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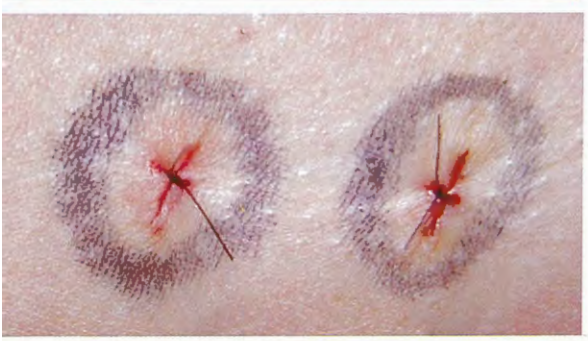

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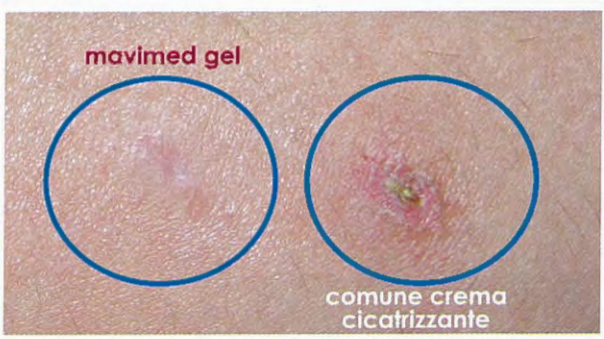

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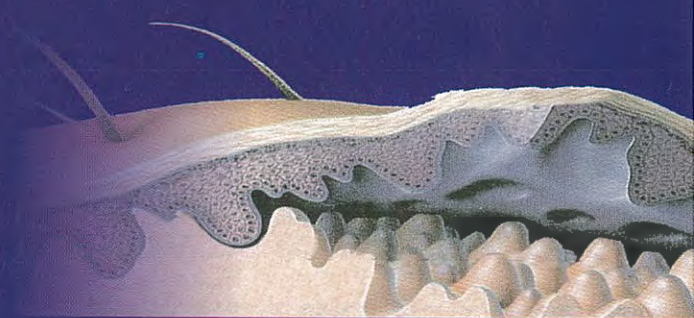

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- **Morganti P. (1998)** Acne therapy : the cosmetic approach. *Soap Perf. & Cosm.*, August 1998, p. 23 - **Morganti P. (1999)** Phospholipid *focus SPC Asia* **18**: 16-18 - **Morganti P., Morganti G. (2000)** The Acne onset at puberty : the need for innovative cosmeceuticals. *Eurocosmetics*, **3**: 27-28 - **Morganti P., (2000),** I Fosfolipidi nella terapia dell'acne, *Cosm. News*, **XXIV (137/01)**, pp.89-92 - **Morganti P, Guarneri F, Morganti G, (2001),** Botanicals in Acne Therapy, *Eurocosmetics*, **9** (n.6), 24-2 - **Morganti P. Fabrizi G. Bruno C. James B. (2002)** A new approach to

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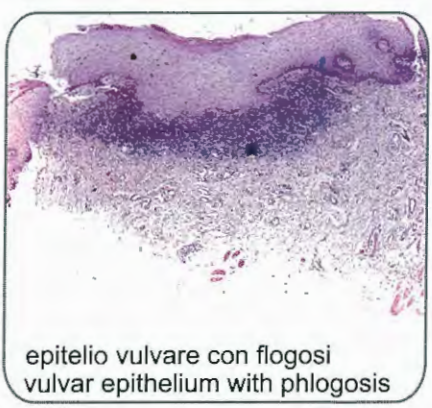

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- **Jarrett A. (1986)** Ageing of the Mucous Membranes, *Cosmetic Dermatology*, Vol. 2, Ed. by P. Morganti, F.J.G. Ebling, Rome - **- Bruno I, Fischetti C, Inghirami P, Senatori R, Bacicalupi A. (1991)** CO2 laser surgery of the lower genital tract in women: ults of post operative treatment with vitamin A+E gelatin mixture *J. Appl. Cosmetol.*, **9**, 73-76 - **3) - Morganti P, Lanzone A, eri L. (1998)** A new diffusion system through the mucous membranes, skin and hair, *J. Appl. Cosmetol.*, **16**, 45-50 - **4) - enzano Ferraris AM, Morganti P. (1999)** Essential fatty acids for the epidermal barrier homeostasis : stability and safety., *C & Worldwide*, **8**, 32-34 - **5) - Cornelli U. (2000)** Fluid diffusion System in the treatment of aging mucous membranes, In: New nds in Cosmetic Science, Conference Proceedings, Verlag, H. Ziolkowsky GmbH, pp. 44-50 - **6) - Beyer N, Driller H, Bünger (2000)** Ectoin an innovative, multifunctional active substance for the cosmetic industry, *SOFW- Journal*, **126**, 26-29 - **7) - glana F, Dionisi B, Lippa P, Ronca S. (2003)** Fisiologia e Omeostasi del distretto vulvo-vestibolo-vaginale, In *Trattato di ologia Vulvare*, vol. I, pag.47-60, SEE- Firenze

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"IN VITRO" DETERMINATION OF INTEGRATED AND ERITEMATIC SOLAR PROTECTION FACTOR PREPARATIONS

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Key words: *Photoprotection; SPF; Polymers;*

Summary

"*In vitro*" Solar Protection Factor (SPF) is determined by measuring the intensity of radiation penetration through a layer of photoprotector formulations prepared with different solar filters (Eusolex® 232, Eusolex® 6300 and Neo Heliopan" AV) of a different chemical nature, hydrosoluble and liposoluble, solid and liquid, with a common silicon vehicle and different concentrations of polymers (aquacoat", kollicoat", kollidon" and aquateric"), were prepared in order to test the influence that such polymers exerts upon SPF. SPF values for Eusolex® 232 are generally lower than those presented by others filters. This is also consistent with the values obtained for these substances on integrated protection factor, in which no significant differences with regard to type of polymer used or its concentration were found. Consequently, we believe that these factors will not have any significant influence on erythematic protection factor.

Riassunto

È stato determinato *in vitro* il fattore di Protezione Solare (SPF) di diverse formulazioni che basate su un unico veicolo siliconico, utilizzavano filtri di diversa natura chimica sia idro che liposolubili, allo stato solido o liquido, associati con polimeri a diversa concentrazione (aquacoat, kollicoat, kollidon e aquateric).

Si è voluta controllare l'influenza esercitata da questi polimeri sull'SPF determinato. In realtà l'SPF

ottenuto con l'uso dell'Eusolex® 232 è risultato sempre più basso quando si utilizzavano tali polimeri, e comunque, il loro uso non ha mai dato luogo ad una riduzione significativa dell'indice eritemigeno ottenuto.

INTRODUCTION

Skin possesses natural photoprotection against actinic aggression. Hair, the thickness of the skin's corneal layer, urocanic acid from sweat, superficial cutaneous lipids, such as carotenoids and above all melanin (1, 2), may absorb or reflect incident solar radiation. All of these induced modifications are brought about by solar radiation at molecular level leading to major alterations in different cutaneous components (3, 4).

These alterations depend on three fundamental factors: The nature of the radiation (wavelength), length of exposure times and the personal characteristics of the individual. Minimum Erythematogenic Dose (MED) is a term used to describe the potential erythematogenic reaction from UV radiation, and is defined as the effective dosage of UV radiation required to produce such an effect in human skin that has not been subjected to previous exposure. However, because of the different degrees of natural protection present in different skin types, different subjects present varying degrees of sensitivity to identical levels of UV radiation, and MED is therefore, a variable factor in any given population (5-7).

The treatment of sun-induced dermatitis involves both its treatment, as well as the appropriate preventative measures taken to avoid the harmful radiation on skin or the use of photoprotection. In general, the last of these measures consists of the application of a substance to act as a barrier between the skin and radiation source, which either absorbs (filters) or reflects (screens) such radiation (8). Substances that protect skin may be either of a natural source, such as melanin, carotenes, or corneal layer, etc, (natural photoprotection) or foreign to the organism (artificial photoprotection) (9).

Solar protection Factor (SPF), is the scale used to assess the degree of protection afforded by any particular type of photoprotective media against immediate erythema (observed after 20±4 hours of exposure). A SPF (10) is a dimensionless number obtained from laboratory measurements and its value is determined in terms of the proportion of UVB radiation that a determined product is capable of filtering. Although SPF is usually applied to topical sun protectors, similar indexes have also been used for clothing materials or optical lenses.

SPF can be determined "*in vitro*" by measuring the intensity of the radiation that penetrates a layer of material in relation to the intensity to which it is exposed (a 2% penetration corresponds to a SPF of 50 (100%/2%). SPF is an integrated value for the entire spectral interval under consideration. Consequently, in order to determine its value, it is necessary to compare the spectrum of incident UVB radiation with the spectrum of erythematic reaction (11).

Solar photoprotectors are widely used to protect skin from the harmful effects of ultraviolet rays. Not only do they prevent sunburn, but they also prevent photocarcinogenesis and photoageing. Sun protection creams may act either physically or chemically. Physical barriers act as mechanical screens, which avoid skin penetration through reflection or dispersion. Chemically inert substances are used, consisting of finely ground opaque powders of optimal granule size, to ensure a high degree of surface dispersion. Among the substances used are: Zinc Oxide (ZnO) and Titanium (TiO_2), Talc and Kaolin (12). Chemical protectors are those that absorb a specific area of the spectrum and are therefore called "sun filters". These are made up of chromophoric chemical substances (unsaturated molecules, rich in conjugated double bonds), which selectively absorb radiation of a determined wavelength and prevent its absorption by the skin. Furthermore, the substance subsequently releases energy, without inducing the formation of harmful free radicals (13). One of the pro-

blems associated with the use of solar filters as photoprotectors, is that these penetrate the skin causing irritation. This undesirable effect can be minimised or even completely neutralised using nanoparticles.

The increase in cases of skin cancer in recent years has brought about the need for effective protection systems, as a means to minimising the harmful effects of the sun. The objective of this study was to determine erythematic SPF and to test different sun protection formulations, made up of micro- and nano-particles of different polymers, as transport systems for chemical filters, in order to study the differences between the different polymers used and their influence on photoprotection. The Protection Factor of the different formulae was determined *in vitro*, according to two different methods. The first of these was carried out by measuring the spectral radiation from each of the formulations, in order to study the influence exerted by the vehicle and to obtain an integrated SPF value. In the second method, solution spectrometry (14) was used, in which photoprotector solutions in a suitable organic solvent were prepared, and the transmittance or absorbance in terms of wavelength were measured, in order to obtain an erythematic SPF. This technique is used by the SAA (Standard Association of Australia).

INVESTIGATIONS, RESULTS AND DISCUSSION

Determination of Integrated Solar Protection Factor

In vitro solar protection factor was determined by measuring the intensity of the radiation that penetrates a layer of different photoprotective formulations prepared from three solar filters, Eusolex® 232, Eusolex® 6300 and Neo Heliopan® AV, a common silicone vehicle at different concentrations (10 and 25%) of the polymer dispersions Aquacoat®, Kollicoat®, Aquateric® and Kollidón® respectively. Analyses were carried out to ascertain the extent to which these dispersions exert an influence on protection factor and the results were expressed in relation to the intensity of the radiation to which it is exposed. Figure 1 shows the emissions curve of the UVA lamp used.

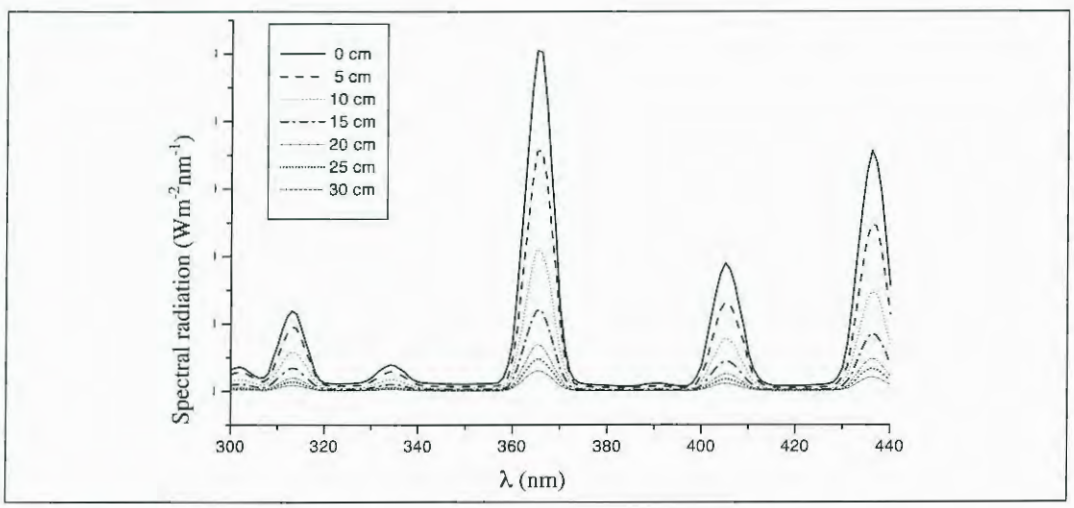

Fig. 1 Emissions curve of the UVA lamp used.

The curve was obtained through extrapolation of the measurements carried out a different distances from the source (0 to 30cm).

For the remaining measurements, the lamp was situated at 15cm from the spectroradiometer.

Spectral radiation measurements were carried out on each of the formulations. Before each measurement was taken, spectral radiation absorbed by the surgical tape used as carrier was calculated, so as to be able to take into account the following factors: the small fluctuations produced by the lamp through out the length of time it was in use, light variations occurring in the room at different times of day, the small imperfections that may be present in the fragments of tape to be used, etc.

