidermis and dermis level to define the appropriate target to act on in order to minimize or reverse aging phenomenon.

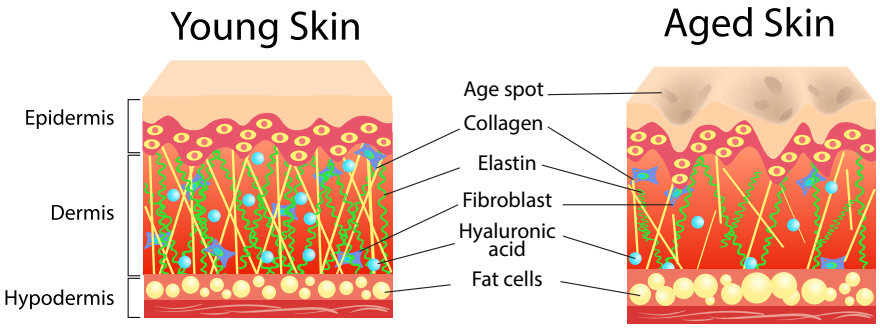


Figure 1 - Schematic representation of young and old skin.

The metabolic imbalances that cause the visible signs of aging occur in the various skin layers, particularly in the epidermis and dermis. At epidermal level, impaired melanin synthesis and decreased keratinocytes turnover lead to abnormal pigmentation called age spots, or lentigines which are a common symptom accompanying aging skin (Figure 1).

At the dermal level, several studies have shown that aging processes affect the activities related to synthesis, remodeling, and catabolism of the extra cellular matrix (ECM) components of the dermis such as collagen and elastin fibers. As a result, not only does the aging process induce a reduction of the ECM density but its architecture and quality are also affected leading ultimately to impaired skin biomechanical properties (D'Aloiso *et al*, 2020). In the end skin becomes wrinkled and skin firmness, density and elasticity decrease.

Skin aging and age spots

Irrespective of skin type, all individuals complain about dark spots and uneven skin color, but in different ways according to their ethnic origin (Vashi *et al*, 2016). In South East Asia, many women prefer fair over tanned skin. Overall, uneven skin tone is a great concern and studies have shown that visible skin color distribution plays an important role in the perception of attractiveness.

Facial skin hyperpigmentation caused by chronological aging and chronic sun exposure is a major skin complaint (Takahashi *et al*, 2017). Clinically, the phenotype of age pigmented skin has a mottled, uneven color, primarily due to age spots (Kang *et al*, 2021). Uneven pigmentation might be attributed to the hyperactivation of melanocytes, altered distribution of pigment (Vashi *et al*, 2016), and melanocyte turnover. Basically, melanin pigmentation plays a critical role in protecting the skin from the harmful effects of ultraviolet (UV) radiations. The accumulation of oxidative stress/ROS in the skin during aging facilitates the progression of the aging process and aging-related pigmentation (Yonei *et al*, 2016).

Skin pigmentation is largely determined by melanin synthesis in melanocytes, melanosome transfer to keratinocytes, and melanosome degradation. Melanin synthesis is restricted to melanosomes, which contain tyrosinase, the key regulatory enzyme of melanogenesis, and tyrosine-related proteins (TRPs). Tyrosinase is involved in the early steps of melanin synthesis by transforming L-tyrosine to dopaquinone, which are common to two types of melanin, brown-black eumelanin and yellow-red pheomelanin. Thus, decreasing tyrosinase activity, is one of the main ways to decrease melanin production and skin hyperpigmentation (Lee, 2021; Goelzer Neto *et al*, 2022).

Skin wrinkling and aging

Appearance of fine lines and wrinkles are irrespective of skin type and are a source of concern to everyone.
Skin wrinkling is due to progressive loss of ECM components over time in the dermis (Cipriani *et al*, 2016). ECM components are produced by fibroblasts. In aged skin, within dermal fibroblasts there is an up-regulation of proteolytic enzyme secretion (Shin *et al*, 2019). Enhanced expression of proteolytic enzymes like matrix metalloproteinases (MMPs) and elastases, contributes to the destruction of the dermal fibrous collagen-elastin matrix (Panwar *et al*, 2020).
Several kinds of wrinkles can be distinguished including wrinkles related to skins texture impairment and expressional wrinkles. Expressional wrinkles are those lines forming on the face where skin has to adapt to the facial muscle's movements and gravity. Indeed, the facial muscles are located just below the skin. Consequently, the skin moves together with them. These expression lines are visible at the age of 30, but they do not increase in number during the years; instead, they become deeper (Cipriani *et al*, 2016).
For wrinkles, compounds increasing ECM proteins are the most common ingredients used.
For expressional wrinkles, consumer sometimes use ingredients which are able to relax the skin such as some toxins which are able to inhibit contraction.

Skin elasticity and aging

As aging occurs, changes in the dermal structures of collagen and elastic fibers occur, and fibers become thin and fractionated; this provokes a loss in the mechanical properties of skin, and elasticity gradually decreases, (Kim *et al*, 2018; Escoffier *et al*, 1989).

Elastic fibers representing 3 to 4% of ECM of the dermis are tight, three-dimensionally interlaced of fibrils, intimately linked with collagen fibers. Elastic fibers, composed of an elastin core (90%) surrounded by fibrillin-rich microfibrils (10%), are essential ECM macromolecules endowing extensible tissues with critical mechanical properties such as elasticity, flexibility and resilience (Fhayli *et al*, 2020; Schmelzer and Duca, 2021). Elastic fibers provide recoil to tissues that undergo repeated stretching.
Elastin, the major component of elastic fibers, has been shown to decrease during aging. Elastin's overall quantitative and qualitative decline, including the loss of structural integrity due to proteolytic enzymatic activity and lack of production by fibroblasts, contributes to the aging of skin (Panwar *et al*, 2020)
Elastic fiber genesis includes the formation of a microfibrillar scaffold, deposition and integration of tropoelastin monomers into the scaffold, and cross-linking (Weihermann *et al*, 2017) (Figure 2).
After synthesis in endoplasmic reticulum and secretion out of the cell, tropoelastin has the ability to undergo self-aggregation of monomers initiated by the Lysyl oxidase family of enzymes and subsequent phase separation in a process called coacervation (Yanagisawa and Davis, 2010). Elastic fiber assembly always occurs in the presence of microfibrils. which are primarily composed of fibrillin polymers with associated glycoproteins and serve as a scaffold for elastin deposition (Yanagisawa and Davis, 2010).

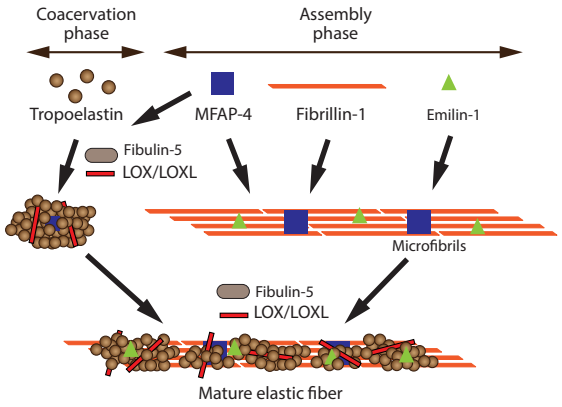


Figure 2 - Mature elastic fiber formation (Kasamatsu *et al*, 2011).

Among the main partners involved in mature elastic fiber formation, some are particularly relevant:

Fibulin-5 (FBLN5) contributes to the formation of elastic fibers in both phases (Figure 2). Fibulin-5 has two main roles in elastogenesis; first role is to limit the extent of aggregation of tropoelastin monomers and/or coacervates and aid in the incorporation of elastin into the microfibril bundles, and secondly to potentially assist in the activation of LOXL-1 (Choi *et al*, 2009). Finally, FBLN5 content in the reticular dermis was described as decreasing with age (Kadoya *et al*, 2005).

Microfibrillar-associated protein 4 (MFAP4) is one of the most important microfibril-associated glycoproteins. MFAP4 has been found to bind with elastin microfibrils and interact directly with Fibrillin-1, and then aid in elastic fiber formation (Lin *et al*, 2020). MFAP4 binds tropoelastin and promotes tropoelastin coacervation which is a crucial step before elastic fiber assembly. Additionally, MFAP4 interacts with fibrillin-1, contributing to the development of microfibrils involved in proper elastic fiber organization (Kasamatsu *et al*, 2011).

Fibrillin-1. Fibrillins are the major component of microfibrils and include fibrillins-1, -2 and -3. Fibrillins show tissue-specific expression, and fibrillin-1 the most abundant fibrillin isoform is expressed in the dermis (Kadoya, 2016).
Fibrillins form a template for elastin deposition and provide a platform for microfibril-elastin binding protein to interact during elastic fiber assembly (Godwin *et al*, 2019).

