Formulating “Clean Beauty” cosmetics with natural origin ingredients

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Introduction:

The skin, like the other organs, is subject to a complex physiological process of aging[1, 2]. Intrinsic or chronological aging is the consequence of a genetically programmed senescence and of biochemical alterations due to endogenous factors. The aging process is characterized by a slow-down in the regeneration of cells and extracellular matrices, a gradually loss of dermal collagen and elastic fibers resulting in dermal and epidermal atrophy, dryness, a reduction in elasticity and firmness of the skin, the appearance of fine lines and wrinkles hyperpigmentation or hypopigmentation blemishes. Extrinsic aging, on the other hand, is due to environmental attack such as pollution, sun light irradiation (including UV radiation) and diseases. This is superimposed on intrinsic aging at zones which are chronically exposed to these attacks, such as photo-aging. Most consumers are concerned with the characteristics of their skin aging, like wrinkles and loss of firmness. Dry skin is a very common skin condition characterized by a lack of the appropriate amount of water in the most superficial layer of the skin. It may occurs depending on hereditary, acquired or environmental factors regardless aging [3].

The “Clean Beauty” claims are spreading across beauty categories in recent years. Although there is no consistent definition on “Clean Beauty”, most attributed meanings are around products with non-toxic, cruelty-free, hypoallergenic, and free-of claims. To fit current trend on “Clean Beauty”, a cosmetic formula was developed, deriving both sensorially-appealing, skin hydration and anti-aging efficacy, with a proprietary natural ingredient blend.

The research data showed the lotions prepared could increase skin collagens expression in ex-vivo human skin biopsy experiments, and superior skin moisturization and anti-aging benefits in clinical studies. Besides, it also provides customizable sensory experience and superb smoothness to skin care products.

Materials & Methods:

Immunostaining evaluation on ex vivo skin
Skin biopsies were obtained with a 6 mm diameter punch (pfm medical) from a plastic surgery intervention. They were cultivated on culture medium containing DMEM 1 g/L glucose (Lonza) and Ham’s-F12 (Lonza) (1:1), supplemented with 10% of FBS (Gibco), 2 mM of L-glutamine (Lonza) and 100 µg/ml of Primocin* (InvivoGen). Skin biopsies were treated, in duplicate, with PBS (Lonza) in control condition or with the blend at 1%, twice a day, for 48 hours. 20 µl of solutions were applied on the top of the biopsies.

Clinical studies
Skin hydration and TEWL (transepidermal water loss)
A 6-hour double-blind comparative study on the“Clean Beauty” product was carried out on 24 Asian subjects, both male and female. The subjects have dry skins and the hydration value on the volar side of forearm is below 40. Test zones are treated with test products (5% blend and placebo) as per randomization and at a dose of 2mg/cm^2. Test time points is T0, T1, T6 (hours). Skin hydration value was measured by Corneometer CM825 (Courage + Khazaka), skin trans-epidermal water loss (TEWL) value with Tewameter TM300 (Courage + Khazaka). Student’s T test (one-tailed) or Wilcoxon signed rank test (one-tailed) were used depending on whether the data were normally distributed.

Skin wrinkle reduction & firmness improvement
The studies were randomized, double-blind and split-face test on 30 Asian subjects, 35-59 years old, both male and female, for 28 days. All subjects have fine wrinkles skin, sagging, and lack of elasticity. The cream was applied on face at 2mg/cm2 by themselves three times a day, in the morning, noon and at night respectively. The volunteers visited the lab three times for testing: at D0, D7 and D28. Skin wrinkles are measured by VISIA-CR. Skin Elasticity and firmness are measured by Cutometer(Courage&Khazaka) [4]. Student’s T test (one-tailed) or Wilcoxon signed rank test (one-tailed) were used depending on whether the data were normally distributed.

Skin sensory evaluation
Double blind randomized contrast tests were carried out by 20 sensory evaluation experts on the back of the hand. The circles with a diameter of 5cm were the evaluation area. The sample amount of the left and right sides was 50 uL. The skin texture of formula was evaluated for spreadability, hydration feeling and fresh feeling during application (rub-in for 15 cycles), slipping feeling (0 minute) and glossiness after application (0, 5 minutes). Sensory Attributes & Scales are shown in Table 1.

Phases	Sensory Attributes	Scales
Rub out Evaluation (after 15 circles)	Spreadability	0 (hard) - 100 (easy)
	Hydration Feel	0 (none) – 100 (great)
	Fresh feel	0 (greasy) – 100 (fresh)
After Feel (after 0&5 min)	0min Gloss	0 (dark) -100 (bright)
	0min Slipperiness	0 (none) – 100 (great)
	5min Gloss	0 (dark) -100 (bright)

Table.1. Sensory Attributes & Scales

Results & Discussion:

Immunostaining evaluation on ex vivo skin
There are 4 types of collagen in the skin: collagen I is the most abundant collagen in the dermis as fibrillar collagen, provides skin strength & integrity; collagen III is always found together with type I collagen, and related with skin regeneration and dermis repair; collagen IV forms a network structure to connect the dermis and epidermis and makes DEJ structure stable; while collagen XVII is a transmembrane collagen, which mediates adhesion of keratinocytes to the underlying DEJ membrane[5]. The level of collagen IV were analyzed by immunostaining (Volocity* image analysis) in ex vivo human skin biopsy model, treated with/without 2% blend for 16 hours. On analyzation of the level of collagen XVII, the skin biopsy model was treated with/without 1% blend for 24 hours. As shown in Fig.1, the active treated ex vivo skin showed better green immunostaining, which represents increased expression of collagen IV & XVII in DEJ (dermal-epidermal junction) structure.