Radiation measurements were carried out at wavelength intervals of between 300-440 nm and the results are shown in figures 2 to 7. Transmisivity values for any one given solar filter can be seen to vary in accordance with polymer dispersions used and their concentrations within the photoprotective formulation. This same result can be seen in subsequent values obtained for integrated protection factor.

In the determination of protection factors (Table I) a weighted integration was used with respect to the spectrum of action of the reaction to be considered in the expression:

$$FPS = \frac{\int_{250}^{390} I_\lambda \, \varepsilon_\lambda \, d_\lambda}{\int_{250}^{390} I_\lambda \, \varepsilon_\lambda \, T_\lambda \, d_\lambda}$$

In this expression, protection factor is represented by the factor of decrease in erythematic effect after radiation penetration through the transmissible material T.

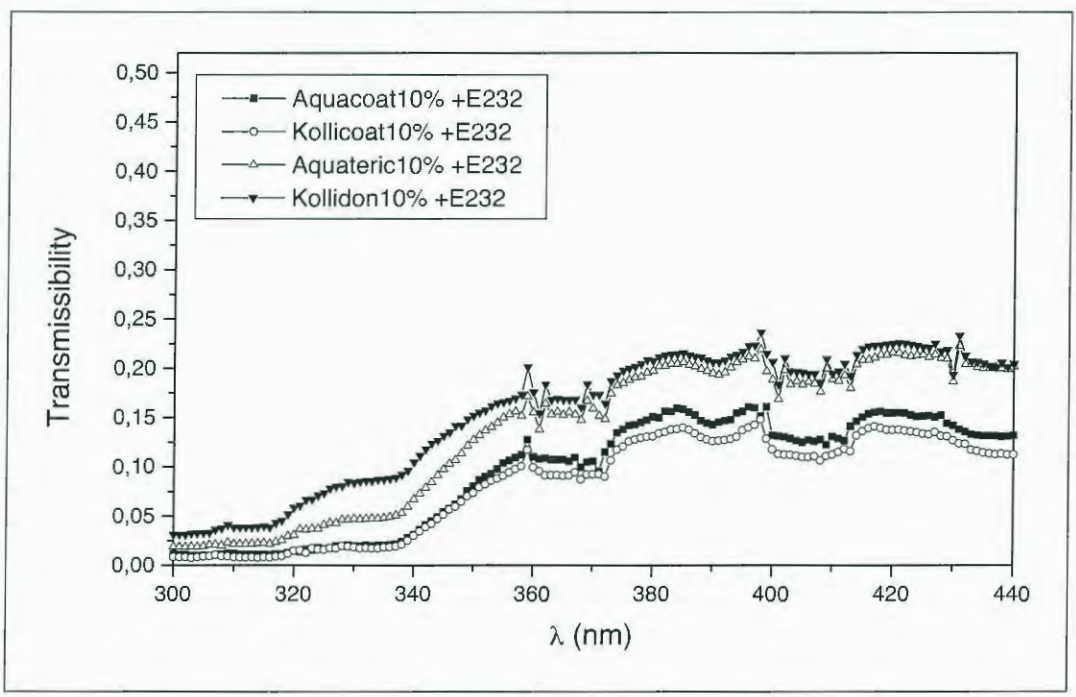

Fig. 2 Transmisivity values at wavelength intervals of between 300 - 440 nm for complexes polymer (10%) with Eusolex® 232.

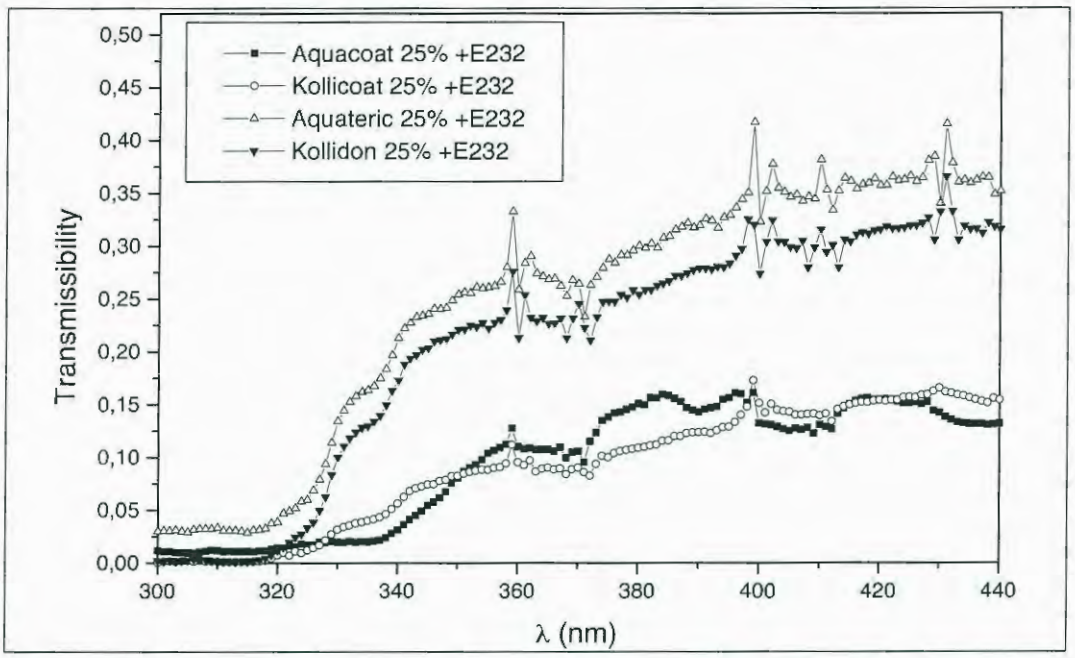

Fig. 3 Transmisivity values at wavelength intervals of between 300 - 440 nm for complexes polymer (25%) with Eusolex® 232.

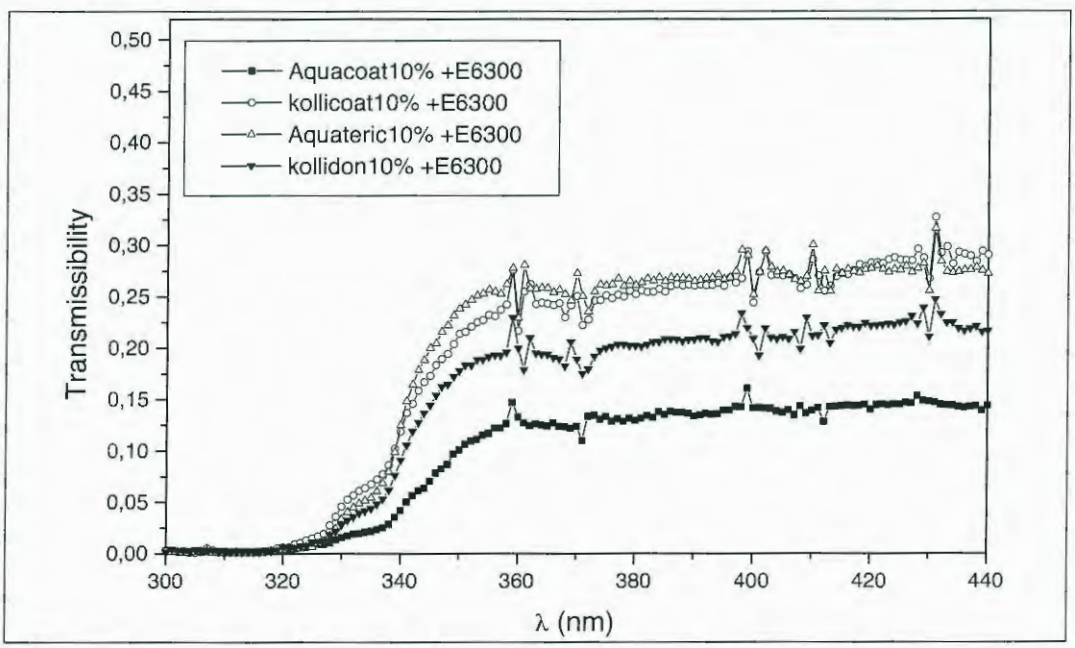

Fig. 4 Transmisivity values at wavelength intervals of between 300 - 440 nm for complexes polymer (10%) with Eusolex® 6300.

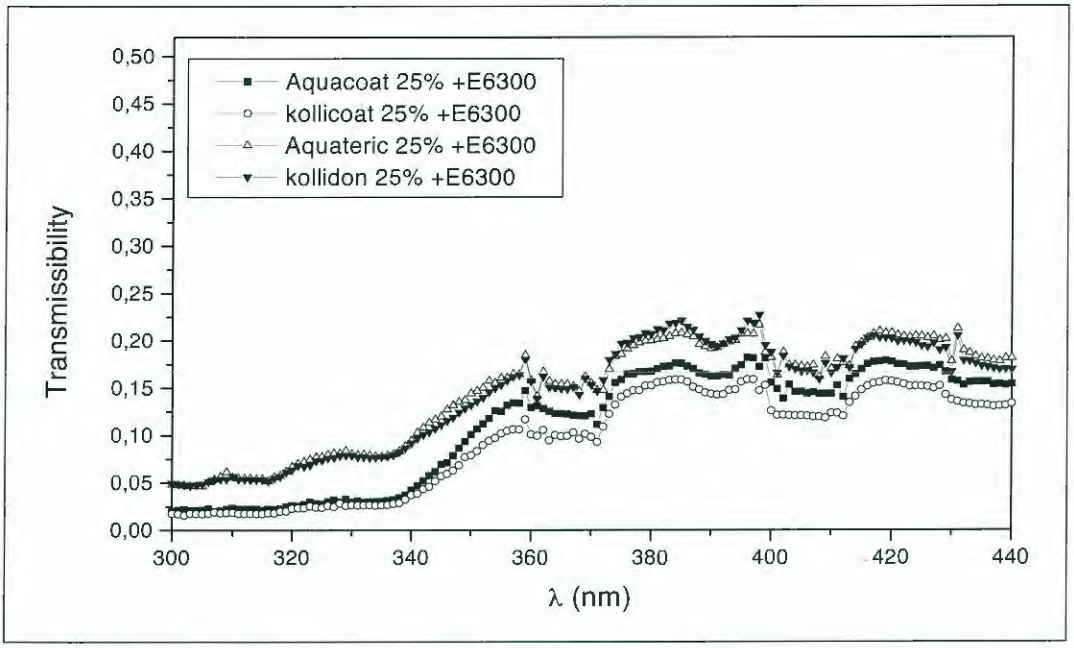

Fig. 5 Transmisivity values at wavelength intervals of between 300 - 440 nm for complexes polymer (25%) with Eusolex® 6300.

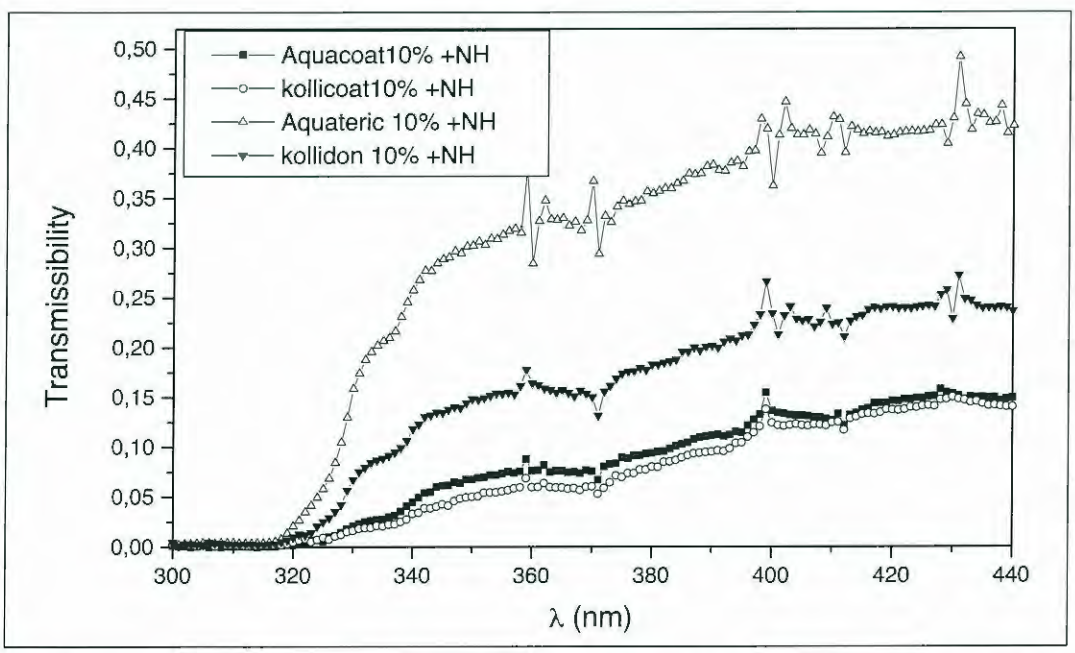

Fig. 6 Transmisivity values at wavelength intervals of between 300 - 440 nm for complexes polymer (10%) with Neo-Heliopan® AV.