Elastin microfibril interface–located protein 1 (EMILIN-1). In skin, Emilin-1 locates in the dermis, up to the basement membrane, interacting with components of the extracellular matrix but also with the anchoring complex. These interactions are important for cell adhesion, migration, proliferation and would suggest that Emilin-1 might be important for maintaining the 3D structure of the extracellular matrix (Fitoussi *et al*, 2019).
Regarding elastogenesis, Emilin-1 plays a role in the fiber assembly phase by aiding the fibrillin-microfibril fibers to be more ordered (Randell *et al*, 2017) (Figure 2). Little is known about the exact function of EMILIN-1. However, this protein seems to be important as it is involved in microfibrils strengthening and its deficiency leads to serious skin disorders (Schiavinato *et al*, 2016). Upon intrinsic and UV induced aging Emilin-1 is drastically reduced and disorganized (Fitoussi *et al*, 2019).

POSTBIOLIFT, POSTBIOTIC TECHNOLOGY FROM THE SKIN YOUTH BACTERIUM *LACTOBACILLUS CRISPATUS*

Lactobacillus crispatus, a youth bacteria identified from skin microbiota

With the objective of exploring skin microbiota and its evolution over age, we have conducted an exploratory study to compare facial skin microbiota of a young cohort aged from 18 to 35 yo with wrinkle grade of 0-1 to that of an old cohort aged above 55 yo with wrinkle grade 5-6. A particular focus was made on the wrinkle areas. Microbial samples were taken from 3 different zones: within the wrinkle hollow, the crow's feet and undereye zone, and the cheek area adjacent to the earlobe as control. DNA was then extracted from the samples and sequenced using Whole-Genome Sequencing protocol and taxonomic analysis was performed using available databases.

The results confirm already reported data about the shift in skin microbiota diversity and some species on old skin (data not shown), (Shibagaki *et al*, 2017; Dimitriu *et al,* 2019). Analysis of results also revealed that in the old cohort compared to the young one, there was a decrease of both prevalence and abundance of the lactic acid bacteria, namely Lactobacilli, in the 3 sampled zones (Figure 3 and 4). In older skin, among the 4 more abundant lactic acid bacteria present, *Lactobacillus crispatus* was one of the most decreased especially in the undereye zone and was not detectable in the wrinkle hollow (Figure 3 and 4). These results evidenced that aged skin is correlated with some changes in skin microbiota composition, particularly a decrease in prevalence and abundance of *Lactobacillus crispatus*. This allowed us to hypothesize that *Lactobacillus crispatus* is a youth bacterium and that its metabolites are potentially beneficial to fight skin aging.

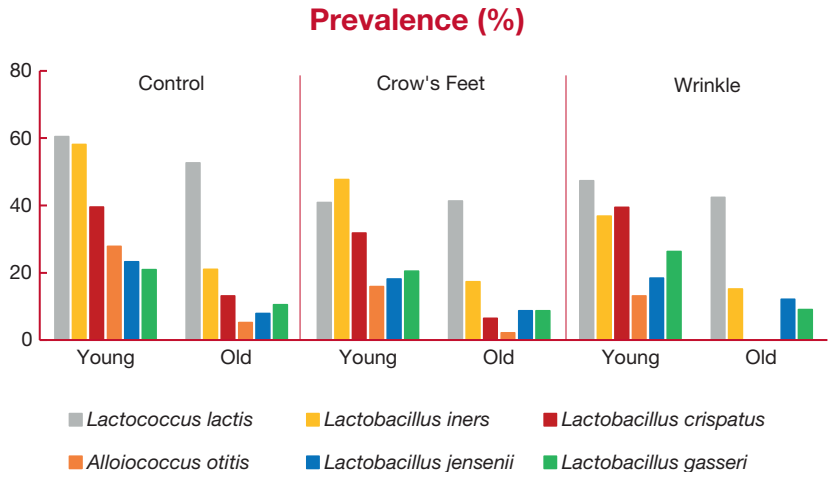


Figure 3 - Lactic acid bacteria prevalence in wrinkles area of the face of young cohort compared to that of old cohort.

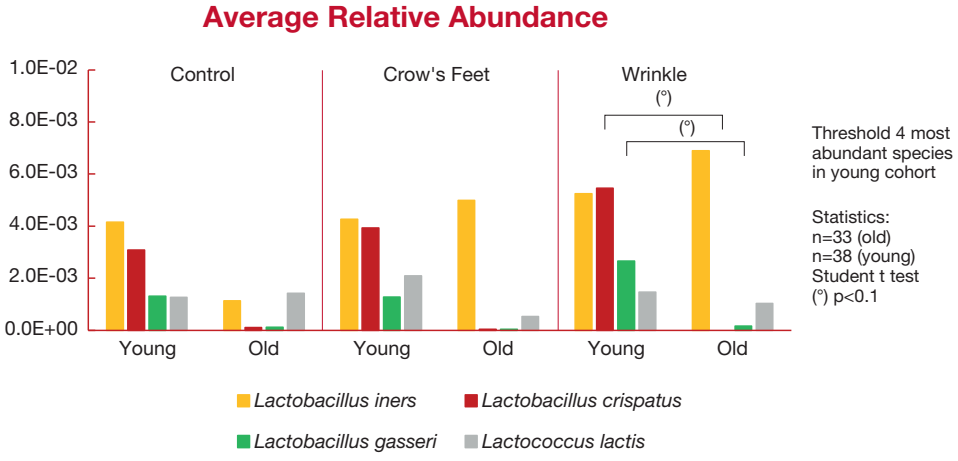


Figure 4 - *Lactobacillus* abundance in wrinkles area of the face of young cohort compared to that of old cohort.

Lactobacillus species show a metabolic versatility and several exploitable functional properties which make them good candidate as probiotic or relevant for food fermentation to develop functional food (D'Alessandro *et al*, 2021).

Lactobacilli have a long history of safe use in fermented food, thanks to their crucial role in the production of fermented products (Rossi 2019). The genus Lactobacillus known as natural inhabitant of oral, gastrointestinal and urogenital tract is essential to modern food and feed technologies (Bernardeau *et al*, 2006); fermentation has recently re-emerged as an approach for improved functionality of food products in addition to the traditional roles such as shelf life, taste, and texture increase (Lee and Lee, 2019).

Previous studies have reported the beneficial effects of lactic acid bacteria, namely Lactobacillus, their extracts or ferments on skin health, including improvements in skin conditions and the prevention of skin diseases (Huang *et al*, 2022). Thus, Lactobacilli derived product have been used in skin care products. Several *in vitro* and *in vivo* studies show that Lactobacillus is a genus that has been proven to hydrate the skin improving the barrier function, reduce inflammation and improve the entire functioning of the skin (Cinque *et al*, 2017; La Khmaladze *et al*, 2019; Lee *et al*, 2015; Tsai *et al*, 2021)

Lactobacillus crispatus is a Gram-positive rod shape anaerobic bacterium. *Lactobacillus crispatus* has been described as a natural inhabitant of some body mucosa (Pan *et al*, 2020). Its presence on the skin has been scarcely described.

As other lactobacilli, *Lactobacillus crispatus* has been reported to show some valuable and host beneficial properties even if it is less studied than other like *Lactobacillus plantarum*. *Lactobacillus crispatus* have shown *in vitro* and *in vivo* antimicrobial activities through its metabolites (Abdul Rahim *et al*, 2021), some immunomodulatory and anti-inflammatory properties (Wang *et al*, 2021); as well as potential uses in functional food (Siroli *et al*, 2017). Biosurfactants produced by *Lactobacillus crispatus* has been reported to improve skin permeation (Abruzzo *et al*, 2021).

Postbiolift, a postbiotic ingredient obtained by *Lactobacillus crispatus* fermentation process

Postbiolift is one of the two newly developed biotic ingredients Probiolift and Postbiolift to support healthy and graceful skin aging. Unlike other biotics existing on the market, the newly developed actives are the first to use a bacterium naturally present on the skin: *L. crispatus*, a bacterium which has been found to decrease with age.

L. crispatus strain used to develop Postbiolift has been isolated from healthy human skin. After swabbing the skin of volunteers and successive plating and scraping on MRS (Man Rugosa Sharp) agar plate enriched medium (with amino acid, then carbohydrates), the isolated strain was identified by PCR. This strain is a wild type, not genetically modified. Isolated strain safety was assessed through its genome analysis. Virulence analysis was based on its full genome using Virulence Factor Database (VFDB). The results of this analysis showed that *L. crispatus* strain was non-pathogenic, non- biofilm forming, non-sporulating and non-toxin producer. No antibiotic resistance has been evidenced. When spread on a surface, cleaning and decontamination can be achieved using 0.5% Hypochlorite solution or 70% ethanol; This strain as other lactobacilli is classified Biosafety Level 1 (BSL1).