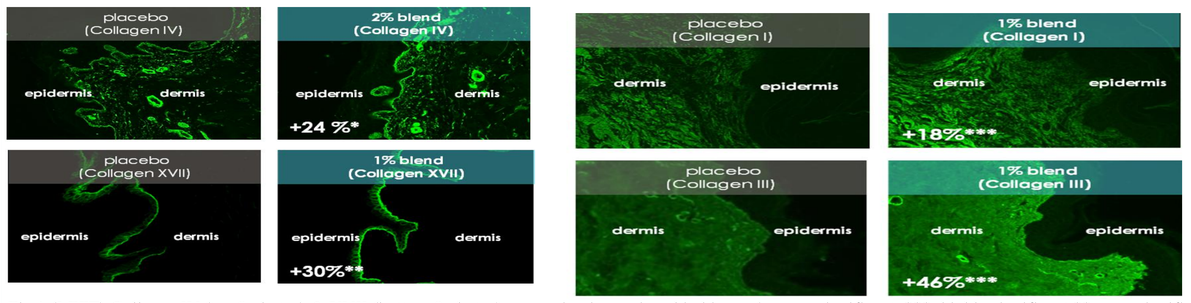

Fig.1 (LEFT) Collagen IV (top 2 photos) & XVII (bottom 2 photos) expression in ex vivo skin biopsy (ns: non significant, ***: highly significant,**: very significant, *: significant with Student’s t-test) Fig.2 (RIGHT) Collagen I (top 2 photos) & III (bottom 2 photos) expression in ex vivo skin biopsy

Clinical studies
Skin hydration and TEWL (transepidermal water loss)

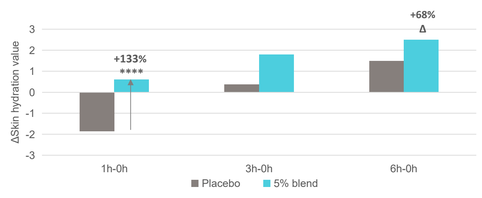

Fig.3 Skin hydration evaluation results

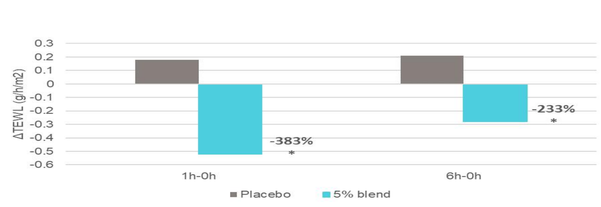

Fig.4 TEWL evaluation results

As shown in Fig.3, the formula with 5% blend significantly increased skin water content by more than 133% and 68% at 1 hour and 6 hours after application, with high and directional significance. Fig.4 showed that the formula with 5% blend reduced the transepidermal water loss (TEWL) and repaired the skin barrier function compared to the placebo.

Skin wrinkle reduction & firmness improvement evaluation

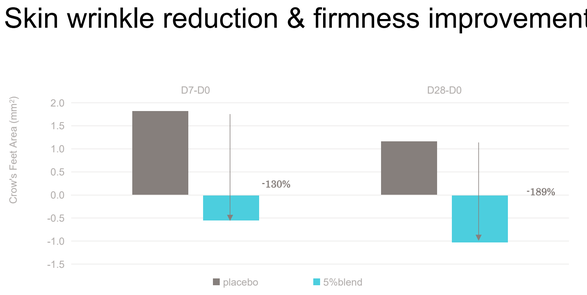

Fig.5 Cow’s feet reduction evaluation

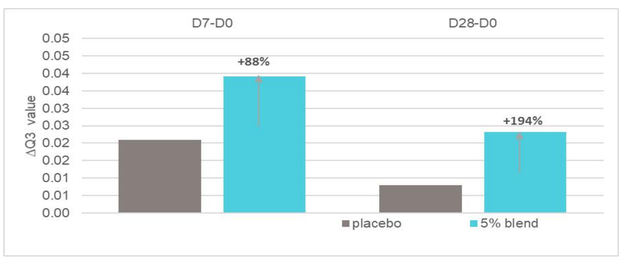

Fig.6 Skin firmness improvement evaluationSkin sensory evaluation

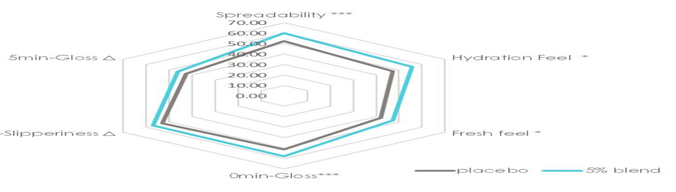

Fig.7 Skin sensory evaluation results

(NS: Not significant, p>0.1, △: Directional, 0.05<p<0.1, *: Significant, p<0.05, **: Very significant, p<0.01, *: Highly significant, p<0.005, ****: Extremely significant, p<0.001)**

Conclusions:

In conclusion, in order to fit the current “Clean Beauty” trend, we have studied a cosmetic formula with proprietary natural ingredients through conducting three clinical experiments, from skin hydration and transepidermal water loss and skin wrinkle reduction & firmness improvement evaluation to sensory evaluation. We can clearly conclude from those results that the formula we have developed has a promising prospect in the cosmetic field in the near future.

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