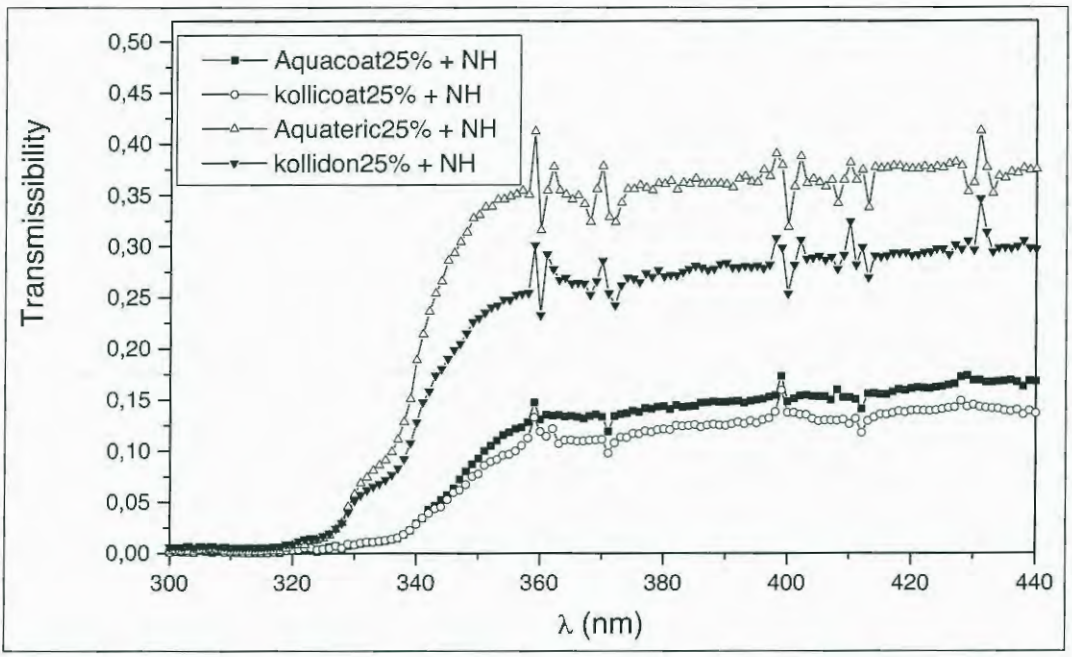

Fig. 7 Transmisivity values at wavelength intervals of between 300 - 440 nm for complexes polymer (25%) with Neo Heliopan® AV.

TABLE I

Integrated Protection factors respect to the spectrum of action of the radiation solar, for different formulations.

	EUSOLEX® 232	EUSOLEX® 6300	NEO HELIOPAN® AV
AQUACOAT® 10%	71	363	162
AQUACOAT® 25%	40	314	182
KOLLICOAT® 10%	88	212	97
KOLLICOAT® 25%	49	339	189
AQUATERIC® 10%	25	25	97
AQUATERIC® 25%	18	64	69
KOLLIDÓN® 10%	40	94	108
KOLLIDÓN® 25%	18	117	64

I_λ represents spectral radiation within the range considered, T_λ is the spectral transmisivity of the solar preparation used and E_λ is the normalised spectrum action of the reaction to be considered against radiation I_λ, which is calculated from the following expressions, in accordance with wavelength value:

$$\varepsilon_\lambda = 1.0 \qquad \text{for } 250 < \lambda < 298 \text{ nm}$$

$$\varepsilon_\lambda = 10^{0.094\,(298 - \lambda)} \qquad \text{for } 298 < \lambda < 328 \text{ nm}$$

$$\varepsilon_\lambda = 10^{0.015\,(139 - \lambda)} \qquad \text{for } 328 < \lambda < 390 \text{ nm}$$

It can be observed from these expressions that the "*in vitro*" determination of SPF varies in accordance with the polymer used and its level of concentration within the photoprotective formulation, given that the different values obtained for the same solar filter are dependant on the components of the formulation.

The higher the SPF value obtained, the lower is the percentage of radiation penetration into the skin. If the results obtained for each active-cosmetic are analysed, it can be seen that independently of the polymer used, values for solar protection factor are highest for Neo Heliopan AV, followed by Eusolex® 6300 and lastly Eusolex® 232.

A possible explanation for this could be that Neo Heliopan® AV, has a structure derived from cinnamic acid in which a 2-ethyl-hexil substitutes the hydrogen of the hydroxyl group of the carboxyl and a methoxy group in position. This would facilitate radiation absorption within the interval of wavelengths studied to a greater degree than that derived from alcanfor (Eusolex® 6300), which showed an almost 100% absorption between 280 and 310nm, 95% at 320nm, and 50% at 330nm, but very low absorption values for the remaining wavelength intervals.

The composition of the Eusolex® 232 filter is made up of a soluble salt from 2-phenyl benzimidazol-5-sulphonic acid, which permits greater Irradiation penetration within the interval of wavelengths studied (330-440nm). This salt presents high absorption in the UVB region but diminishing absorption in UVA. Maximum absorption is obtained at a wavelength of 302nm, which is why values for the intervals studied are lower.

With regard to the presence of the polymer, in general it can be seen that the preparations containing Aquateric® present the lowest degrees of protection, followed by Kollidon®. This may be attributable to the consistency of these preparations, which are more fluid than those containing Aquacoat® or Kollicoat®, and may allow a greater degree of radiation penetration to occur.

Different combinations of polymers were observed to have little influence on integrated SPF, given that only in isolated cases was an increase in protection was observed, as in the case for example of Kollicoat®, Kollidón® and Aquateric® with Eusolex® 6300, or Aquacoat® and Kollicoat® with Neo Heliopan® AV. However, no such effect was observed in any other combination. We believe that the concentration of the polymer in the formulation does not have a significant influence on integrated solar protection factor.

In conclusion, the formulation that afforded highest protection was that containing Eusolex® 6300-Aquacoat® 10% (fig. 3), and that giving lowest protection was Eusolex® 232-Aquateric® 25% and Eusolex® 232-Kollidón 25% (fig. 2), with values that were considerably lower.

Determination of Erythematic Solar Protection Factor

The determination of this parameter was carried out on all of the photoprotection formulations, using solution spectrophotometry. By obtaining absorption spectra from the different formulations, together with a value for absorbance at 308.8nm, we were able to extrapolate values for

in vitro erythematic SPF. There shown in table II. All values oscillate from between 0.66 to 1.17. SPF values for Eusolex® 232 are generally lower than those presented by Eusolex® 6300 and Neo Heliopan® AV. However, little difference between these last two polymers can be observed. It should be remembered that Eusolex® 232 presents different physical-chemical properties, given that it is a hydrosoluble substance, while the other two filters are liposoluble. This could provide an explanation for the differences in the data obtained.

This is also consistent with the values obtained for these substances in the previous section on integrated protection factor, in which no significant differences with regard to type of polymer used or its concentration were found. Consequently, we believe that these factors will not have any significant influence on erythematic protection factor.

The erythematic SPF values obtained are in general low, due to the fact that the concentration of solar filter used in the formulations was also low (1%). An increase in filter concentration will however result in higher values in solar protection factor. For example, after selecting a formulation containing Aquacoat® at a concentration of 10% and adding different concentrations of the filter Neo Heliopan® AV at 1%, 2%, 4%, 6% and 8% (fig. 8), SPF values were found to oscillate from between 0.87 to 6.92.

TABLE II

Values obtained for erythematic SPF for different formulations.

	EUSOLEX® 232	EUSOLEX® 6300	NEO HELIOPAN® AV
AQUACOAT® 10%	0'91	1'04	0'96
AQUACOAT® 25%	0'78	1'12	1'02
KOLLICOAT® 10%	0'97	1'08	1'06
KOLLICOAT® 25%	0'85	1'24	1'22
AQUATERIC® 10%	0'72	0'81	1'12
AQUATERIC® 25%	0'80	0'83	0'98
KOLLIDÓN® 10%	0'66	1'17	1'15
KOLLIDÓN® 25%	0'72	1'16	0'82

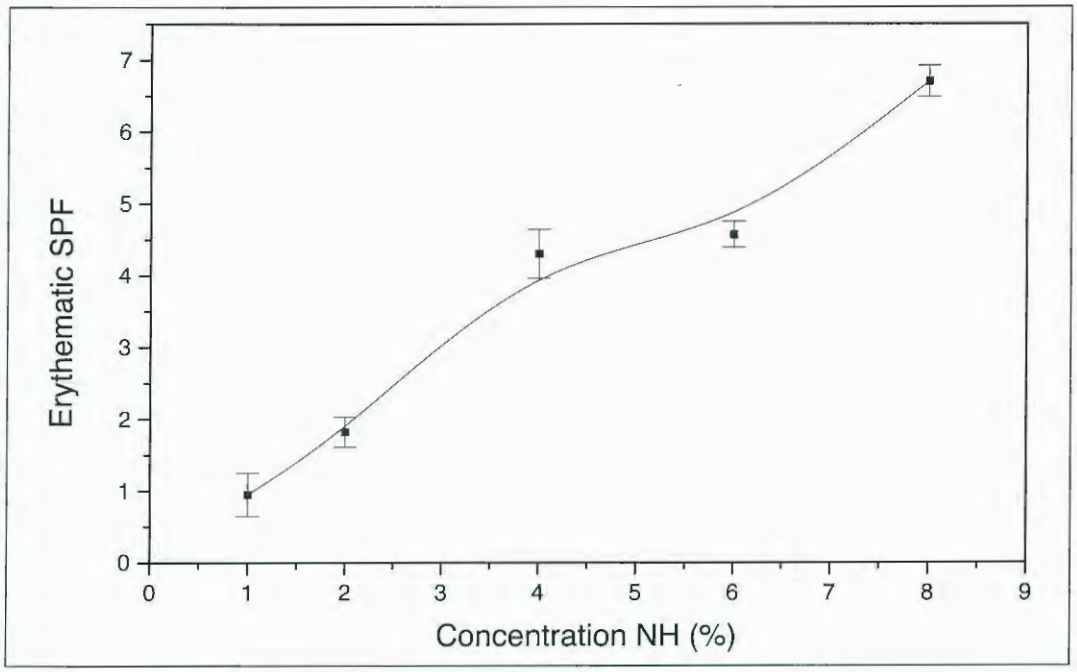

Fig. 8 SPF values at different concentrations of the filter Neo Heliopan® AV (1, 2, 4, 6 and 8%).

EXPERIMENTAL

Materials

4-methoxycinnamate of 2-ethylhexyl (Neo-Heliopan® AV). Supplied by Haarmann & Reimer, S.A.E. (España). Liposolnble. Absorbance in UVB range.

3-(4-methylbenzylidene) camphor or 1,7,7-trimethyl-3-(4-methylbenzylidene)-norbornane-2 one (Eusolex® 6300) supplied by Merck. Liposoluble. Absorbance in UVB range.

2-phenylbenzimidazole-5-sulphonic acid (Eusolex® 232). Supplied by Merck. Hydrosoluble. Absorbance in UVB range.

Dow Corning 245 (D.C$_{245}$) Supplied by Dow Corning (Belgium).

Abil EM 90. Supplied by Goldschmidt. A/O non-ionic silicone emulsifier.

n-decane. Supplied by Merck.

Aquacoat® Manufactured by FMC Corporation (USA) and supplied by Foret S.A. (Spain).

Kollicoat®. Supplied by Basf (Germany).

Aquateric® Manufactured by FMC Corporation (USA) and supplied by Foret S.A. (Spain).

Kollidón® SR. Supplied by Basf (Germany).

Distilled water

Methods
Preparation of formulations

The vehicle, chosen on the basis of data obtained from previous studies (15), was a silicone latex made up of the following composition:

$D.C_{245}$	25%
n-decane	0'5%
Abil EM 90	5%
Distilled water	c.s

The preparation technique consisted of adding aquaeous phase (distilled water) to the oil phase ($D.C_{245}$, n-decane & Abil EM 90), followed by agitation at 3000 rpm for 20 minutes with a mechanical shaker (Ultra-Turrax T25 Janke & Kunkel Ika®-Labortechnik).

Once the vehicle had been prepared, the photoprotective substances at 1% and the polymer nanoparticle (16) at 10 and 25% were added.

DETERMINATION OF INTEGRATED PROTECTION FACTOR

The determination of *in vitro* integrated protection factor was carried out through the measurement of spectral radiation with a Licor 1800 spectroradiometer. This instrument operates within a spectral interval of 300 to 1100 nm, with a precision of 6 nm. The optical receptor is a Teflon diffuser with a field of vision of 2 πsr. The monochromer is a holographic network, which disperses the radiation within its spectral components. At the entrance of the monochromer, there is a wheel with seven filters and an opaque disk. The detector, situated at the exit window of the monochromer, is a photodiode of silicone.

The measurements were carried out with a 2nm step, and the scanning time for the whole interval of wavelengths was 27 seconds.

Spectroradiometer commands were performed using a portable PC operated with simple software provided by the manufacturer. Two scans were carried out at each measurement and the average value obtained was recorded directly. The time required to perform this process was less than 90 seconds for each sample (17).

A commercial Osram Ultravitalux lamp (manufactured by Osram Germany), normally destined for commercial nse in UVA treatment parlours, was used as emissions source.

The prior determination of transmisivities was necessary, in order to obtain the spectral curve of the emissions from the ultraviolet lamp (not supplied by the manufacturer). This was carried out experimentally by measuring spectral radiation at different distances from the lamp, I_λ (x), and the ordinate at the origin of such adjustments $I_{o\lambda}$, was determined through lineal regression for each wavelength.

In order to measure the transmisivity of the solar formulations under study, a commercial 3M transpore surgical tape was used as a support, on which transmissivity within the range of 300-440 nm was also determined.

Formulations over the surgical tape were applied manually. In order to achieve a uniform thickness of layers throughout all trials, 0.09 g of the cream was applied as evenly as possible.

figure 9 shows a diagram of the experimental slide used.

DETERMINATION OF ERYTHEMATIC PROTECTION FACTOR

The solution spectrometry method is used to determine *in vitro* erythema protection factor, because the relationship between solar protection factor and absorbance is lineal. For this reason, we have used a lineal regression method. The SPF of the formulations under study, were obtained by interpolating on the regression straight a series of different standard emulsions with different known SPFs.