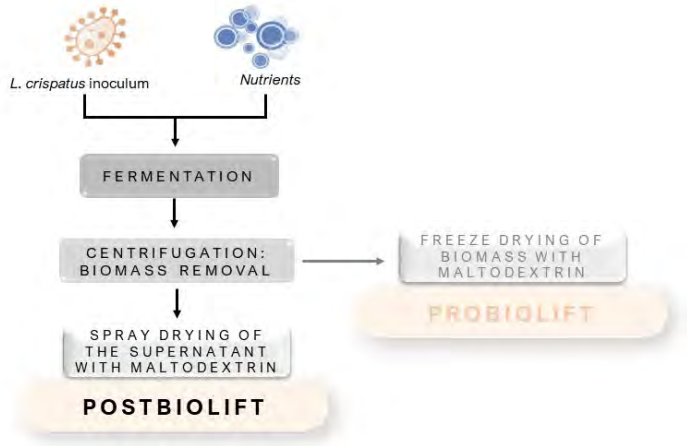


Figure 5 - Schematic process of Postbiolift.

The 2 products Probiolift and Postbiolift are simultaneously produced from the same fermentation process: Probiolift, the probiotic with dormant cells of living *L. crispatus* (biomass) and Postbiolift, the supernatant containing the metabolites secreted by *L. crispatus* (Figure 5). With this approach we ensure that no additional resources is required to produce the 2 products, inputs are mutualized during the process, in addition we also minimize the production of waste (as the non-valorized part would have been discarded).

The fermentation conditions of *L. crispatus* were optimized in terms of temperature, pH and inputs for the growth of biomass, and the production of small metabolites. The highest production of small organic acid metabolites namely lactic acid and derivatives was obtained under these optimized conditions with total consumption of the carbon source nutrient. After recovery of *L. crispatus* biomass for Probiolift, the supernatant rich in essential metabolites (amino acids, peptides, minerals & organic acids) is spray dried over maltodextrin to give Postbiolift as a powder (INCI: Maltodextrin, Lactobacillus Ferment) titrated in lactic acid under lactate form.

Postbiolift is characterized by its 5-7% (w/w) content in lactic acid, under lactate form at the pH of 5.0-7.0. Beside lactic acid other small organic acids were identified: small aliphatic organic acids such as propionic acid and 2-hydroxyisocaproic acid , and lactic acid derivatives such as hydroxyphenyl lactic acid, 3-phenyl lactic acid and quantified around 0.1-0.5% w/w in total.

Lactic acid is a molecule naturally present in the skin. As an alpha hydroxy acid, it is also commonly used in cosmetics to gently accelerate cell renewal and to give radiance to the skin; some authors have shown that lactic acid participates in the synthesis of epidermal and dermal compounds for skin rejuvenation, (Yamamoto *et al*, 2006; Smith *et al*, 1996). Finally, lactic acid helps to increase production of ceramides in keratinocytes thus reinforcing the skin barrier and preserving skin moisturization (Rawlings *et al*, 1996).

Lactic acid derivatives have been reported to control some opportunistic pathogenic growth (Jung *et al*, 2019).

Other lactic acid derivatives have been described as melanin synthesis inhibitors *in vitro* (Kim *et al*, 2011).

Safety / tolerability of the product

Postbiolift was tested to ensure its safety under the recommended conditions of use. Postbiolift does not irritate the eyes or skin and no indication of skin sensitization was observed.

Description

Postbiolift is a postbiotic fermentation supernatant with the secreted metabolites of the skin bacterium *Lactobacillus crispatus*. Titrated in lactate form (5-7%), it is available as a preservative-free powder.

INCI name: Maltodextrin (and) Lactobacillus Ferment

Naturalness origin content (according to ISO 16128): 100% from natural origin

Dose of use: 1%

China IECIC listed

Example: illustration of a commercial sample of Postbiolift, with an emulsion and hydrogel containing it at 1%.

DEMONSTRATED EFFICACY

Postbiolift biological properties and efficacy have been proven *in vitro* and *in vivo*. Clinically Postbiolift improved skin elasticity, decreased skin melanin content and smoothed wrinkles, thus promoting healthy skin aging.

Proven *in vitro* performance

Evaluated at protein level, Postbiolift proved its efficacy *in vitro* to stimulate elasticity targets:

- it increased elastin synthesis by fibroblasts, contributing thus to the improvement of skin elasticity and resilience,
- it boosted Emilin-1, Fibulin-5 and MFAP4 synthesis by fibroblasts in monolayer culture; Fibrillin-1 and Emilin-1 in reconstructed skin also contributing to the improvement of skin elasticity.

Postbiolift showed a capacity to inhibit tyrosinase activity and subsequently melanin synthesis.

Interestingly, Postbiolift contributed to skin relaxation by decreasing contraction frequency of neuromuscular cell culture *in vitro*; this activity contributes to a decrease in wrinkle appearance.

***In vivo* performance**

Postbiolift demonstrated its ability to:

- increase skin elasticity thanks to the increase of several measured parameters,
- improve skin tone by decreasing the skin melanin content,
- smooth crow's feet wrinkles.

EFFICACY

Stimulation of skin elasticity targets

Stimulation of elastin synthesis by fibroblasts

in vitro

OBJECTIVES

During aging, ECM components decrease in terms of quantity and their organization is impaired. Elastin, the main protein responsible for skin elasticity and resilience is decreased and become more susceptible to degradation.
The aim of this study was to assess Postbiolift capacity to enhance elastin synthesis in dermal fibroblast monolayers using Western blot for quantification.

RESULTS AND DISCUSSION

After 96h of culture, the positive control TGFβ at 10ng/mL significantly increased elastin production (data not shown). The results presented in Figure 6 show that after the incubation period Postbiolift at 0.05% significantly stimulated fibroblast synthesis and secretion of elastin by 28%.

CONCLUSION

In vitro Postbiolift showed a capacity to boost the synthesis of elastin by fibroblasts, and thus its potential to rejuvenate the skin and to improve skin elasticity.

Elastin synthesis stimulation

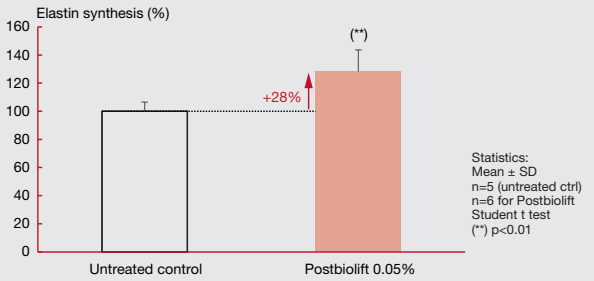


Figure 6 - Effect of Postbiolift on elastin synthesis on fibroblast monolayer.

MATERIALS&METHODS

Cell culture

Normal human fibroblasts (woman 62 years old) were seeded in a 12 well plate at 50000 cells/cm^2 and were cultured in a defined medium (Fibroblast Growth Medium, FGM) at 37°C under 5% of CO_2 until confluence.
Once the monolayer reached confluence, cell layer was incubated for 96 hours in the presence of Postbiolift at 0.05%, the positive control TGFβ at 10ng/mL or in absence of any compound for the untreated control.

Western blot quantification of elastin

After treatment, culture media were recovered to perform a western blot analysis on elastin. Protein concentration was determined by BCA assay. All the samples were adjusted to the same protein concentration to deposit the same quantities in each capillary.
A biotinylated molecular weight ladder, streptavidin-HRP, DTT, molecular weight fluorescence standards, luminol-S, hydrogen peroxide, sample buffer, running buffer, stacking matrix, separation matrix, wash buffer, and matrix removal buffer were purchased from ProteinSimple (Santa Clara, CA, USA). Antibody diluent, goat-anti rabbit secondary antibody, was also purchased from ProteinSimple. The capillaries were obtained from ProteinSimple containing a proprietary UV-activated chemical linked coating.

Elastin was identified by a capillary electrophoresis-based protein analysis system (ProteinSimple Sally Sue Instrument, ProteinSimple, San Jose, California, USA) using primary anti-elastin antibody immunoprobed with a horseradish peroxidase-conjugated secondary antibody and chemiluminescent substrate. Both antibodies were incubated 30 minutes. After 40 min to allow the separation, the resulting chemiluminescent signal was detected and quantified by Compass Software version 2.7.1 (ProteinSimple).

Results and statistics

The results are expressed in percentage, as the mean ± standard deviation (SD) compared to the untreated control standardized to 100%. Each condition was carried out in n=5-6. Statistical analysis *vs* the untreated control was done using One Way Anova analysis (Dunnett's method) after normal distribution comparison of the values (Shapiro-Wilk test) following Sigmaplot software recommendations (Systat Software Inv. USA). The threshold of significance was set to 5% ($p<0.05$).

EFFICACY

Stimulation of skin elasticity targets

Stimulation of elastin partners synthesis by fibroblasts

in vitro

OBJECTIVES

Elastic fibers consist of an elastin core covered by a sheath of microfibrils, which are composed of several distinct glycoproteins. Mature elastic fibers synthesis and functioning need intervention of several elastin partner proteins among which MFAP4, Fibulin-5 and Emilin-1 are the major one. A multi-target western blot assay focused on the above-mentioned proteins involved in skin elasticity was used to demonstrate the effect of Postbiolift on their synthesis in dermal fibroblasts.

