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Potential preventive performance

It is not easy to measure the preventive efficacy of skin care products, but Claire Mas-Chamberlin, Philippe Mondon, François Lamy, Karl Lintner, Claire Jossan and Frédérique Girard report on an accelerated skin ageing-type process used to investigate active efficacy

Many health care providers and specialists agree that preventive medicine is preferable to curative medical care; in the long run it is more efficacious, more cost effective and often much less painful. But the message is difficult to convey successfully to the general population. Much the same is true for the cosmetic skin care field, and for the way products in this market are promoted. Protecting the skin against excessive sunlight (UVA and UVB), against chemical and environmental aggression (tobacco smoke, detergents, pollutants etc) from an early age, would be so much more beneficial to the beauty of the skin than to hope later, when the damage is done, for miracles in a jar, a laser probe or a scalpel.[1]

But prevention is a difficult sell. The use of sunscreens is certainly increasing as a consequence of great efforts in teaching consumers about the dangers (cancer) and the deleterious effects (wrinkles) of exposure to the sun on the beach or on the slopes. While this awakening of the public conscience in this particular application is a good thing, it is far from enough, as several authors confirm.[2, 3]

Pernicious UVA radiation in particular, for a long time considered harmless, seems to be the cause of slowly causing what is for a long time invisible deterioration of the skin tissue. And UVA exposure accumulates and leads to more or less irreversible cascade effects that only much later in life become evident to the consumer.[4]

Although the skin has its own defence systems against these aggressions they are inadequate to cope with the stresses of our modern lifestyles. A simple example illustrates the problem: in the upper layers of the epidermis, including the stratum corneum, the skin harbours detoxifying enzymes such as superoxide-dismutase (SOD), catalase and glutathione-peroxidase.[5] These enzymes, acting in cascade fashion to eliminate superoxide anions and hydrogen peroxide, are there to protect the skin against some of the aggressive free radicals generated mostly by UVA irradiation. It turns out, however that these same enzymes are heat and UV sensitive, ie they are not stable under conditions of sun exposure. Maes and colleagues[6] have demonstrated and quantified the U-shaped curve of cutaneous catalase activity between the months of January and December; in summer, when we need protection most, almost no catalase activity can be detected in the skin! Talk about a paradox... unless we take into account the fact that biological and cultural evolution of humankind have not gone hand in hand.

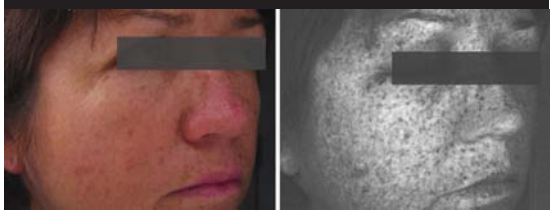
Prevention is better than cure but educating consumers on the benefits is a hard task
Aveda

The consequence of this discrepancy is premature skin ageing: drying out, thinning, loss of elasticity and radiance, changes in melanisation.[7] To prevent this, consumer education is necessary, beyond the standard admonition of staying out of strong sunlight, of using sunscreens and protective clothing. Indeed every day the exposed skin experiences UVA exposure, every

day it needs supplemental protection against these influences, thus age 'prevention' rather than 'treatment'.

Yet how can we convey this message to the consumer if we do not have convincing evidence - visual if possible - that prevention can provide benefits that do not need 20 years to become perceivable? Certainly a number of papers describe investigations of the differences in skin appearance and condition between old and young, between exposed (hands, face) and unexposed (lower back) sites.[8,9] But none to our knowledge show how quickly, and measurably, the unprotected skin ages.

Figure 1 - 'Sun spots', only visible under 'Wood' light; indicative of UV induced skin damages (right)



The study presented here attempts to show that there are ways to address this problem.

The starting point was the idea of an analogy. Cosmetic formulations that are expected to be stable for at least 30 months on-shelf have rarely undergone 30 months of ageing studies. Rather the formulator employs techniques of accelerated ageing (eg freeze-thaw cycles, storage at elevated temperatures for shorter times, centrifugation etc) to extrapolate stability over longer periods of time. As it is economically and logistically very difficult to do studies over very long periods with defined protocols, controlled conditions of cosmetic usage and confined to a single product (compliance would be the major problem),[10] a strategy of accelerated ageing of the skin was used in order to demonstrate what happens on unprotected skin as compared to skin that uses preventive/protective ingredients (rather than post-damage treatment actives).