In order to obtain the straight line, the SPFs of the different polydispersa systems are placed on the ordinate axis and on the abscissas axis the corresponding absorbance values for each formulation are shown.

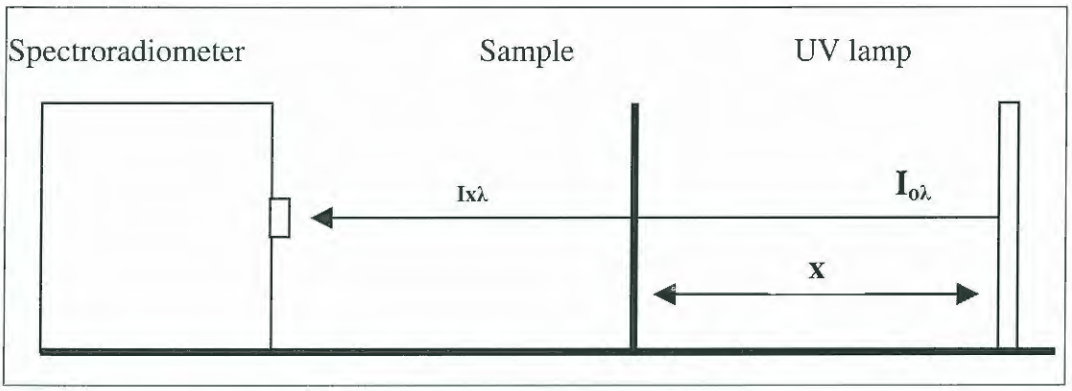

Fig. 9 *Diagram representation of the experimental slide used for the determination of protection factor.*

In this way, because the absorbance of the test sample is known, SPF can be determined by carrying out a simple interpolation (18, 19).

In order to determine erythematic SPS *in vitro* a 127 Perkin Elmer Running Lambda 2 spectrophotometer was used. Samples were weighed to 0.1 g added to 25 ml of methanol, and subsequently shaken and then filtered with a Millipore® (0.45 μm) filter over a 100 ml flask. This extraction process was repeated three times. The 0.1% solution was obtained by levelling the flask at 100ml; 3ml of this solution was then diluted with 10ml of methanol, giving a solution at 0.03%, which is read in the UV spectrometer at a wavelength of 308.8 nm (the wavelength at which solar erythema occurs). Before carrying out a reading, a blank-basing had to be carried out.

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References

1) **Riley, PA. (1992)** Material melanic: further dark thoughts. *Pigment Cell Res.*, **5:** 101-106.
2) **Riley PA. (1997)** Melanin. *Int. J. Biochem. Cell Biol.* **29:** 1235-1239.
3) **Herlihy E, Gies P, Roy C. and Jones M. (1994)** Personal dosimetry of solar UVR for different outdoor activities. *Photochem. Photobiol.* **60:** 288-293.
4) **Gies, P, Roy, C, Javorniczky, J, Henderson, S, Lemus-Deschamps, L. and Driscoll, C. (2004)** Global solar UV index: Australian measurements, forecasts and comparison with the UK. *Photochem. Photobiol.* **79:** 32-39.
5) **Taylor S. and Diffey BL. (2002)** Simple dosage guide for sunscreens will help users. *Br. Med. J.* **324:** 1526.
6) **Gies P, Javorniczky J, Roy C, Henderson S. (2006)** Measurements of the UVR Protection Provided by Hats Used at School. *J. Photochem. Photobiol.* **82:** 750-754.
7) **Schrader A, Jukupovic J. and Baltes W. (1994)** Photochemical studies on trac-3-methylbutyl 4-methoxycinnamate. *J. Soc. Cosmet. Chem.* **45:** 43-47.
8) **Kielbassa C. and Epe B. (2000)** DNA damage by UV and visible light and its wavelength dependence. *Methods Enzymol.* **319:** 436-445.
9) **Ratan KC, Lascu Z, Puccetti G, Deshpande A, Paknikar SK. (2006)** Design of a Photostabilizer Having Built-in Antioxidant Functionality and Its Utility in Obtaining Broad-spectrum Sunscreen Formulations [dagger]. *Photochem. Photobiol.* **82:** 823-829.
10) **Gonzenbach H, Hill TJ, and Truscott TG. (1992)** The triplet energy levels of UVA and UVB sunscreens. *J. Photochem. Photobiol. B* **16:** 377-379.
11) **Martínez-Lozano JA, Utrillas MP. Tena F. (2001)** Fotoprotección frente a la radiación UVA: primeros resultados de una experiencia docente de laboratorio. *Revista Española de Física,* **15:** 43-47.
12) **Schulz J, Hohenberg H, Pflücker F, Gartner E. (2002)** Distribution of sunscreens on skin. *Advanced Drug Delivery Reviews,* **54:** 157-163.
13) **Sheldon R, Pinnell MD. (2003)** Cutaneuos photodamage, oxidative stress, and topical antioxidant protection. *J. Am. Acad. Dermatol.*, **48:** 1-19.
14) **Sayre RM. (1990)** A method for the determination of UVA protection for normal skin. *J. Am. Acad. Dermatol.*, **23:** 429-432.
15) **Gallardo V, Montoya R, Ruiz MA. (2001)** Study of Silicone Vehicles for Aloe Vera. *J. Cosmet. Sci.*, **52:** 255-263.
16) **Ruiz MA, Martínez MT, Zouaki J, Gallardo V. (2002)** Latex as a sunscreen carrier in a silicone vehicle. *Int. J. of Cosmet. Sci.*, **24:** 235-239.
17) **Martínez-Lozano JA, Utrillas MP, Tena F. (1995)** Spectral Solar irradiance in the range 300-1100 nm measured at Valencia, Spain. *Renewable Energy,* **6:** 997-999.
18) **Gallardo V, Rniz MA, Parera A, Hernández A. (2000)** Radiaciones solares: tipos y efectos. *Ars Pharm.*, **41:** 167-176.
19) **Ruiz MA, Hernández A, Gallardo V. (2000)** Methods to determine the Protection Factor of Sunscreen Formulations. *Dermatol. Cosmet.* **10:** 7-10.

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CUTANEOUS ABSORPTION OF NANOSTRUCTURED CHITIN ASSOCIATED WITH NATURAL SYNERGISTIC MOLECULES (LUTEIN)

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Summary

The present investigation of chitin nanofibrils highlighted the hydrophilic properties of chitin (a nanostructured extracellular material) and its effect on cell behaviour as a microenvironmental stimulus. In this respect chitin is similar to dextran and its derivatives, which are capable of "trapping" growth factors (heparin binding factor, TGF-β1, FGF-2) and stimulate cell proliferation. Thus, chitin is also to be considered as a bioactive polymer capable of promoting correct cutaneous trophism. Therefore chitin, albeit indirectly, also controls in the skin epithelium-mesenchyma molecular relationships and the hair follicle cycle. In addition, the influence of polysaccharides on cell biology is related to the osmotic stress generated by their hydrophilicity.

These consideration led us to devising a morphofunctional experimental investigation into the dermal-epidermal penetration of chitin nanofibril solutions, which, in the near future, will be an important vehicle for the diffusion of dermato-cosmetological molecules biologically active.

Riassunto

Nel presente studio, rivolto alle nanofibrille di chitina (quale materiale nanostrutturato extracellulare), si evidenzia come la loro idrofilicità abbia la capacità di influenzare il comportamento delle cellule presenti quale stimolo microambientale, similmente al destrano, ai suoi derivati etc., che sono in grado di "intrappolare" vari fattori di crescita (heparin binding factor, TGF-b1, FGF-2) i quali stimolano successivamente la proliferazione cellulare. Anche la chitina va pertanto considerato un polimero bioattivo utile ed efficacie nel favorire e realizzare un corretto trofismo cutaneo. Perciò, seppur indirettamente, essa ha pure, nella cute, un controllo sui rapporti molecolari tra epitelio-mesenchima e ciclo del follicolo pilifero. Inoltre, l'influenza dei polisaccaridi sulla biologia della cellula, è noto, è legata allo "stress osmotico" che la loro idrofilicità finisce col generare.

Alla luce di ciò abbiamo condotto uno studio morfo-funzionale atto a valutare sperimentalmente, la penetrazione epidermico-dermica di soluzioni contenenti nanofibrille di chitina, che in un futuro prossimo rappresenteranno un importante veicolo di diffusione di molecole dermato-cosmetologiche biologicamente attive.

INTRODUCTION

A recent report on nanomaterials (1) by Friends of the Earth, an environmental group, has called for a moratorium on the liberalization of products containing nanomaterials (including sunscreens and cosmetics in general), as they have been the object of various safety investigations in terms of both human health and the environment. In contrast to other researchers, Friends of the Earth, claim that nanomaterials should be considered as novel chemical substances, in that they may have substantially different properties from those of the same materials in macroscopic form (2).

Cells interact with the extracellular environment (be it natural or artificial) via surface proteins such as integrins, which trigger various metabolic pathways with important roles in processes such as cell shape, mobility and proliferation (3-5). Adequate extracellular inputs therefore prompt a local cellular response that may be accompanied by a diffuse response. Endoplasmic reticulum (ER) and associated organelles are generally uniformly distributed in the cytoplasm, forming a network, at least in steady-state in vitro conditions. An extracellular stimulus inducing intercellular adhesion, cell proliferation and migration can however lead to their dynamic rearrangement (6-7).

Chitin is a hydrophilic material, a property it shares with several other polysaccharides. Chitin nanofibrils (an extracellular nanostructured material) can constitute a microenvironmental stimulus, thereby influencing cell behaviour, as also recognised in the literature (8). For instance dextran and its derivatives are capable of "trapping" various growth factors (heparin binding factor, TGF-β1, FGF-2), stimulating cell proliferation. These polysaccharides should thus be considered as bioactive polymers capable of promoting correct cutaneous trophism (8). Therefore polysaccharides, albeit indirectly, also control epithelium-mesenchyma molecular relationships and the hair follicle cycle (9).

The influence of these molecules on cell biology is also related to the osmotic stress induced by their hydrophilicity (10). When cells are exposed to a hypotonic extracellular fluid they initially swell up with water, but subsequently shrink back to close their previous volume (a mechanism called regulatory volume decrease-RVD) through the inflow of organic ions (mainly Cl^- and K^+) and osmolytes, which induce water outflow (10). A hypotonic extracellular environment also results in remodelling of the actin cytoskeleton by activation of Rac and Cdc42 (11).

In the light of these considerations an experimental morphofunctional study was devised to assess the dermal-epidermal penetration of solutions containing chitin nanofibrils, which will become an important vehicle for the diffusion of dermato-cosmetological molecules biologically active in the near future.

MATERIALS AND METHODS

Materials

The following solutions were tested:
- Saline (Solution A).
- Chitin nanofibrils (Solution B)
- Chitin nanofibrils/ Solvent/ (Solution C)
- Chitin nanofibrils/Solvent/Lutein (Solution D)

Solutions B-D contained an aqueous solution of chitin nanofibrils (2-2.5g/L). Nanofibrils were prepared as described previously (12). Lutein was dissolved in diethylene glycol monoethylic ether (solvent) (MAVI SUD Srl, Aprilia, Italy).

Experimental Procedure

Twelve male Wistar rats weighing 200±20 g were used. Animals were subjected to a general veterinary examination before being placed in

individual cages. Amoxicillin was administered 2 days before the experiments. All work was performed at INRCA (Ancona), which holds all the certifications required for all types of animal experiments. Throughout the study rats were kept in a standard environment at 20-21°C, relative humidity 55% and a normal light/dark cycle. They received a pellet diet and had ad libitum access to water. Experiments were conducted under general anaesthesia by intraperitoneal injection of 242 ng/kg of 2,2,2 tribromoethanol. Rats were carefully shaved avoiding any cuts. Filters soaked with solutions A, B, C or D were placed each in a separate aluminium chamber measuring 8 mm in diameter (area 50 mm^2; volume ca. 20-25 µl) of a 4-chamber device (Finn Chambers - Epitest Ltd Oy, Tuusula, Finland) (fig.1) and taped on the dorsal area 24 h later using Scanpor tape (Actavis Norway, Norgesplaster AS), which is endowed with protective paper backing. Solutions were left in contact with the epidermis for 4 h.

One group of 4 animals was euthanized at the time of chamber removal (T_0), another at 24 h and the third at 48 h.

Tissue was collected from the areas in contact with the solutions and divided into two fragments; one was frozen for morphological and immunohistochemical investigations, the other was resin-embedded for light microscopy.

Fig. 1 Finn chambers.

Morphological and Immunohistochemical Studies

Skin fragments were frozen in liquid nitrogen and stored at 70°C; 6-mm-thick sections were obtained with a cryotome (Leica CM 1900, Leica Microsystems, Cambridge UK), left to dry overnight and then fixed in acetone for 10 min. Some sections were stained with haematoxylin/eosin The remaining sections were incubated overnight with anti-CD34 (dil. 1:20, BD Biosciences, Belgium), anti-CD4 (dil. 1:50, Santa Cruz, CA, USA) and anti-CD8 (dil. 1:50, Dakocytomation, Denmark) monoclonal antibodies at 4°C and processed with the streptavidin-biotin peroxidase method. They were finally incubated with 3,3 diaminobenzidine (Sigma-Aldrich, Italy), stained with Mayer's haematoxylin and mounted in Paramount. Antibody activity was evaluated with a Nikon Eclipse (Nikon-Italia, Italy) light microscope.

Semithin Sections Analysis

Tissue fragments were fixed in 2% glutraraldehyde (GTA) in 0.1 M cacodylate buffer, dehydrated in rising ethanol concentrations and araldite-embedded. Semithin sections were obtained with an LKB ultra-microtome, stained with toluidine blue and examined with a a Nikon Eclipse (Nikon-Italia, Italy) light microscope.