RESULTS AND DISCUSSION

A fully automated western blot method which overcomes the obstacles related to reproducibility, sensitivity, quantifiability, and speed seen in traditional western blotting was used to assess effect of Postbiolift on the selected targets.

TGFβ and TNFα known to stimulate the synthesis of respectively MFAP4, Fibulin-5 and Emilin-1 were used as positive control to validate the experiments (data not shown).

Figure 7 shows the effects of Postbiolift on the synthesis of the elastic partners proteins MFAP4, Fibulin-5 and Emilin-1.

As shown in figure 7 A, MFAP4 an important glycoprotein involved in elastin formation, maturation and interaction with microfibrils was significantly upregulated by 60% after incubation with Postbiolift at 0.05%.

Postbiolift at 0.1% stimulated significantly by 50%, the synthesis of Fibulin-5, a key component involved in tropoelastin and elastic fiber assembly (Figure 7B).

Emilin-1, a ligand of microfibrils involved in proper elastic fiber organization, was significantly stimulated by 61% at 0.05% (Figure 7C).

The significant increase in MFAP4, Fibulin-5 and Emilin-1 synthesis revealed the potential of Postbiolift to improve the elastic fibers synthesis and assembly which are impaired with aging. As a consequence, Postbiolift may help to promote a strong and functional elastic fibers network.

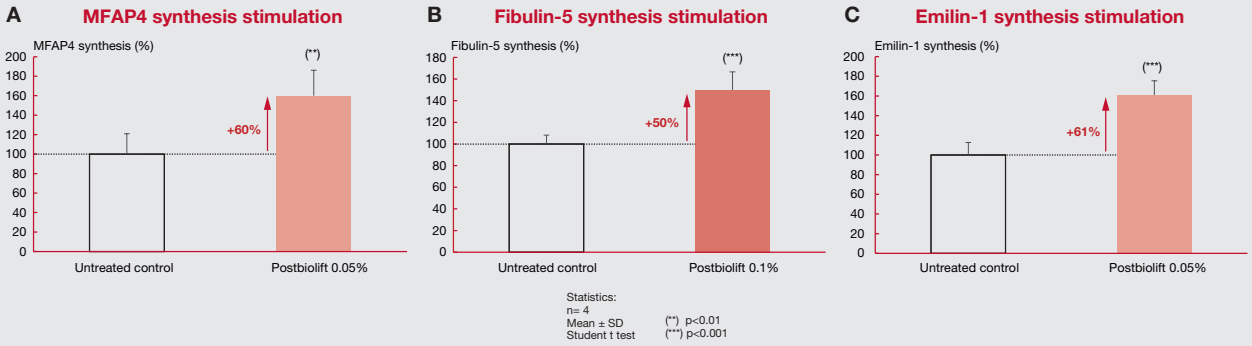


Figure 7 - Effect of Postbiolift on the protein expression of elasticity protein targets. **A**- MFAP4 synthesis. **B**– Fibulin-5 synthesis. **C**– Emilin-1 synthesis.

CONCLUSION

In vitro Postbiolift showed the ability to boost the synthesis of the elastic fiber partners MFAP4, Fibulin-5 and Emilin-1 by fibroblasts, and thus its potential to improve elastic fiber synthesis and maturation for an improved skin elasticity.

MATERIALS&METHODS

Cell culture

Normal human fibroblasts from a 19 year old donor were seeded at 50000 cells/cm^2 and cultured in a defined medium (Fibroblast Growth Medium, FGM) at 37°C under 5% of CO_2 until confluence. Once confluence is reached cells were incubated for 48 hours for Fibulin-5 and Emilin-1, and 96h for MFAP4 in the presence or absence of Postbiolift at 0.05 or 0.1%. TGFβ was used as positive control of Fibulin-5 and MFAP4 stimulation synthesis while TNFα was used for Emilin-1. Culture medium was used as untreated control.

Western blot on the elastin partners proteins MFAP4, Emilin-1 and Fibulin-5

After treatment, culture media were recovered to perform a western blot analysis on MFAP4 and Emilin-1 and the cells were harvested and lysed with a specific lysis buffer to evaluate Fibulin-5 content. Protein concentration was determined by BCA assay and then the samples were kept frozen at -80°C until use. All the samples were adjusted to the same protein concentration to deposit the same quantities in each capillary.

A biotinylated molecular weight ladder, streptavidin-HRP, DTT, molecular weight fluorescence standards, luminol-S, hydrogen peroxide, sample buffer, running buffer, stacking matrix, separation matrix, wash buffer, and matrix removal buffer were purchased from ProteinSimple (Santa Clara, CA, USA). Antibody diluent, goat-anti rabbit secondary antibody, was also purchased from ProteinSimple. The capillaries were obtained from ProteinSimple containing a proprietary UV-activated chemical linked coating.

Targeted proteins were identified by a capillary electrophoresis-based protein analysis system (ProteinSimple Sally Sue Instrument, ProteinSimple, San Jose, California, USA) using an incubation of primary antibodies and immunoprobed using a horseradish peroxidase-conjugated secondary antibody and chemiluminescent substrate.

After 40 min to allow the separation, the resulting chemiluminescent signal was detected and quantified by Compass Software version 2.7.1 (ProteinSimple).

Results and statistics

The results are expressed in percentage, as the mean ± standard deviation (SD) compared to the untreated control standardized to 100%. Each condition was carried out in n=4. Statistical analysis *vs* the untreated control was done using One Way Anova analysis (Dunnett's method) after normal distribution comparison of the values (Shapiro-Wilk test) following Sigmaplot software recommendations (Systat Software Inv. USA). The threshold of significance was set to 5% ($p<0.05$).

EFFICACY

Stimulation of skin elasticity targets

Stimulation of elastin partners synthesis by fibroblasts in reconstructed skin model

in vitro

OBJECTIVES

Elastic fibers consist of an elastin core covered by a sheath of microfibrils. Fibrillin-1 is one of the constituents of microfibrils on which elastin fibers deposit; it is the most abundant fibrillin expressed in the dermis.

Emilin-1 plays a role in the fibers assembly phase by aiding the fibrillin-microfibril fibers to be more ordered. In the skin this protein locates in the dermis, up to the basement membrane, interacting thus with components of the extracellular matrix but also with the anchoring complex.

As these two proteins can be stained on the skin, we have developed a reconstructed skin model in which Emilin-1 and Fibrillin-1 will be quantified by staining after incubation with Postbiolift.

RESULTS AND DISCUSSION

Results of toluidine staining indicated that after incubation with Postbiolift reconstructed skin quality was satisfactory; epidermis and dermis integrity was conserved after 2 and 7 days of treatment with Postbiolift (data not shown).

Stimulation of Emilin-1 synthesis and secretion observed in fibroblast monolayer cultures was confirmed in reconstructed skin, as staining intensity in the dermis after one topical application of Postbiolift at 1% was higher compared to that of the untreated control (Figure 8A). Staining quantification showed a 58% ($p<0.01$) increase of Emilin-1 (Figure 8B) throughout the dermis confirming Postbiolift potential to improve elastic fiber organization and functionality.

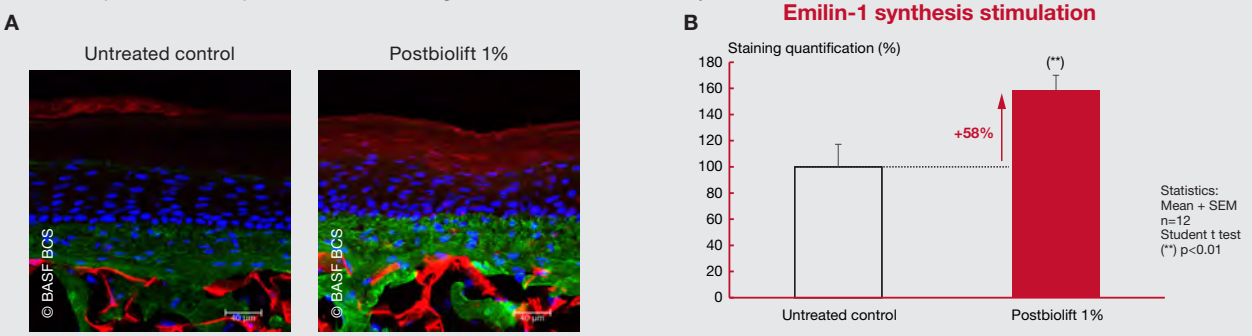


Figure 8 - Effect of Postbiolift on Emilin-1 synthesis on reconstructed skin. Measurement 48h after a single topical application of Postbiolift at 1%. **A**- Blue: dapi nuclei staining; Green: Emilin-1 staining; Red: Blue evans (reconstructed skin morphology). **B**- Quantification of Emilin-1 staining.