The trick employed was simple: carry out a six month study under tropical conditions (the island of Mauritius), on two panels (one using a placebo cream containing the active's excipient only, the other using the same vehicle with protective enzymes incorporated) of volunteers of phototype I-III that had recently (<6 months) arrived on the island. Their skin, not used to almost permanent sunlight, thus underwent accelerated ageing. The purpose of the experiment was to see the differences between the panels under these conditions.

Test protocol

Two skin care creams (formulas in table 1 - SED0503519I and SED0503519J) were prepared and coded. SED0503519I cream contained 5% Venuceane, an enzyme cocktail (Extremozymes) obtained from the fermentation process of *Thermus thermophilus*, extremophile bacteria harvested in the vicinity of hydrothermal vents in the Pacific Ocean. This ferment possesses enzymatic activity that mimics the one found in the stratum corneum,[11] ie SOD-like, catalase-like and glutathion peroxidase-like detoxifying properties. However, these enzymes are highly UV and heat stable, in contrast to the natural (or otherwise biotechnologically derived) similar enzymes.[12] The other cream was the placebo.

Two groups of 25 volunteers each were chosen (aged 30-50 years), of phototype I-III, recently (<6 months) arrived on the island of Mauritius. Usual exclusion criteria applied.

Measurements and skin evaluation were carried out on day 1 (August 2005) and on day ~180 (February 2006), with certain parameters also evaluated after 4 weeks and 12 weeks. The creams were randomly assigned to the two groups; they were to be applied twice a day on face and volar forearm. Although panellists were advised not to use other skin care products on the face during the study period, they were not induced to change any other habit or parameter of lifestyle. In particular panellists could go to the beach, use sunscreens to their liking and otherwise engage in their usual activities, the aim of the protocol being to approach real life conditions with little constraints. End-points included the measurement of TEWL using a Tewameter (Courage&Khazaka), skin relief quantification by Laser Profilometry, electron microscopy analysis of stratum corneum strippings (on 2x10 panellists) and dermatological evaluation of skin hydration, wrinkles, skin texture, solar spots by clinical scoring and on photographs obtained with the Visiasystem. Questionnaires were given to the volunteers to evaluate organoleptic, but also efficacy criteria of the creams.

Results & discussion

TEWL - Table 2 shows that the TEWL values on the placebo treated panel increase with time, the increase being small but significant (p=0.058). This result in itself merits attention. Although it is possible to measure certain skin damage parameter changes after even shorter periods of UV exposure (lipoperoxydation,[11] carbonylation of proteins, mitochondrial DNA mutations), we cannot expect to see dramatic macroscopic skin deterioration in six months, even in a tropical climate and under normal lifestyle conditions. It is thus remarkable to observe a measurable, significant change in a skin condition that is usually associated with reaction to severe macroscopic stress (chemical aggression, inflammation, stripping) and/or decade long-term ageing.

Satisfyingly, the panel using the cream containing Venuceane does not follow the same pattern as

Table 1 - Formulations for SED0503519I and SED0503519J

Ingredients	% SED0503519I	%SED0503519J
Phase A		
Cetearyl ethylhexanoate (Crodamol CAP, CRODA)	5.00	5.00
Cetearyl alcohol, dicetyl phosphate, ceteth 10 phosphate (Crodafos CES, CRODA)	4.00	4.00
Sorbitan stearate (Crill 3, Croda)	0.50	0.50
C12-C15 Alkyl benzoate (Crodamol AB, CRODA)	2.00	2.00
Caprylic/capric triglyceride (Crodamol GTCC, CRODA)	4.00	4.00
Phase B		
Potassium sorbate	0.20	0.20
H_2O	qs to 100	qs to 100
Phase C		
Glycerin	3.00	3.00
Methylparaben, ethylparaben, propylparaben	0.40	0.40
Phase D		
Sodium hydroxyde 30 %	0.20	0.20
H_2O	2.00	2.00
Phase E		
Thermus thermophilus ferment, Glycerin (Venuceane, Sederma)	5.00	
H_2O		5.00