RESULTS

Gross Examination

After 4 h the solutions were completely absorbed.

Morphological Investigations

T_0

Skin treated with solutions B, C and D did not display histological or structural changes compared with control epidermis, except for a partial disorganization of the horny layer.

24 h

In skin fragments treated with the solution containing chitin nanofibrils and lutein displayed sparse, roughly rectangular areas with regular borders (fig. 2).

48 h

Epidermis

Examination of skin treated with Solution A (saline) (fig. 3b) showed a modest swelling of the epithelium and immediately underlying collagen bundles, but not of deeper dermal layers, in saline-treated compared with control skin (healthy cutis) (fig. 3a).

In skin fragments treated with Solution B (chitin nanofibrils) (fig. 3c) the epidermis appeared to be constituted of cells with a denser cytoplasm compared with the specimens treated with solution A (fig. 3b) and with control skin (fig. 3a), with modest epidermal thinning being a possible consequence of osmotic-mechanical stress also induced by dermal turgidity.

The dermal-epidermal changes observed in cutis treated with Solutions C and D were also less significant than those noted in tissue treated with Solution B.

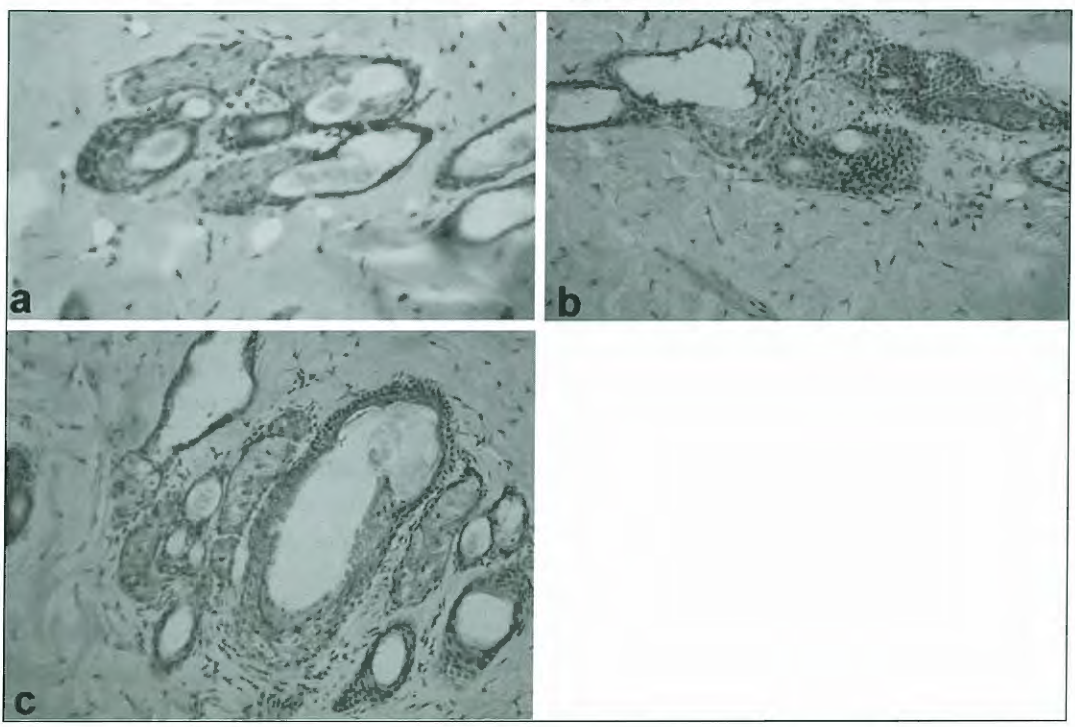

Fig. 2 Haematoxylin-Eosin sections of skin treated for 24 h with a) Solution A (saline); b) Solution B (chitin nanofibrils); c) Solution D (chitin nanofibrils + solvent + lutein).

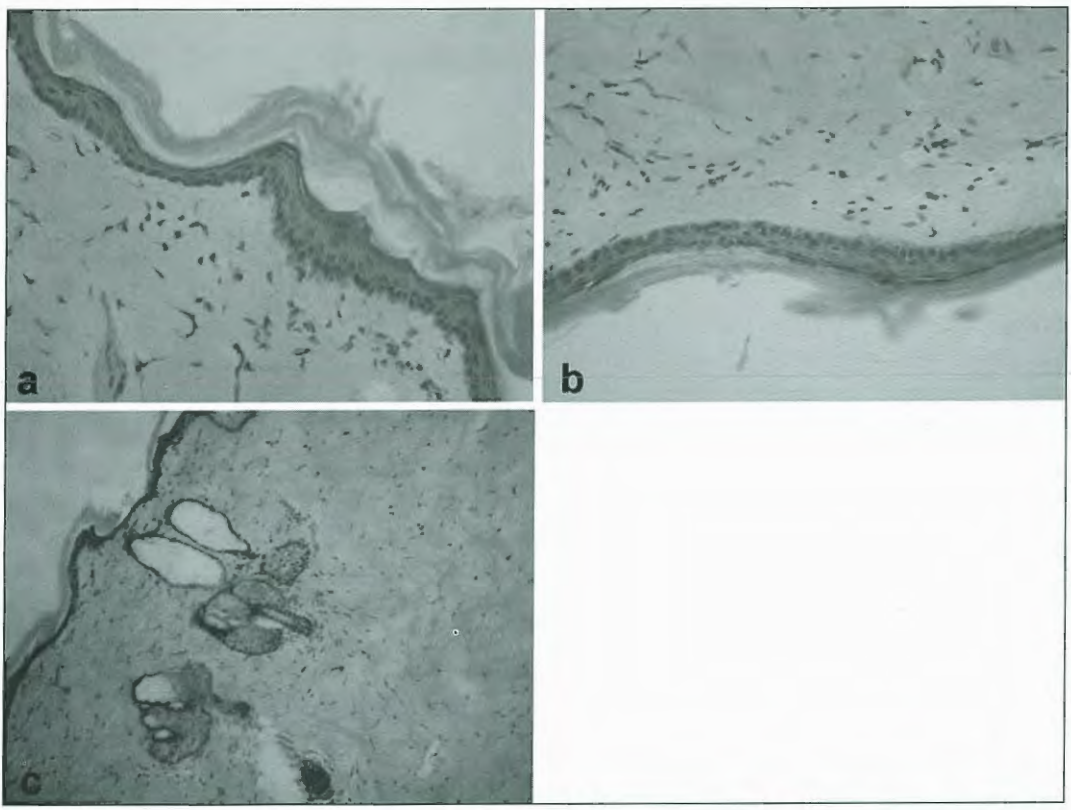

Fig. 3 Haematoxylin-Eosin sections of a) normal skin (control) and skin fragments treated for 48 h with b) Solution A (saline) and c) Solution B (chitin nanofibrils).

Dermis

More compact hair root components and collagen bundles in surrounding dermis were observed in control skin. A modest inflammatory infiltrate was noted (fig. 4a).

At 48 h saline (Solution A) did not appear to induce significant changes in hair roots or collagen bundles in the surrounding dermis (fig.4b), despite a modest subepithelial oedema.

In Solution B treated skin slightly enlarged intercellular spaces and cells whose cytoplasm had a different density, interpreted as a possible "modulated" outcome of extracellular hypo-osmolarity, were noted in hair roots among follicular sheets. The connective bundles around the roots also appeared to be less compact compared with control cutis. Chitin nanofibril aggregates were not clearly detectable. There were few inflammatory cells; vessels were slightly and locally dilated (fig. 4c).

In skin treated either with Solution C or D morphostructural changes were less marked than in skin fragments treated with Solution C (fig. 4d).

At 48 h, chitin nanofibrils alone (Solution B) appeared to induce greater dermal soaking compared with preparations containing solvent (Solution C) or solvent and lutein (Solution D).

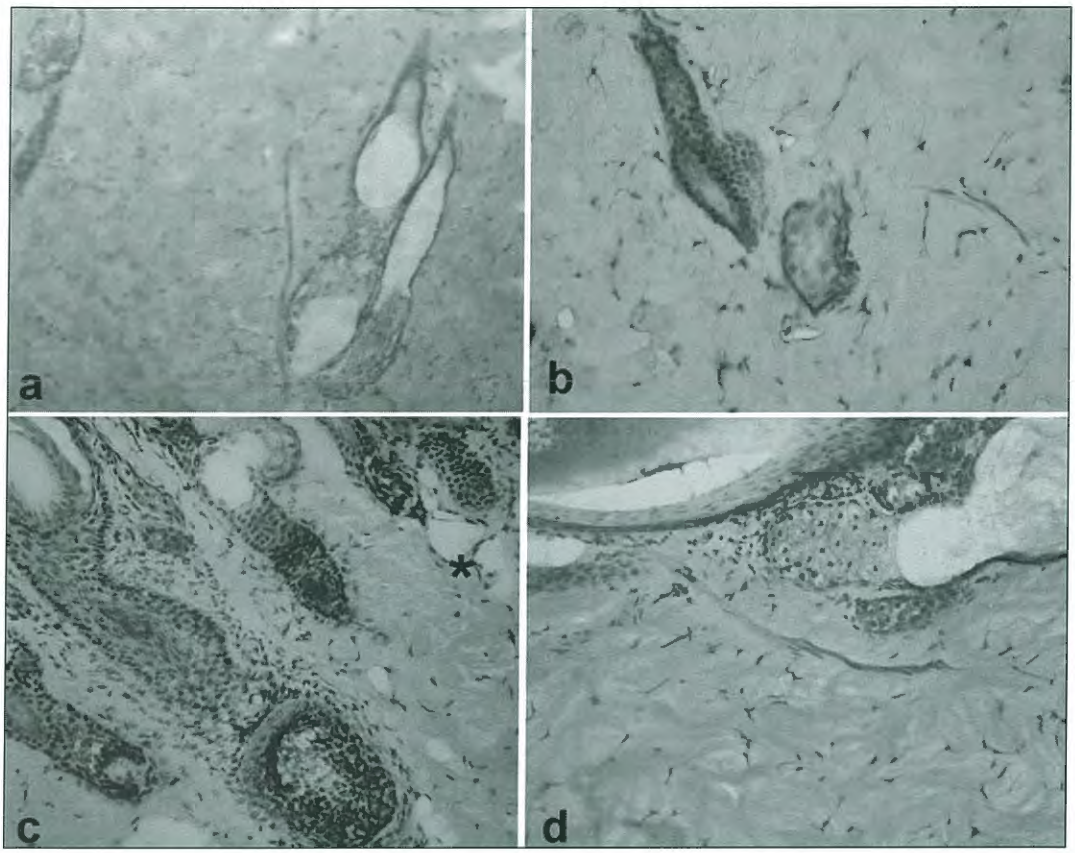

Fig. 4 Haematoxylin-Eosin sections of a) normal skin (control) and skin fragments treated for 48h with b) Solution A (saline); c) Solution B (chitin nanofibrils). (= small dilated vessel); and d) Solution D (chitin nanofibrils + solvent + lutein).*

Immunohistochemical Studies

CD4-CD8

The immunohistochemical study for CD4 and CD8 in skin treated with Solution B (chitin nanofibrils) did not evidence significant lymphocyte chemotaxis. Similar immunohistochemical features were noted in sections from rats treated with Solution C (chitin nanofibrils /solvent) and with Solution (chitin nanofibrils /solvent/lutein).

The CD4 and CD8 lymphocyte infiltration found especially in skin treated with Solution C was thus comparable to that seen in untreated skin fragments (healthy cutis), further highlighting the lack of adverse effects of the latter solution (Table 1).

Similar findings were noted in specimens from areas treated with saline (Solution A).

CD34

The immunohistochemical study (Table I) demonstrated that CD34-positive cells with the characteristics of stem cells appeared to be more expressed (although not significantly so) in skin treated with chitin nanofibrils (figs 5c,d), espe-

cially with the solution containing solvent, than in untreated cutis (fig. 5a) or in saline-treated skin (fig. 5b).