Figure 9 showed an increase in Fibrillin-1 staining after 7 days of incubation with topical application every two days of Postbiolift at 1% (Figure 9 A). Fibrillin-1 staining was equally distributed throughout the dermis. Signal quantification gave a 74% ($p<0.001$) increase in Fibrillin-1 staining (Figure 9B), showing Postbiolift potential to strengthen the microfibrils network on which elastin fibers will deposit.

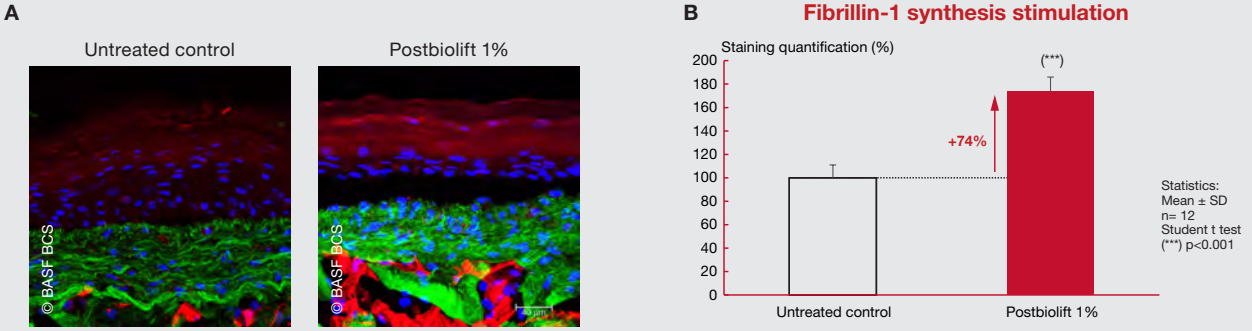


Figure 9 - Effect of Postbiolift on fibrillin-1 synthesis on reconstructed skin. Measurement after 7 days, with topical application every two days of Postbiolift at 1%. **A**- Blue: dapi nuclei staining; Green: Fibrillin-1 staining; Red: Blue evans (reconstructed skin morphology). **B**- Quantification of fibrillin-1 staining.

CONCLUSION

Postbiolift stimulated Emilin-1 and Fibrillin-1 synthesis in 3D model of reconstructed skin, and thus aiding the assembly of elastin fibers for an improved skin elasticity.

MATERIALS&METHODS

Cell culture

Fibroblasts from a 18 years old donor were seeded on collagenous sponge and cultured in DMEM/HAM-F12 medium for 28 days. Then keratinocytes from a 37 years old donor were added and cultured in keratinocyte medium based on DMEM/HAM-F12 for 7 days and were allowed to differentiate at air liquid interface for 16 days. After differentiation step, 130µL of a 1% Postbiolift solution was applied topically every two days on the reconstructed skin samples up to 7 days.

Staining assay of Emilin-1 and Fibrillin-1

The quality of the epidermis and the dermis of the reconstructed skin was checked by toluidine blue staining. Emilin-1 was stained after 48h of incubation; visualization was made using confocal microscopy after incubation 60 minutes at room temperature with anti-Emilin-1 antibody.

Fibrillin-1 was stained after 7 days of incubation; visualization was made using confocal microscopy after incubation 60 minutes at room temperature with anti-Fibrillin-1 antibody.

For staining of Emilin-1 and Fibrillin-1 green signal quantification was made using Image J software.

Counterstainings were made with DAPI a blue-fluorescent DNA stain was used to visualize cell nuclei in reconstructed skin and Blue Evans to visualize reconstructed skin morphology.

Results and statistics

After staining quantification, results were expressed as mean staining percentage ± standard error on the mean (SEM).

Each condition was carried out in n=12. Statistical analysis *vs* the untreated control was done using Student t test after normal distribution comparison of the values (Shapiro-Wilk test) following Sigmaplot software recommendations (Systat Software Inv. USA). The threshold of significance was set to 5% ($p<0.05$).

EFFICACY

Inhibition of tyrosinase activity

in vitro

OBJECTIVES

Tyrosinase is an enzyme which plays a key role in the melanogenesis process. Inhibiting its activity is one of the main pathways used to decrease melanin within the skin.

Our aim in this study was to evaluate the potential of Postbiolift to decrease skin hyperpigmentation associated with aging skin by inhibiting *in tubo* human tyrosinase activity.

RESULTS AND DISCUSSION

In this study we used human tyrosinase extracted from melanocytes. Kojic acid a well-known depigmenting molecule was used as a positive control in the experiment and successfully inhibited tyrosinase activity (data not shown).

Postbiolift totally inhibited tyrosinase, with 100% decrease of tyrosinase activity at 0.05% (Figure 10). This activity gave an indication that Postbiolift can inhibit melanin synthesis. This effect will be checked afterward.

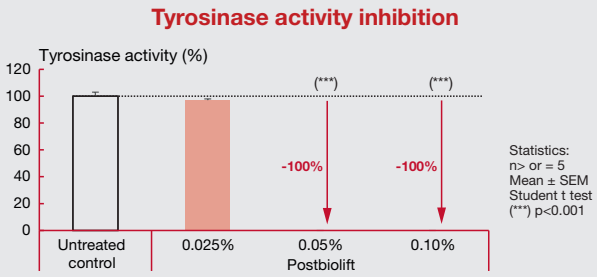


Figure 10 - Effect of Postbiolift on human tyrosinase activity.

CONCLUSION

Postbiolift inhibited human tyrosinase activity thus showing a potential to decrease melanin synthesis.

MATERIALS&METHODS

Tested products

The used tyrosinase was extracted from normal human melanocytes.

Kojic acid at at 8 mM was used as positive control and inhibited tyrosinase activity by 100%. Postbiolift was used at 0.025, 0.05 and 0.1%.

Tyrosinase inhibition assay

Products were pre-incubated with the extracted enzyme for 10 minutes on ice. Then, the tyrosinase substrate, L-Tyrosine at a final concentration of 1 mM was added to the medium and the plates were incubated at 37°C for 24 hours.

The same enzyme extract without addition of any product with ethanol was used as a negative control (Control).

Enzyme activity was assessed by measuring tyrosine transformation by optical density (OD) at 540 nm using a microplate reader.

Optical density measurements were also performed without the addition of substrate (n=1) to detect possible interference from the product at each concentration tested.

Results and statistics

The percentage inhibition of tyrosinase activity was calculated according to the following formula:

Inhibition percentage: 100 - [OD value/Mean OD of control x 100].

Results were expressed as mean percentage of Inhibition ± Standard error on the mean. Each condition was tested in triplicate. Intergroup comparisons were performed by an unpaired Student t test. The threshold of significance was set to 5% ($p<0.05$).

EFFICACY

Inhibition of melanin synthesis

in vitro

OBJECTIVES

Melanin pigmentation plays a critical role in protecting the skin from the harmful effects of ultraviolet (UV) radiation. With aging, uneven pigmentation and dark spots appear on the skin due to the hyperactivation of melanocytes, altered distribution or turnover of melanin pigment.

In this study, we aimed to assess the effect of Postbiolift on melanin synthesis on melanocytes and keratinocytes coculture, using melanin quantification by spectrophotometry.

RESULTS AND DISCUSSION

In this experiment, melanin synthesis assessment was made under stimulation induced by 40µM of oleic acid to evidence more easily melanin synthesis inhibition. 4-butyresorcinol, a known depigmenting agent, was used at 0.002% (w/w) as positive control. It significantly inhibited melanin synthesis by 28% (data not shown), thus validating the assay.

After incubation with Postbiolift, melanin synthesis was significantly decreased from 0.05% to 0.1% doses, with respectively 18% and 32% of inhibition ($p<0.01$) (Figure 11). As expected from its tyrosinase inhibition property Postbiolift has demonstrated its ability to effectively inhibit the synthesis of melanin, a process that is hyperactivated or dysregulated with aging.

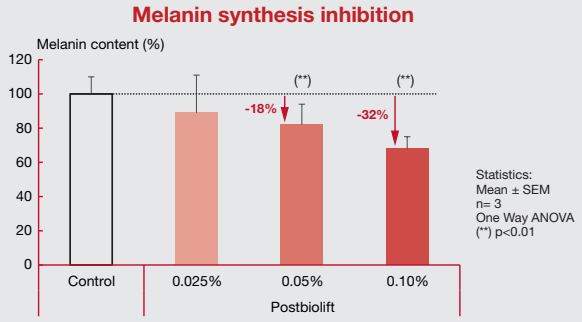


Figure 11 - Effect of Postbiolift on melanin synthesis.

CONCLUSION

Postbiolift showed significant inhibition of melanin synthesis by melanocytes in presence of keratinocytes. This shows its potential to fight and decrease age-related melanogenesis dysregulation such as aged spots.

MATERIALS&METHODS

Tested products

Normal human keratinocytes and neonatal human melanocytes were seeded at 20,000 cells/cm^2 and 10,000 cells/cm^2, respectively in 24 wells plates, and then cultured for 7 days at 37°C under 5% CO_2 and 95% relative humidity in a mixture of 50% of melanocyte culture medium and 50% of keratinocyte culture medium supplemented with growth factors.