Table 2 - TEWL variations compared to the initial state

Tested product	Kinetics	Variations in g.m^{-2}.h^{-1}(mean ± SEM)	Variations in % (mean)	Significance
SED0503519I				
(verum)	W4/W0	-1.0 ± 0.7	-6%	No (p=0.192)
	W12/W0	+0.8 ± 0.9	+5%	No (p=0.391)
	W24/W0	+0.2 ± 0.9	+1%	No (p=0.855)
Tested product	**Kinetics**	**Variations in g.m^{-2}.h^{-1}(mean ± SEM)**	**Variations in % (mean)**	**Significance**
SED0503519J				
(placebo)	W4/W0	+0.9 ± 0.8	+7%	No (p=0.258)
	W12/W0	+1.3 ± 0.7	+10%	No (p=0.081)
	W24/W0	+1.4 ± 0.7	+11%	Limit (p=0.057)

TEWL does not show notable change over the same period. A clear protective effect can thus be attributed to the active ingredient.

DSQUAM strips - This first observation is corroborated by the stripping experiment. From 10 randomly chosen volunteers in each panel, tape-strips (Dsquam) of the stratum corneum of the volar forearm were obtained on day 1 and on day ~180. These were then fixated and prepared for SEM analysis. Figure 2 illustrates this. From an initial loose and dry cutaneous surface, the treatment with Venuceane leads to a compact, defensive and intact stratum corneum after 24 weeks, whereas no such improvement can be observed in the placebo treated panel. The barrier protection (and repair) effect certainly contributes to the better water retention capacity of the treated skin (table 2).

The compactness of the cutaneous barrier is clearly evident from representative pictures in figure 2; initially, there was no observed difference between the two groups of panellists.

While a number of other skin parameters (skin surface relief, texture, sun spots) undergo further analysis (to be published), it is interesting to note that the subjective evaluation by the volunteers also indicates a difference in the efficacy of the two creams.

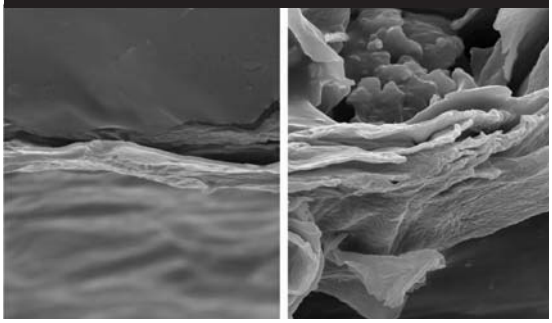
The data in table 3, obtained from panellists who had no information on the products, show a clear advantage for the Venuceane containing protective cream in all six criteria. Next to visual proofs of activity, consumer perceived benefits of cosmetic products are surely the most important arguments for promoting the idea of preventive cosmetic anti-ageing concepts.

The clinical study presented here can only be the beginning of more detailed investigations. It is now clear that preventive/protective cosmetic activity on human volunteers can be demonstrated in relatively short (six months) trial periods with macroscopic parameters and end-points, which are accessible and comprehensible to the consumer. Further experiments under similar conditions, with further refined measurement techniques, lifestyle controls and larger panels, appear useful and desirable and these will be reported in due course.

Table 3 - Panellists self-evaluation

Product efficacy	SED0503519I (verum)			SED0503519J (placebo)		
	W4	W12	W24	W4	W12	W24
Global improvement of the state and the aspect of the skin	54%	81%	81%	61%	61%	74%
More precisely moisturised skin	46%	69%	81%	57%	61%	65%
Smooth skin	50%	81%	81%	52%	48%	70%
Comfortable skin	54%	73%	77%	43%	57%	65%
Nourished skin	50%	69%	73%	39%	57%	61%
Skin more beautiful	62%	69%	62%	43%	52%	61%

Figure 2 - DSQUAM strips after 24 weeks of treatment with Venuceane (left) or placebo vehicle (right)



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