TABLE I
% positive cells

	CD4	CD8	CD34
Healthy cutis	10	9	7
Sol. A	12	12	4
Sol. B	10	9	15
Sol. C	13	15	19
Sol. D	9	16	12

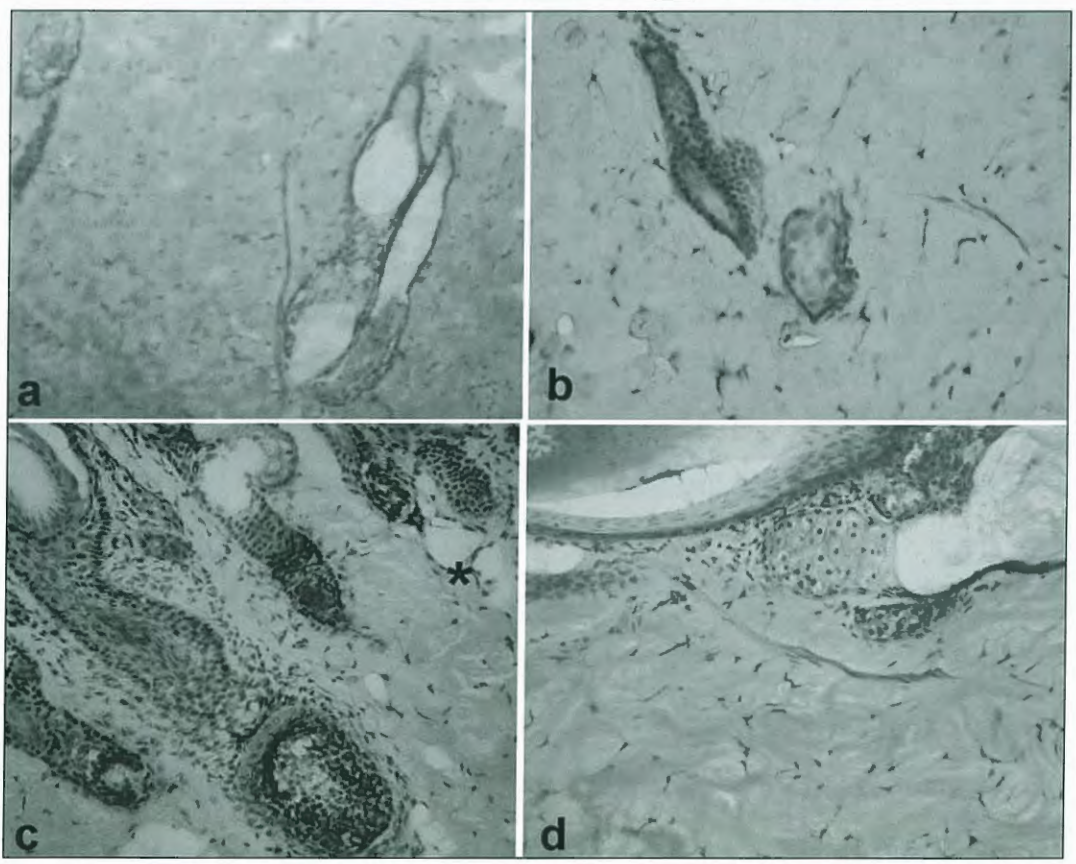

Fig. 5 *Immunohistochemical detection of CD34 in a) normal skin (control) and skin treated with b) Solution A (saline); c) Solution B (chitin nanofibrils); and d) Solution D (chitin nanofibrils + solvent + lutein).*

Semithin Sections

48 h

The interweaving connective structure of the dermis gives rise in vivo to a 3D porous scaffold in which molecules penetrating through the epidermis are filtered before entering the peripheral circulation.

Oedema of the subepithelial connective tissue, albeit without necrosis or inflammation, was clearly evident in semithin sections of skin treated with Solution B (fig. 6). Comparison with saline-treated skin revealed that Solution A also induced the onset of subepithelial oedema, in this case accompanied by gross loosening of collagen fibres and small erythrocyte clusters.

The addition of solvent (Solution C) or solvent and lutein (Solution D) did not appear to exert specific effects compared with the solution containing chitin nanofibrils alone.

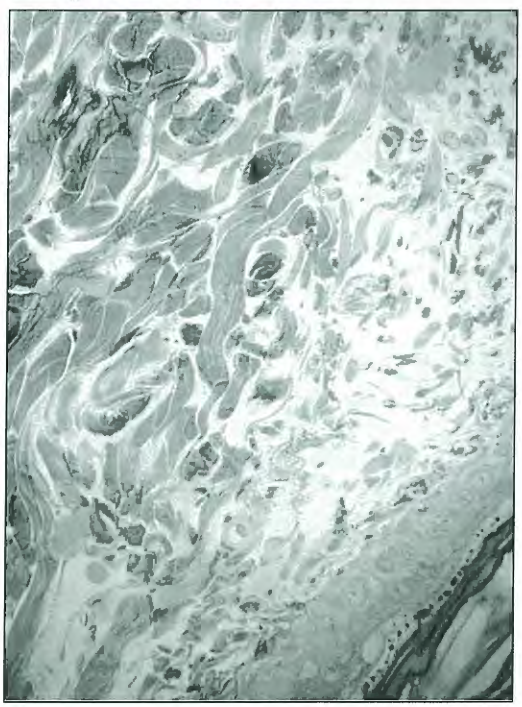

Fig. 6 *Semithin section of skin treated for 48h with Solution B(chitin nanofibrils).*

DISCUSSION

In vitro studies performed to document possible adverse effects of nanostructured molecules, such as chitin nanofibrils, used in dermato-cosmetology meet the need for identifying actions exerted by these molecules towards cells other than resident cell populations.

This requires first of all to evaluate any distress that the nanostructure may exert on resident epidermal-dermal cells (keratinocytes, fibroblasts, endothelial cells, Langerhans cells, melanocytes, lymphocytes). The possible local release of cytokines then entails testing for any chemotactic activity of resident cells and for the extravasation of blood subpopulations that enhance specific local immunoreactivity (13)

Such functional modulation is currently difficult to assess exhaustively in skin equivalent devices, making in vivo experiments necessary, at least in the short term. It should also be noted that skin equivalent devices do not have the hair component.

Topically applied substances penetrate through the cutis in three different ways: transcellular, intercellular and follicular. In particular, hair follicles are viewed as important drug delivery pathways. A recently devised class of liposomes is able to penetrate to a depth of 84% into the hair follicle of experimental animals thanks to a cationic charge on their surface; penetration is demonstrated by markers like carboxyfluorescein and curcumin (14). This significant technological advance will enable better testing of intradermal penetration of dermato-cosmetic products with a nanostructured component of chitin nanofibrils associated with molecules aimed at specific dermato-cosmetological targets (15).

In neglected cutis the more superficial dermal-epidermal layers exhibit a structural instability that on the one hand impairs the skin's role as a defensive barrier but on the other allows easier

penetration of "regenerative" molecules to deeper layers. In this context the production of "regenerative" compounds capable of crossing the skin barrier is especially important, *making the production of nanostructured products an advantage*.

The combination of a nanofibril structure and hydrophilicity (8) promises to enhance the penetration of chitin and of molecules associated to it.

Our data document that intraepidermal and dermal penetration of soluble nanostructured chitin molecules enables their extracellular accumulation and does not appear to induce inflammatory–reactive phenomena or scarce tolerance to chitin itself. Chitin biodegradablility prevents intracutaneous persistence, which in turn could induce a non-self inflammatory reaction.

The absorption of chitin nanofibrils at the level of root, follicular and perifollicular sheets, determine a modification in the architecture these structures, although not markedly. This effect may partially be attributable to an osmotic stress mechanism as well as to the recall of molecular trophic factors (growth factors), which favour hair regrowth. The risk of small subepidermal vessels constituting a pathway for the migration and diffusion of nanostructured molecules to the circulation is attenuated by chitin's biodegradable nature and thus low biological persistence.

Our data also confirm that the absorption of chitin nanofibrils alone or associated with solvent or solvent/lutein does not induce significant non-self dermal-epidermal reactions (15-16). The chitin nanofibril solution also proved to be an interesting stimulus for CD34-positive radicular-follicular (i.e. stem) cells (17,18). The evidence for biofunctional CD34 cell activation in the presence of chitin nanofibril solutions opens interesting prospects for the use of such vehicle in dermato-cosmetology and is expected to result in clinical-dermatological benefit.

These and previous findings may enable future research into the degree of penetration of dermato-cosmetic factors with trophic-regenerative action, using chitin nanofibrils as the vehicle. These researchers may be carried out almost exclusively in skin equivalent devices (19), further to curb animal experiments.

References

1) **Maynard AD. (2006)** Nanotechnology: assessing the risks. *Nano Today*, **1:** 22-33.
2) **Sealey C. (2006)** Getting under your skin. *Nano Today*, **1:** 1.
3) **Toh YC, Ng S, Khong YM, Zhang X, Zhu Y, Lin PC, Te CM, Sun W, Yu H. (2006)** Cellular response to nanofibrous enviroinment. *Nano Today*, **1:** 34-43.
4) **Rosso F, Giordano A, Barbarisi M, Barbarisi A. (2004)** From cell-ECM interactions to tissue engineering. *J Cell Physiol.*, **199:** 174-80.
5) **Ng S, Wu YN, Zhou Y, Toh YE, Ho ZZ, Chia SM, Zhu JH, Mao HQ, Yu H. (2005)** Optimization of 3-D hepatocyte culture by controlling the physical and chemical properties of the extra-cellular matrices. *Biomaterials*, **16:** 3153-63.
6) **Tran H, Pankov R, Tran SD, Hampton B, Burgess WH, Yamada KM. (2002)** Integrin clustering induces kinectin accumulation. *J Cell Sci.*, **115:** 2031-40.
7) **Ong LL, Er CPN, Ho A, Aung MT, Yu H. (2003)** Kinectin anchors the translation elongation factor-1 delta to the endoplasmic reticulum. *J Biol Chem.*, **278:** 32115-123.
8) **Frank L, Lebreton-Decoster C, Godeau G, Coulomb B, Jozefonvicz J. (2006)** Dextran derivatives modulate collagen matrix organization in dermal equivalent. *J Biomater Sci Polym*, **17:** 499-517.
9) **Botchkarev VA. (2003)** Neurotrophins and their role in pathogenesis of alopecia areata. *J Investig Dermatol Symp Proc.*, **8:** 195-8.
10) **Kippenberger S, Loitsch S, Guschel M, Muller J, Kaufmann R, Bernd A. (2005)** Hypotonic stress induces E-cadherin expression in cultured human keratinocytes. *FEBS Lett.*, **579:** 207-14.
11) **Carton I, Hermans D, Eggermont J. (2003)** Hypotonicity induces membrane protrusions and actin remodeling via activation of small GTPases Rac and Cdc42 in Rat-1 fibroblasts. *Am J Physiol Cell Physiol.*, **285:** C935-44.
12) **Muzzarelli RAA, Morganti P, Morganti G, Palombo P, Palombo M, Biagini G, Mattioli Belmonte M, Giantomassi F, Orlandi F, Muzzarelli C. (2007)** Chitin nanofibrils/chitosan glycolate composites as wound medicaments. *Carbohydrate Polymers*, **70:** 274-284.
13) **Tada Y, Riedl E, Lowenthal MS, Liotta LA, Briner BM, Crouch EC, Udey MC. (2006)** Identification and Characterization of Endogenous Langerin Ligands in Murine Extracellular Matrix. *J Invest Dermatol.*, **126:** 1549-1558.
14) **Jung S, Otberg N, Thiede G, Richter H, Sterry W, Panzner S, Lademann J. (2006)** Innovative Liposomes as a Transfollicular Drug Delivery System: Penetration into Porcine Hair Follicles. *J Invest Dermatol.*, **126:** 1728-1732.
15) **Gupta P, Freyschmidt-Paul P, Vitacolonna M, Kiessling S, Hummel S, Hildebrand D, Marhaba R, Zöller M. (2006)** A Chronic Contact Eczema Impedes Migration of Antigen-Presenting Cells in Alopecia Areata. *J Invest Dermatol.*, **126:** 1559-1573.
16) **Hodak E, David M, Maron L, Aviram A, Kaganovsky E, Feinmesser M. (2006)** CD4/CD8 double-negative epidermotropic cutaneous T-cell lymphoma: an immunohistochemical variant of mycosis fungoides. *J Am Acad Dermatol.*, **55:** 276-84.
17) **Kaur P. (2006)** Interfollicular Epidermal Stem Cells: Identification, Challenges, Potential. *J Invest Dermatol.*, **126:** 1450-1458.

18) **Cotsarelis G. (2006)** Epithelial Stem Cells: A Folliculocentric View. *J Invest Dermatol.*, **126:** 1459-1468.

19) **Zghoul N, Fuchs R, Lehr CM, Schaefer UF. (2001)** Reconstructed skin equivalents for assessing percutaneous drug absorption from pharmaceutical formulations. ALTEX., **18:** 103-6.

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CLINICAL APPLICATIONS OF A NEW DEVICE FOR FRACTIONAL PHOTOTHERMOLYSIS

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Summary

Fractional photothermolysis, is a novel concept for treating the sequelae of cutaneous photoaging, skin pigmentation disorders, superficial vascular malformations, acne scars and stretch marks or striae. It creates a pattern of microscopic zones of tissue coagulation that heal over several weeks while the skin retains a normal appearance. Aim to achieve homogeneous thermal damage at a particular depth within the skin, fractional photothermolysis creates microscopic thermal wounds (microscopic treatment zones) and specifically spares tissue surrounding each wound. Fractional photothermolysis is a promising new modality that, based on this preliminary report, produces a consistent level of efficacy for the tested treatments with significantly reduced side effects.

Riassunto

La fototermolisi frazionale è una nuova tecnica per trattare le sequele dell'invecchiamento cutaneo, le alterazioni della pigmentazione cutanea, le malformazioni vascolari superficiali, le cicatrici esito di acne e le smagliature. Questa nuova tecnica laser permette di creare una griglia di microscopiche aree di coagulazione cutanea che si rigenerano in alcune settimane lasciando alla cute un aspetto normale in ogni fase. Invece di creare un danno termico omogeneo, la fototermolisi frazionale crea piccolissime aree di danno risparmiando il tessuto intorno ad ognuna di esse, in modo da avere dei serbatoi cellulari da cui far partire la rigenerazione.
Basandosi sui risultati di questo e di altri studi preliminari la nuova metodica sembra produrre miglioramenti consistenti per tutti gli inestetismi trattati con una frequenza molto bassa di effetti collaterali.

INTRODUCTION

Fractional photothermolysis, is a novel concept for treating the sequelae of cutaneous photoaging, skin pigmentation disorders, superficial vascular malformations, acne scars and stretch marks or striae. It creates a pattern of microscopic zones of tissue coagulation that heal over several weeks while the skin retains a normal appearance. Rather than creating a global tissue effect at the surface of the target tissue, or in the dermis alone, this treatment creates injury in a tiny fraction of the skin treated, coagulating multiple columns of tissue of about 100 μm in diameter, spaced 500 μm and extending through the epidermis and deeply into the dermis for about 500-700 μm. The number of microscopic thermal wounds can vary from 400 to 750 every spot. The laser tested in this study is an ER:Glass (erbium glass) laser whose wavelength of 1540nm is absorbed by the water of the skin cells and by means of its special lens array, it can stimulate the deep, the superficial dermis and epidermis at different temperature grades. It produces an evenly low level of thermal neocollagen and elastin stimulation on all the treatment areas and, in addition, a high level thermal heating and coagulation within the fractional areas.