After one week of culture, the culture medium was replaced with a mixture of antibiotic-free DMEM (Dulbecco's modified Eagle's minimum essential medium) culture medium and growth factor-free melanocyte medium and supplemented with 40µM oleic acid.

This culture medium was used as an untreated control (Control).

Postbiolift was added at concentrations of 0.025, 0.05 and 0.10% (w/w).

0.002% of 4-butylresorcinol (w/w) was used as a positive control condition.

Melanin quantification assay

Products were incubated for 72h at 37°C under 5% CO_2 and 95% relative humidity, then the culture medium was renewed. After 48h of additional culture, the medium was then removed, and the cells rinsed with PBS (phosphate buffer). A 1N sodium hydroxide solution containing 10% Dimethylsulfoxide (DMSO) was added to each well of the plate. 200µL of each well was transferred to a transparent plate. The Optical Density reflecting the amount of melanin was read at 475nm.

Results and statistics

The results were expressed as the mean percentage of melanin quantity ± standard error on the mean compared to the untreated control normalized to 100%. Statistical analysis of the results was done against the untreated control using the One-Way ANOVA test after normal distribution comparison of the values (Shapiro-Wilk test) following Sigmaplot software recommendations (Systat Software Inv. USA). The threshold of significance was set to 5% ($p<0.05$).

EFFICACY

Skin relaxation

Inhibition of cell contraction

in vitro

OBJECTIVES

Expressional wrinkles appear at around 30 years old on the face where skin has to adapt to the facial muscle's movements and gravity. Facial movements are based on repeated subcutaneous muscle contraction and create with aging expressional wrinkles which are difficult to remove. The goal of this experiment was to evaluate Postbiolift capacity to decrease frequency of cell contraction, in a coculture model of cellular motor neurons and muscle cell.

RESULTS AND DISCUSSION

The positive control α-bungarotoxin at 1µM significantly inhibited cellular contraction starting at 2 hours of incubation; this result validated the experiment.

Postbiolift induced a significant inhibition of the contraction frequency as early as 2 hours after treatment at 0.002% (Figure 12). The significant effect observed from two hours persisted until 24 hours of incubation.

Postbiolift observed efficacy is comparable to that of the α-bungarotoxin positive control of inhibition which acts by binding specifically to acetylcholine receptors.

Cellular contraction inhibition

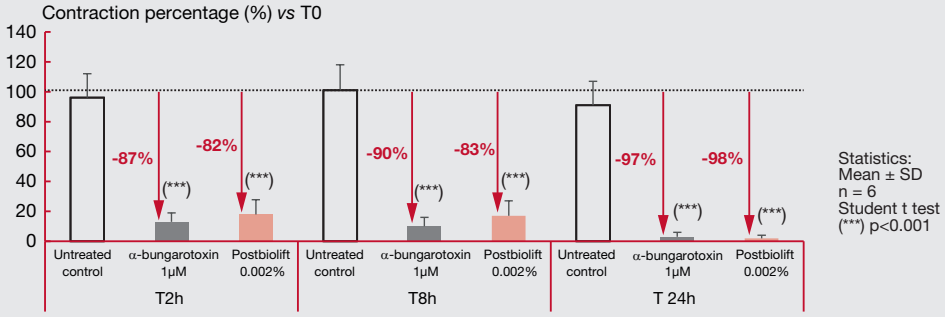


Figure 12 - Effect of Postbiolift on cellular contraction frequency.

CONCLUSION

Postbiolift showed a significant inhibition of cell contraction, and thus its potential for an improved skin relaxation to lower appearance of expression wrinkles.

MATERIALS&METHODS

Cell culture

Human Induced Pluripotent cells (hiPS) derived from reprogrammed human fibroblasts were seeded in 6-well plates coated with a thin layer of Matrigel (Dutscher), in a growth medium for hiPS cells. This medium was then changed by a succession of 3 differentiation media. These media are supplemented with different factors inducing a specific differentiation towards the motor neuron pathway. The cells were maintained in culture at 37°C and 5% CO_2 for 10 days.

After 9 days of differentiation in the different culture media, the differentiating hiPS cells were recovered and seeded in 96-well plates on top of a mat of human muscle cells from an adult donor seeded 24 hours before on a Matrigel coated medium. These cells grew in the differentiation medium.

Under these conditions, after 3 weeks of co-culture, the muscle cells contract spontaneously under the impulse of the motor neurons. These contractions can be blocked by a-bungarotoxin (Santhanam *et al*, 2018) at 1µM.

Cells' contraction registration

At this stage, the cells were placed under an InCell 2200 automated microscope (GE Healthcare) in a controlled atmosphere at 37°C. For each culture well, the areas where muscle fibers are contractile were identified; on all identified areas, a 30 second movie and cells 's contraction frequency were recorded (basal contraction conditions).

At the end of the first recording, the compounds or α-bungarotoxin were incubated. The following treatment conditions were performed:

- control medium,
- control medium + Lactobacillus Ferment equivalent to Postbiolift at 0.002%,
- control medium + α-bungarotoxin at 1µM as positive control.

Each condition was repeated 6 times.

By experimental condition, contraction frequencies were analyzed before treatment and then after 2 hours, 8 hours, and 24 hours of treatment.

Statistics

Results were expressed as mean ± SD percentage of contraction frequency which was calculated relative to the frequency of contractions before incubation with tested compounds. The basal contraction condition was considered as the maximum frequency (100%). Intergroup comparisons were performed by an unpaired Student t test. The threshold of significance was set to 5% ($p<0.05$).

In vitro conclusion

We evidenced *in vitro* the antiaging and rejuvenating properties of Postbiolift.

Postbiolift activated extracellular matrix component participating to skin elasticity and resilience through:
- the increase in elastin synthesis in fibroblasts monolayer (+28%),
- the stimulation of MFAP4 (+60%) and Fibulin-5 (+50%) production in fibroblast monolayer, all involved in elastic fiber assembly.
- the increase in Emilin-1 synthesis in fibroblast monolayer (+61%) and in reconstructed skin (+58%) involved in elastin and microfibrils proper assembly.
- the boosting synthesis of one of the major proteins of microfibrils, Fibrillin-1 in the dermis in reconstructed skin (+74%).

Postbiolift decreased the melanin synthesis which is dysregulated during aging by:
- inhibiting tyrosinase activity (up to 100% of inhibition),
- inhibiting melanin synthesis by melanocytes cocultured with keratinocytes (-32%).

Postbiolift relaxed the skin by inhibiting cells contraction frequency (-98%).

Altogether, Postbiolift showed *in vitro* several biological properties involved in skin rejuvenation for potential improvement of skin elasticity, skin tone homogeneity and wrinkles appearance.

EFFICACY

Improvement of skin elasticity, wrinkle reduction and melanin decrease with Postbiolift for anti-aging efficacy

in vivo

OBJECTIVES

The objective of the study was to evaluate in a double-blind, randomized, split-face, placebo-controlled clinical study the anti-aging effect of Postbiolift at 1%. This study was conducted on 30 healthy female volunteers (Chinese origin), aged 40-50 years old, presenting crow's feet wrinkles and pigmentation spots.

The anti-aging effect was quantified by instrumental measurement:
- Cutometer for skin elasticity,
- VisioScan VC20 Plus for wrinkles,
- Visia-CR for illustrative images,
- Mexameter for melanin measurement,

and by self-assessment, before and after 28 and 56 days of treatment.

Figure 13 - Clinical study of Postbiolift on skin aging.

RESULTS AND DISCUSSION

1. Skin elasticity improvement:

Skin elasticity before and after Postbiolift or placebo application was measured using several clinical parameters which were registered by a Cutometer:

- R2 for Gross elasticity
- R5 for Immediate elasticity
- Q1 for the Total recovery.
- F3/F4 for skin elasticity thanks to skin fatigue and deformability.

An increase of these parameters, which decrease with age, correspond to an improvement of skin elasticity, (Ma *et al*, 2017; Muggli *et al*, 2005).

On Gross elasticity parameter R2 (Figure 14), Postbiolift at 1% significantly showed an increase compared to baseline by 4.4% ($p<0.001$) after 28 days of treatment, up to 12.1% ($p<0.001$) after 56 days of use. These increases are significant *vs* the placebo, by 3.3% ($p<0.05$) after 28 days of treatment, up to 9.5% ($p<0.001$) after 56 days.

On Immediate elasticity parameter R5 (Figure 14), Postbiolift at 1% significantly showed an increase compared to baseline by 5.8% ($p<0.01$) after 28 days of treatment, up to 11.7% ($p<0.001$) after 56 days of use. These increases are significant *vs* the placebo, by 10.1% ($p<0.01$) after 56 days.

On Total recovery parameter Q1 (Figure 14), Postbiolift at 1% significantly showed an increase compared to baseline by 4.7% ($p<0.001$) after 28 days of treatment, up to 12.5% ($p<0.001$) after 56 days of use. These increases are significant *vs* the placebo, by 9.8% ($p<0.001$) after 56 days.

Finally, on the elasticity parameter F3/F4 (Figure 14), Postbiolift at 1% significantly showed an increase compared to baseline by 7.1% ($p<0.01$) after 28 days of treatment, up to 18.8% ($p<0.001$) after 56 days of use. These increases are *vs* placebo close to significance by 5.2% ($p<0.1$) after 28 days, up to significant, by 13.2% ($p<0.001$) after 56 days.

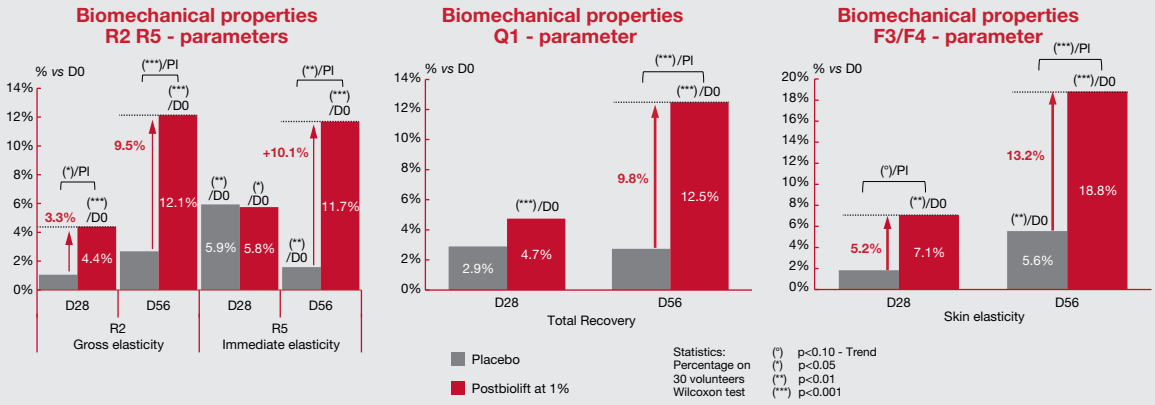


Figure 14 - Effect of Postbiolift on skin elasticity measured by a Cutometer.

These increases of the different skin elasticity parameters demonstrate an improvement of the face skin elasticity due to the Postbiolift treatment.

in vivo

2. Pigmentation decrease of age spots

As shown by the decrease of the melanin index (Figure 15) on the age spots, Postbiolift at 1% significantly showed an anti-dark spot effect compared to baseline by 3.1% ($p<0.001$) after 28 days of treatment, up to 5.5% ($p<0.01$) after 56 days of use. These decreases are significant *vs* the placebo, by 3.0% ($p<0.001$) after 28 days of treatment, up to 6.5% ($p<0.01$) after 56 days.

This decrease of the melanin index on the pigmented area demonstrates an improvement of the age spots for an even skin tone.

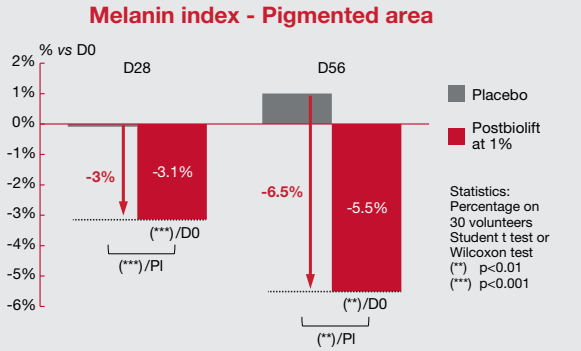


Figure 15 - Effect of Postbiolift on skin melanin content of pigmented area of the face.

3. Smoothing Crow's feet wrinkles

After 56 days of treatment, Postbiolift at 1% significantly showed a decrease of crow's feet average relief compared to baseline by 8.2% ($p<0.001$) and compared to the placebo by 6.6% ($p<0.01$) (Figure 16).

This decrease of the crow's feet relief demonstrates a smoothing effect on skin wrinkles appearance (Figure 16).

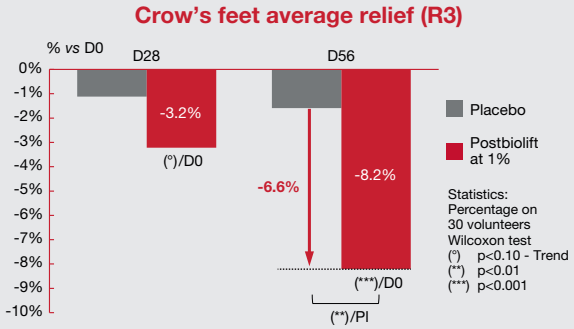


Figure 16 - Effect of Postbiolift on crow's feet wrinkles relief.

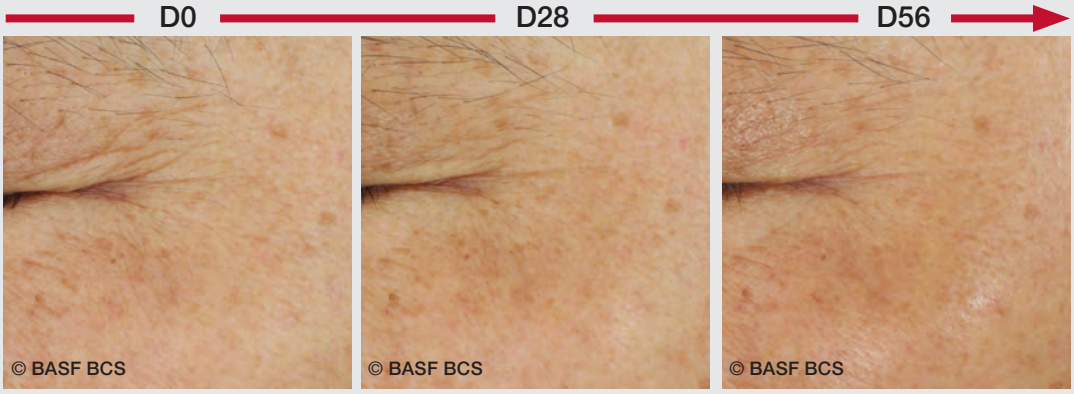


Figure 17 - Illustrative image by VISIA of Postbiolift effect on crow's feet wrinkles .

CONCLUSION

Postbiolift at 1% increases elasticity parameters *vs* Placebo from 1 month demonstrating its ability to increase skin elasticity and resilience.

Postbiolift at 1% significantly decreases the melanin index *vs* Placebo from 1 month demonstrating a capacity to decrease aged spot pigmentation for an even skin tone.

After 2 months of application, Postbiolift at 1% smoothed crow's feet wrinkles with a significantly higher efficacy compared to the placebo.

Taken together, the clinical results show the capacities of Postbiolift to increase skin bouncy and to decrease aged spots and wrinkles for a healthy aging.

MATERIALS&METHODS

Study design

The clinical study was carried-out as a double-blind, randomized, split-face, placebo-controlled clinical study. The efficacy of the formulation containing Postbiolift at 1% was compared to the baseline (before treatment, D0) and to that of a placebo formulation. The formulation is detailed in Annex 2. The study was conducted for a period of 56 days with check points at D0, D28 and D56.

Inclusion criteria

The study was done on 30 healthy Chinese female volunteers, aged from 40 to 50 years old with crow's feet wrinkle and age spot on face.

Application modality

The products (a formulation containing Postbiolift at 1% or a placebo formulation) were applied by the volunteers twice a day on each half of the face for 56 days, under normal conditions of use.

Evaluation methods

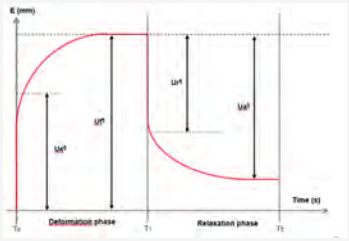
Skin elasticity measurement

Measurements were taken using the Cutometer (Courage+Khazaka). The principle of this technique is based on the creation of negative pressure on the surface of the skin and the measurement of induced vertical deformation by two optical prisms.

The application of a constant negative pressure was followed by a withdrawal of the strain, relaxation period, using the 2 mm suction probe.

The skin deformation produced by the 2 mm probe corresponds typically to the deformation of upper dermis.

Following parameters can be measured.

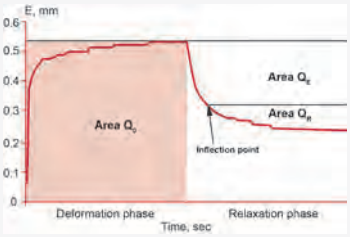


R2= Ua/Uf represents Gross elasticity.

The higher the R2, the better the Gross elasticity

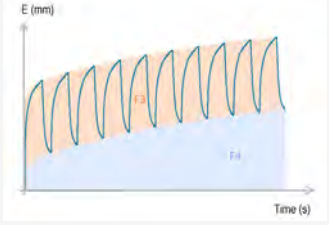
R5=Ur/Ue represents Immediate elasticity.