In contrast to ablative skin resurfacing and non ablative dermal remodeling techniques which aim to achieve homogeneous thermal damage at a particular depth within the skin, fractional photothermolysis creates microscopic thermal wounds (microscopic treatment zones) and specifically spares tissue surrounding each wound. This laser treats about 20% of the skin with each session.

MATERIALS AND METHODS

Fifty five healthy subjects of Fitzpatrick skin type II–V received treatments with the Matisse® laser (Quanta System S.p.a., Italy). Matisse® laser (Quanta System S.p.a., Italy), uses fractional photothermolysis to achieve its clinical effect. 30 patients (25 females, 5 males aged 40-70 years, mean age 55 years) for treating the sequelae of cutaneous photoaging and pigmentation disorders, 10 patients (8 females, 2 males aged 20-35 years, mean age 26 years) for superficial low flow capillary vascular malformations or generalized telangiectasia syndrome, 5 patients (3 females, 2 males aged 25-50 years, mean age 34 years) for acne scars, 10 patients (7 females, 3 males aged 20-30 years, mean age 24 years) for stretch marks/striae.

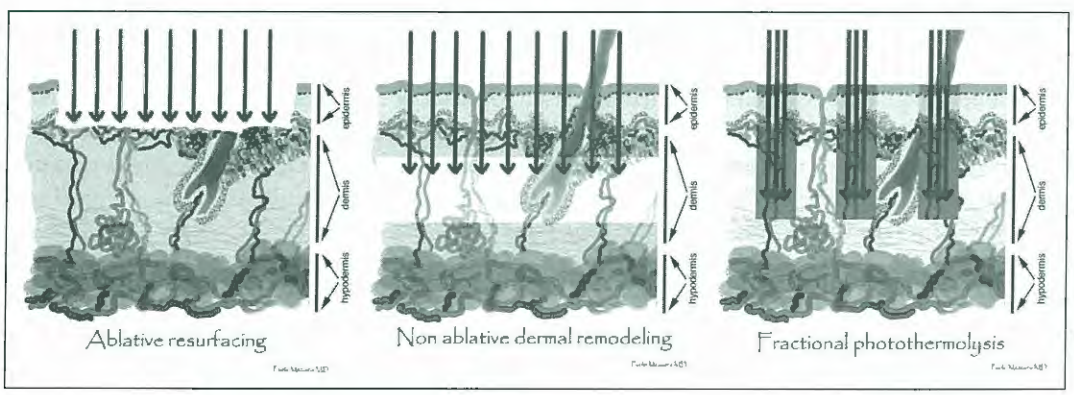

Fig. 1

Exclusion criteria were history of keloid formation, history of isotretinoin use within the last 6 months, current systemic infections, pregnancy and severe systemic or dermatologic diseases. No anesthesia was used to perform the exposures and for pain control was used the cooling device on the machine handpiece. Patients were photographed and evaluated preoperatively and at 1, 2, 4, 8, and 12 weeks after the last session. At each visit patients were asked to evaluate any difference noted. 3 months after the last session evaluation of results were made on a scale of 1±9, with 1 representing no results and 9 representing excellent results by a blinded physician and by patients themselves. Silicone negative imprints of the right cheek were made to the patients treated for sequelae of cutaneous photoaging.

Photoaging and skin pigmentation disorders (Fractional Rejuvenation or Fractional Resurfacing).

Photoaging refers to the clinically visible changes of skin chronically exposed to UV light. Mottled pigmentation in the form of both hyper and hypopigmentation, telangiectasia, coarsened texture, dull or sallow coloration, enlarged pilosebaceous units, wrinkles, and benign and malignant neoplasm are the clinical findings in photoaged skin. It has been well established that the wavelengths of UV radiation (UVA) are responsible for much of the visible changes of photoaging.

Until the middle of the 20th century, treatment options were limited to application of various natural and synthetic preparations to the skin, chemical peels or dermabrasion. The development of the pulsed CO2 laser/computerized pattern generator in the early 1990's, and the Erbium (Er:YAG) laser made it possible to obtain more consistent and predictable results than with the peels and dermabrasion, and led to the widespread acceptance of Ablative Laser Resurfacing by cosmetic surgeons and patients. Although highly effective for wrinkles, brown spots, and even sagging skin, the disadvantages of the Ablative Laser Resurfacing include discomfort, oozing wound as well as the long-term risk of scarring and changes in skin pigmentation.

During the late 1990's, Non-Ablative Laser Resurfacing became popular. Using a variety of laser wavelengths, or intense pulsed light sources some acting more towards the skin surface, some deeper in the collagen layers, Non-Ablative techniques are capable of improving skin texture, pigment abnormalities, and even some tightening occasionally, with no or minimal risk and without any downtime. However, multiple treatments are needed, and with a few, unpredictable exceptions, the results are limited. During each treatment of fractional laser resurfacing millions of microscopic small volumes of thermal damage are distributed within the skin, the epidermal repair is fast, heals within 24 hours by keratinocyte migration into the defect. The barrier function of the epidermis is preserved during this process, no visible wound is created, so no wound care is needed and make-up can be applied immediately.

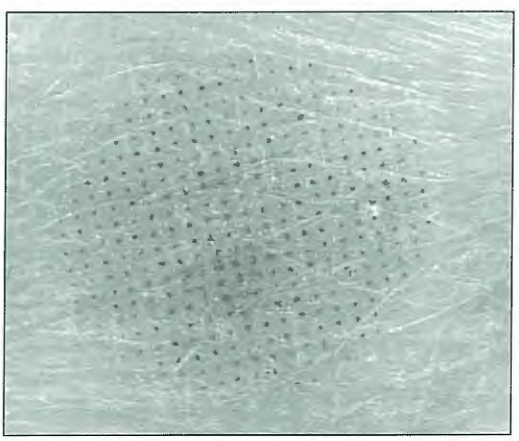

Fig. 2

Patients should avoid taking oral retinoids for at least 3-6 month prior to treatment, and topical retinoids should be discontinued at least 2 weeks before treatment.

The treatment is performed in the office. The treatment area is cleansed with a mild abrasive cleanser. The laser head is moved over the treatment area, using an overlapping technique, so that a total of 2-4 passes are made over each area. The skin is kept cool during the treatment. The actual treatment takes approximately 30 minutes.

The energies and pulse durations were varied based on the grade of photoaging according to Glogau Wrinkle Scale and Fitzpatrick skin type.

Table I

Glogau wrinkle scale

Wrinkle scale	Age (years)	Findings
1, no wrinkles	Early 20s or 30s	Early photoaging: early pigmentary changes, no keratoses, fine wrinkles.
2, wrinkles in motion	30s to 40s	Early to moderate photoaging: early senile lentigines, no visible keratoses, smile wrinkles.
3, wrinkles at rest	50 plus	Advanced photoaging: dyschromia and telangiectasia, visible keratoses, stable wrinkles.
4, only wrinkles	60s or 70s	Severe photoaging: yellowish skin color, previous skin malignancy, generalized wrinkling.

Table II

Treatment Parameters

Wrinkle scale	Age (years)	Laser Parameters
1, no wrinkles	Early 20s or 30s	No treatment needed.
2, wrinkles in motion	30s to 40s	Pulse duration 4-7 ms / 6-11 mJ spot 2/3 passages in overlapping.
3, wrinkles at rest	50 plus	Pulse duration 6-9 ms / 9-12 mJ spot 2 passages in overlapping.
4, only wrinkles	60s or 70s	Pulse duration 6-10 ms / 9-13 mJ spot 2 passages in overlapping.

In Fitzpatrick skin types V-VI, to avoid post-inflammatory hyperpigmentations is better to use the treatment parameters of the Glogau wrinkle scale 2 in every case.

Immediately after treatment a sensation of heat or "sunburn" is common, lasting about 30 minutes, and is easily relieved by cool compresses and only moisturizers and zinc sunscreens are used on the face. In the week after treatment, the skin may become somewhat dry and red. This "bronzed" appearance and dryness is from the surface of each laser "spot" separating and sloughing off. During this time potentially irritating skin products such as AHAs or retinoids should be avoided. In most cases, the redness and bronzing will gradually improve over a week.

The intervals and numbers of sessions vary also with Glogau wrinkle scale.

Table III		
Intervals between sessions and number of sessions		
Wrinkle scale	**Age (years)**	
1, no wrinkles	Early 20s or 30s	No treatment needed
2, wrinkles in motion	30s to 40s	4 sessions/ interval 2-3 weeks
3, wrinkles at rest	50 plus	5 sessions/ interval 2-3 weeks
4, only wrinkles	60s or 70s	6-7 sessions/ interval 2-3 weeks

Skin superficial low-flow capillary vascular malformations and generalized telangiectasia syndrome.

Two common capillary vascular malformations are the salmon patch (naevus simplex) and port wine stain (naevus flammeus). Salmon patch are very common and occur in about 40% of all newborns. They are usually small flat patches of pink or red skin with poorly defined borders. Most lesions will spontaneously disappear within the first year of life.

Port Wine Stain are much less common than salmon patches, occurring in about 0.3% of newborns. A port wine stain is usually a large flat patch of purple or dark red skin with well-defined borders. At birth the surface of the port-wine stain is flat, but in time it becomes bumpy and often more unsightly. The face is most commonly affected although they can occur anywhere on the body. Where present, they generally appear on one side of the body with a sharp mid-line cut-off.

Some port wine stains may fade over time but most remain unchanged or may even deepen in colour. They do not shrink by themselves or disappear spontaneously. If the port-wine stain affects the face and neck, it may have a severe impact on the social, psychological and economic development.

Generalized telangiectasia syndrome refers to telangiectases that develop in the absence of any preceding or coexisting cutaneous or systemic disease.

Different presentations of primary telangiectases have been arbitrarily classified as distinct syndromes, designated by terms that often are descriptive based on inheritance, age of onset, anatomic distribution, morphology, prognosis, or associated findings. No recognized nomenclature exists for these telangiectatic disorders. Generalized essential telangiectasia (GET) refers to one syndrome of acquired primary telangiectases that are so termed because of their widespread anatomic distribution. The pathophysiologic factors causing blood vessel dilatation in GET are yet to be elaborated. Familiar cases have been reported with an autosomal dominant pattern of inheritance. Unlike other laser sources and intense pulsed light devices, which relies on selective absorption of broadband light by hemoglobin, the 1540nm wavelength has a direct thermal effect on the dilated vessels in the upper dermis with a random pattern of coagulation and a progressive discoloration of the vascular lesions. In this way we can avoid an extensive thermal damage to the skin due to the excess of haemoglobin present in these lesions.

The treatment is performed in the office. The treatment area is cleansed with a mild abrasive cleanser. The laser head is moved over the treatment area, using an overlapping technique, so that a total of 3-4 passes are made over each

area. The skin is kept cool during the treatment. The energies and pulse durations varies. 10-13 mJ/spot with a pulse duration of 7-10 ms. The end point after each session is a remarkable discoloration of the vascular lesion followed by a mild redness. The interval between sessions is about 2 weeks and the number of sessions vary from 4 to 7 according to the lesion severity and extension. After the treatment cool compresses must be applied and only moisturizers and zinc sunscreens used.

Acne scars

Acne is one of most common skin conditions in the world. Nearly 80 percent of people aged 11 to 30 years have acne, most often on the face, chest and back. However, acne is not restricted to any age group; adults in their 20s, 30s and even into their 40s can get acne. Most cases of acne responds to treatment and clears up without leaving scars. Healed acne does leave scars in some people, however, and it is not easy to predict who will have scars after acne and who will not. Severe, inflamed, cystic acne always leaves scars after healing, but in some people even superficially inflamed acne can result in scarring.

Whether acne scarring is deep or superficial, extensive or scattered, the esthetic result can be less than desirable and even disturbing. Acne scars can give the skin an "old" look. Scars may also contribute to an appearance of age as the skin loses its elasticity over the years.

A number of treatments are available to remove or improve acne scars.

Acne scars result from two types of tissue response to the inflammation of acne: (1) increased tissue formation, and (2) loss of tissue. Increased tissue formation are scars caused by increased tissue formation by a build up of collagen in the skin. These are called hypertrophic and keloid scars.

Scars resulting from loss of tissue are more common than scars resulting from increased tissue formation. The last type of scars will benefit of fractional photothermolysis.

The treatment is performed in the office. The treatment area is cleansed with a mild abrasive cleanser and 8% glycolic acid lotion. The laser head is moved over the treatment area, using an overlapping technique, so that a total of 4 passes are made over each area. The skin is kept cool during the treatment. The energies and pulse durations varies. 8-12 mJ/spot with a pulse duration of 6-10 ms. The interval between sessions is about 2 weeks and the number of sessions vary from 4 to 5 according to the lesions severity and extension. After the treatment cool compresses must be applied and only moisturizers and zinc sunscreens used.

Stretch marks or striae

Stretch marks or striae are a form of scarring on the skin with a silvery white hue. Stretch marks are generally associated with pregnancy, obesity, bodybuilding, puberty, and intense physical activity. They result from overstretching of the skin, which disrupts the normal production of collagen, causing a scar. They first appear as reddish or purple lines, but tend to gradually fade to a lighter color. The affected areas appear empty and soft to the touch.

This common condition will benefit of fractional photothermolysis.

The treatment is performed in the office. The laser head is moved over the treatment area, using an overlapping technique, so that a total of 3 passes are made over each area. The skin is kept cool during the treatment. The energies and pulse durations varies. 8-12 mJ/spot with a pulse duration of 6-9 ms. The interval between sessions is about 3 weeks and the number of sessions vary from 3 to 4. After the treatment cool compresses must be applied and elasticizing

ointment was applied daily with a massage.