The higher the R5, the better the Immediate elasticity.



Q1 = $(Q_E+Q_R)/Q_0$ represents Total recovery (elastic + viscoelastic recovery).

The higher the Q1, the better the Total recovery.



F3/F4 represents the skin elasticity thanks to skin fatigue and deformability.

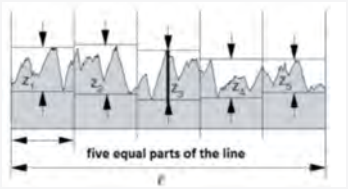
The higher the F3/F4, the better the skin elasticity.

All measurements were made on the cheeks.

Roughness of crow's feet wrinkle by VisioScan VC 20 Plus

In contrary to conventional color skin cameras, the Visioscan VC 20 plus features a unique UV-A LED illumination (proven to present no hazard to normal human skin). The light is homogenously distributed by a reflector. In addition, the camera consists of a high-resolution b/w video sensor chip, an objective and the new "liquid lens technology", an electronically controlled lens which automatically adapts to the skin surface distance and maximizes the range of depth of field.

The results are images of the skin surface (size approx. 10 x 8 mm) displayed in 255 grey levels. Wrinkles and lines appear dark and skin scaliness can be seen as very bright pixels. The software analyses the image regarding a variety of topography parameters. The measurement was performed on both crow's feet.



$$R3 = \frac{Z_1 + Z_2 + Z_3 + Z_4 + Z_5}{5}$$

R3: The average roughness is the arithmetic average of the different segment roughness values. In the DIN norm this parameter is known as Rz.

A decrease in R3 characterizes an anti-wrinkles effect. Unit: a.u.

Measurement of melanin index on skin spot by Mexameter

The Mexameter MX 18 (Courage+Khazaka) enables to measure by reflectance the quantities of the major component responsible for skin color, melanin using specific light wavelengths. A receiver measures the reflection from the skin. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated to determine the melanin index (melanin amount)

The melanin is measured on the cheeks by two specific wavelengths (red: 660 nm and near infrared: 880 nm) chosen to correspond to different absorption rates by the melanin pigments. The highly sensitive measurement gives values on a scale (0-999) for melanin

The unit for the melanin amount is Melanin Index (MI). A MI (melanin Index) decrease on skin spots corresponds to an anti-spot effect.

Statistics

Results are expressed as the mean percentage of change compared to the baseline measurement.

The statistical analysis of the evolution of the parameters as a function of time and of the differences in the studied parameters between the treatment groups, was done after verifying the normality of distribution using Shapiro-Wilk test. The following tests were used afterwards:

- for those comparisons where the normality of both data sets was achieved Student t test was used.
- for those comparisons where normality was invalidated, Wilcoxon test was used.

GENERAL CONCLUSION

Postbiotic technology for healthy aging

- Part of the Biotic rejuvenation range, including also Probiolift as live probiotic, based on youth bacterium
- First natural postbiotic technology from natural endogenous skin bacteria *Lactobacillus crispatus*, that are abundant in young skin and decreased in older skin.
- *In vitro*, stimulates synthesis of elastin and its partners, decreases melanin synthesis, and slows-down cell contractions.
- Clinically proven to support healthy aging by improving skin elasticity, age spots, skin tone eveness and smoothing crow's feet wrinkles.

Aging is an ineluctable process which is often worsen by external environmental factors like UV rays, pollution of even daily habits. The importance of aging for cosmetic market lies in the enormous consumer demand for actives or treatments that can prevent or reverse its signs, among which loss of elasticity, age spots and wrinkles. In all regions, anti-aging skincare demand is growing. At the same time, consumers also recognize the naturalness as well as the health and wellness benefits of biotics; this leads the increasing attractiveness of skincare products with biotic ingredients such as postbiotics.

To meet this growing consumer demand, we developed Postbiolift, the first postbiotic made from a bacterium naturally present on the skin, *Lactobacillus crispatus*, which tends to disappear from the skin surface with aging. Postbiolift is produced by fermentation and characterized by high level of *L. crispatus* metabolites namely small organic acids among which lactic acid and its derivatives.

In vitro performance evaluations evidenced for Postbiolift valuable anti-aging properties. *In vitro*, Postbiolift increases the synthesis of elastin, MFAP4, Fibulin-5, Emilin-1 and Fibrillin-1, all involved in the development of mature and functional elastic fibers. Postbiolift also inhibits tyrosinase and melanogenesis and slows down cell contraction.

Clinical improvements corroborate these anti-aging properties demonstrated *in vitro*. Indeed, in a placebo-controlled study, Postbiolift showed an improvement in skin elasticity, a decrease in skin melanin in pigmented areas of the face and a reduction in crow's feet wrinkle appearance.

Postbiolift – Lactobacillus ferment is the first postbiotic from a natural endogenous bacterium dedicated to skin, meeting consumers quest for safe, natural, and effective active ingredients for healthy skin aging with elastic, smoothed and even skin.

ANNEXES

Annex 1 - Technical data - Available upon request

- Quality and Regulatory Product Information
- Toxicological abstract
- Composition sheet
- Specifications
- Formulation Data Sheet
- Natural content origin (according ISO 16128)
- Product Purity Profile

Annex 2 - Clinical test formula

Trade name	INCI name	Placebo formulation %	Postbiolift formulation %
Glycerin 99-5	Glycerin	5.00	5.00
1,3-Butanediol	Butylene Glycol	10.00	10.00
Rheocare XGN	Xanthan Gum	2.00	2.00
Water	Water	qsf 100	qsf 100
Sodium benzoate	Sodium Benzoate	0.25	0.25
Dermosoft 1388 Eco	Glycerin, Aqua, Sodium Levulinate, Sodium Anisate	1.00	1.00
Dehymuls PGPH	Polyglyceryl-2 Dipolyhydroxystearate	1.00	1.00
Cetiol CC	Dicaprylyl Carbonate	6.00	6.00
Cetiol RLF	Caprylyl Caprylate/Caprate	10.00	10.00
Plantapon LC 7	Laureth-7 Citrate	0.50	0.50
Citric Acid (50% solution)	Citric acid	0.30	0.30
Postbiolift	Maltodextrin, Lactobacillus Ferment	-	1.00

Annex 3 - Formulation example

Probiotic Hydra Serum (SC-FR-22-SH-012601)

Phase	Ingredients	INCI	% by weight	Function
A	Glycerin	Glycerin	5.00	Humectant
	1,3-Butanediol	Butylene Glycol	10.00	Humectant
	Verdessence™ Xanthan	Xanthan Gum	2.00	Stabilizer
	Postbiolift™BC10152	Maltodextrin, Lactobacillus Ferment	1.00	Active ingredient
B	Water, demin.	Aqua	62.95	
	Sodium Benzoate	Sodium Benzoate	0.25	Preservative
	Dermosoft 1388 eco *(Evonik)*	Glycerin, Aqua, Sodium Levulinate, Sodium Anisate	1.00	Auxiliary
C	Dehymuls® PGPH	Polyglyceryl-2 Dipolyhydroxystearate	1.00	Emulsifier (W/O)
	Cetiol® 4 All	Dipropylheptyl Carbonate	3.00	Emollient
	Cetiol® CC	Dicaprylyl Carbonate	3.00	Emollient
	Cetiol® RLF	Caprylyl Caprylate/ Caprate	10.00	Emollient
	Plantapon® LC 7	Laureth-7 Citrate	0.50	Surfactant
D	Citric Acid (50% solution)	Citric Acid	0.30	pH Adjustment

Can be used in a double packaging with Probiolift BC10157 Booster

Microbiome Friendly care emulsion (SC-FR-22-BC-50945-01)

Phase	Ingredients	INCI	% by weight	Function
A	Plantaquat® NC	Cetearyl Alcohol, Lecithin, Sodium Cetearyl Sulfate, Olus Oil [EU], Cetearyl Alcohol, Lecithin, Sodium Cetearyl Sulfate, Vegetable Oil [CTFA]	3.00	Consistency agent
	Cutina® HVG	Hydrogenated Vegetable Glycerides	2.50	Consistency agent
	Cetiol® RLF	Caprylyl Caprylate/ Caprate	6.50	Emollient
	Cetiol® CC	Dicaprylyl Carbonate	4.00	Emollient
B	Water, demin.	Aqua	61.35	
	1,3-Butanediol	Butylene Glycol	10.00	Emollient
	Sodium Benzoate	Sodium Benzoate	0.25	Preservative
C	Glycerin	Glycerin	5.00	Humectant
	Verdessence™ Xanthan	Xanthan Gum	1.00	Rheology modifier
D	Dermosoft 1388 *(Evonik)*	Glycerin, Aqua, Sodium Levulinate, Sodium Anisate	1.00	Auxiliary
E	Water, demin.	Aqua	4.00	
	Postbiolift™BC10152	Maltodextrin, Lactobacillus Ferment	1.00	Active ingredient
F	Citric Acid (20% solution)	Citric Acid	0.40	pH Adjustment

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