RESULTS

No major complications were seen. Complications of fractional laser are rare. Mild blistering, especially on the chin and temples may occur at higher powers and densities, and these have healed nicely.

Scarring from the procedure is virtually unknown. Hyperpigmentations may occur in darker skinned patients, or patients prone to discoloration. The debris from the epidermal wound forms a "button" of Microepidermal Necrotic Debris (MEND), which sometimes give the treated area a bronzed appearance, until they are shed a few days to a week later.

In the group of 30 patients (25 females, 5 males aged 40-70 years, mean age 55 years) treated for photoaging and pigmentation disorders the average results are 8.0 (1 no result, 9 excellent results) in patient self evaluation and 7.5 in physician's evaluation, with remarkable confirmation of skin texture improvement documented with silicone negative imprints. In the group of 10 patients (8 females, 2 males aged 20-35 years, mean age 26 years) treated for superficial low flow capillary vascular malformations or generalized telangiectasia syndrome the average results are 7.5 (1 no result, 9 excellent results) in patient self evaluation and 8.0 in physician's evaluation. In the group of 5 patients (3 females, 2 males aged 25-50 years, mean age 34 years) for acne scars, the average results are 7.5 (1 no result, 9 excellent results) in patient self evaluation and 7.5 in physician's evaluation. In the group of 10 patients (7 females, 3 males aged 20-30 years, mean age 24 years) treated for stretch marks or striae the average results are 7.0 (1 no result, 9 excellent results) in patient self evaluation and 6.0 in physician's evaluation.

DISCUSSION

The 1540-nm is a mid-infrared wavelength of light, largely absorbed by intracellular and extracellular water in the skin, similar to the way that the light from resurfacing lasers is absorbed. This is not a wavelength at which there is high absorption of hemoglobin or melanin (the other main chromophores targeted in other light-based skin therapies).

The penetration of light into the skin is much deeper than with carbon dioxide or Erbium:YAG (Er:YAG) laser wavelengths. Each pulse of laser light fired into the skin creates a column of coagulated tissue, extending from the surface of the epidermis into the dermis. Fractional photothermolysis treats a small fraction of the skin, leaving intact, undamaged skin around each treated area to act as a barrier and a reservoir for rapid healing. Interestingly, healing of the dermal layers seems to lack an "inflammatory" phase, especially at low microscopic treatment zones densities, although there is histological evidence of collagen remodeling and clinical evidence of skin tightening. Preservation of barrier function following treatment may explain the lack of clinically evident oozing and crusting and the absence of skin infections noted. Even more interestingly, although near- and mid-infrared laser wavelengths are poorly absorbed by melanin, there appears to be a controlled melanin release with melanin concentration in the microepidermal necrotic debris that act as "melanin shuttle", which are subsequently shed.

The very good results showed by the use of this laser in the different clinical conditions described above are very good and demonstrate the versatility of this device with almost no risk for major complications. The device is very easy to use. The learning curve is also very shallow. There is almost no downtime whatsoever and almost no patient discomfort during treatment. It's use in superficial capillary vascular malfor-

mations is a new application for fractional photothermolysis.

CONCLUSION

Fractional photothermolysis is a promising new modality that, based on this preliminary report, produces a consistent level of efficacy for treatment of photo aged skin, acne scars, capillary vascular malformations, stretch marks with significantly reduced side effects.

References

1) **Arias GAM. and Ferrando J. (2001)** Intense pulsed light for melanocytic lesions. *Dermatol. Surg.* **27:** 397.
2) **Behroozan DS, Goldberg LH, Dai T, Geronemus RG, Friedman PM. (2006)** Fractional photothermolysis for the treatment of surgical scars: a case report. *J Cosmet Laser Ther.* **8 (1):** 35-8.
3) **Behroozan DS, Goldherg LH, Glaich AS, Dai T, Friedman PM. (2006)** Fractional photothermolysis for treatment of poikiloderma of civatte. *Dermatol Surg.* **32 (2):** 298-301.
4) **Bitter PH. (2000)** Noninvasive rejuvenation of photodamaged skin using serial, full-face intense pulsed light treatments. *Dermatol. Surg.* **26:** 835.
5) **Fisher GH, Geronemus RG. (2005)** Short-term side effects of fractional photothermolysis. *Dermatol Surg.* **31:** 1245-9; discussion 1249.
6) **Geronemus RG. (2006)** Fractional photothermolysis: current and future applications. *Lasers Surg Med.* **38 (3):** 169-76.
7) **Goldberg DJ. and Cutler KB. (2000)** Nonablative treatment of rhytids with intense pulsed light. *Lasers Surg. Med.* **26:** 196-201.
8) **Goldberg DJ. and Samady JA. (2001)** Intense pulsed light and Nd:YAG laser non-ablative treatment of facial rhytids. *Lasers Surg. Med.* **28:** 141-144.
9) **Hantash BM, Bedi VP, Sudireddy V, Struck SK, Herron GS, Chan KF. (2006)** Laser-induced transepidermal elimination of dermal content by fractional photothermolysis. *J. Biomed Opt.* **11 (4):** 411-415.
10) **Hasegawa T, Matsukura T, Mizuno Y, Suga Y, Ogawa H, Ikeda S. (2006)** Clinical trial of a laser device called fractional photothermolysis system for acne scars. *J. Dermatol.* **33 (9):** 623-7.
11) **Kauvar ANB. and Dover JS. (2001)** Facial skin rejuvenation: Laser resurfacing or chemical peel—Choose your weapon. *Dermatol. Surg.* **27:** 209.
12) **Khatri KA. (2001)** The effects of variable pulse width of Er:YAG laser on facial skin. *Dermatol. Surg.* **27:** 332-336.
13) **Kullick MI. (2001)** Photorejuvenation: Using intense pulsed light technology in a cosmetic surgery practice. *Aesthetic Surg. J.* **21:** 255.
14) **Lauhach HJ, Tannous Z, Anderson RR, Manstein D. (2006)** Skin responses to fractional photothermolysis. *Lasers Surg Med.* **38 (2):** 142-9.
15) **Lin JY, Chan HH. (2006)** Pigmentary disorders in Asian skin: treatment with laser and intense pulsed light sources. *Skin Therapy Lett.* **11 (8):** 8-11. Review.
16) **Manstein D, Herron GS, Sink RK, Tanner H, Anderson RR. (2004)** Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. *Lasers Surg Med.* **34 (5):** 426-38.
17) **Negishi K, Tezuka Y, Kushikata N. and Wakamatsu S. (2001)** Photorejuvenation for Asian skin by intense pulsed light. *Dermatol. Surg.* **27:** 627-631.
18) **Negishi K, Wakamatsu S, Kushikata N, Tezuka Y, Kotani Y. and Shiba K. (2002)** Full-face photorejuvenation of photodamaged skin by intense pulsed light with integrated contact cooling: Initial experiences in Asian patients. *Lasers Surg. Med.* **30:** 298-302.
19) **Prieto VG, Sadick NS, Lloreta J, Nicholson J. and Shea CR. (2002)** Effects of intense

pulsed light on sundamaged human skin, routine, and ultrastructural analysis. *Lasers Surg. Med.* **30:** 82-84.

20) **Rokhsar CK, Fitzpatrick RE. (2005)** The treatment of melasma with fractional photothermolysis: a pilot study. *Dermatol Surg.* **31 (12):** 1645-50.

21) **Ross EV, Miller C, Meehan K. (2001)** One-pass CO2 versus multiple-pass Er:YAG laser resurfacing in the treatment of rhytides: A comparison side-by-side study of pulsed CO2 and Er:YAG lasers. *Dermatol. Surg.* **27:** 709-713.

22) **Rostan EF, Fitzpatrick RE. and Goldman MP. (2001)** Laser resurfacing with a long pulse erbium:YAG laser compared to the 950 ms pulsed CO2 laser. *Lasers Surg. Med.* **29:** 136-139.

23) **Weiss RA, Weiss MA. and Beasley KL. (2002)** Rejuvenation of photoaged skin: 5 years results with intense pulsed light of the face, neck, and chest. *Dermatol. Surg.* **28:** 1115-1119.

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Drug Hypersensitivity

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The allergic response may occur when the body is repeatedly exposed to the chemicals with sensitizing potential. If chemicals are administered via the skin, it is called contact sensitization.
The sensitization response differs from irritation, which is a local reaction.
However hypersensitivity reactions (DHRs) are the adverse effects of chemicals/drugs which, taken at a dose tolerated by normal subjects, clinically resemble allergy. And, being drug hypersensitivity a complex and still widely neglected topic, few true epidemiological data are available. Moreover the available information requires a cautious interpretation being the pathogenic mechanism not yet demonstrated by diagnostic tests.
DHRs affect up to 20% of hospitalized patients and up to 7% of out patients. However because both under-and over-diagnosis are often taken into account for the epidemiological data, it is necessary a cautious interpretation before a definitive diagnose.
Therefore, multicenter studies, both in hospital-based population and in general population, using the same methodologies and definitions, would be of great value in order to have an accurate global perspective about risk factors and possible regional differences and to allow the implementation of better preventive measures for patients. These well-designed epidemiological studies on hypersensitivity drug reactions, and future progress in genetics, will be helpful in identifying patients at risk of developing DHRs reactions, in particular severe ones, and in implementing early preventive measures also.
The first 2 chapters represent *Part I* of Epidemiology of Drug Allergies of the book, divided in 5 parts and 33 chapters.
Chapter 1 and **Chapter 2** describe current data on the incidence, prevalence, mortality, and risk factors of DHRs, providing information on the incidence and demography of severe cutaneous adverse reactions (SCARs) also.
Part II has as subject the *Pathomechanisms genetics and Animal Models* comprehensive of 12 chapters.
Hypersensitivity reaction to drugs, generally manifested in skin, are mediated by cytotoxic T-cells and controlled by immunosuppressive regulatory T-cells. However, a crucial yet poorly understood event in the hypersensivities is the activation of the innate immune system and the subsequent induction and polarization of the adaptive immune response.
Thus assay systems for the *in vitro* identification of contact allergens and predictive risk assessment has to be developed for improving the safety of consumer products and help to avoid the presence of allergens as much is possible.

The Encyclopedia of Ultraviolet Filters

By Nadim A Shaath, Ph.D.

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Skin, serving as a barrier against all the external attacks is the potential target organ of environmental oxidative stress, one of the major determinant in skin aging. Moreover since free radicals are known to cause DNA damage, they are thought to play an important role in tumor initiation and in many other diseases. Thus we cannot ignore the main cause of free radical formation represented from ultraviolet radiation (UVR) and *blue light* too.

The cumulative effects of sunlight, in fact, are manifested as skin cancer and aging of the exposed skin.

To prevent or ameliorate these light-induced reactions, effective sunscreens and photo protective measures are essential. These topical preparations formulated in the form of solutions, gels, creams or ointments absorb or filter out 95% or more of UVB and UVA radiations (UVR) and prevent or minimize the deleterious effects on human skin caused by excessive exposure to sunlight.

By varying the concentration of the sunscreen agents, topical sunscreens or oral photo protective compounds are therefore, formulated to: **(a)** screen out either totally or partially UVR and blue light impinging on the skin; **(b)** prevent photochemical and biochemical reactions in the skin (e.g. photodynamic lipid peroxidation, responsible for the induction of sunburn reaction); **(c)** protect DNA of viable cells of the epidermis and dermis against the formation of cyclobutyl pyrimidine dimers and chromosomal damage that eventually contribute to carcinogenic effects of sunlight; **(d)** prevent or modulate the immunosuppressive effects of UVR exposure; **(e)** prevent drug-induced photosensitivity reactions and various other types of photodermatoses; and **(f)** protect epidermal (keratin) and dermal proteins photo-oxidation (collagen and elastin) against the formation of cross-links responsible for photoaging of skin.

The UV-absorbing or scattering topical sunscreens are usually applied in the form of an invisible film and has to be cosmetically acceptable to most normal individuals and patients providing they are non- irritant, non-sensitizing, stable to UVR, non-volatile, non-staining, non-comedogenic, non-mutagenic, and non-carcinogenic. Moreover the developing of systemic photoprotectants useful to avoid the limitations associated with topical application of photoprotective agents has been formulated also: **(a)** to attenuate the level of actinic insult by acting as a further barrier to UVR and blue light rays; **(b)** to compete with the target molecules for the damaging products of the offending agent and, therefore, the use of the antioxidants compounds; **(c)** to restore the action of the immunocompetent cells, by the use of immunomodulator compounds; and to suppress the various stages of the inflammatory response.

Many replies to all these described problems are reported on this interesting book constituted by 6 comprehensive and well described chapters.

After an interesting introductory overview on the solar spectrum radiation and the relative effects on the skin (**Chapter 1**), **Chapter 2** reports the main problem relating the efficacy of the sunscreen products, as the mechanism of UV absorption, fundamental to understand their structure activity relationship.

Armed with knowledge outlined the resonance delocalization, isomerization and the basic requirements for ultraviolet absorption, the cosmetic chemist can proceed to design ultraviolet filters with required characteristics for the purpose his product has to have. He has select molecules capable to absorb or scatter the harmful short- wave (high-energy) UV rays (280 to 400 am), converting the remaining energy into innocuous longer wave (lower energy) radiation as the organic UV absorbers, or reflecting it, as the inorganic particulates. However the ultraviolet filters used has to possess a broad-spectrum protection versus both UVB and UVA region and cover possibly the harmful activity of blue light, also. Of course all have to be safe, non comedogenic, non sensitizing or phototoxic and must be approved worldwide by official regulatory agencies.

Thus on **Chapter 3** the *Worldwide regulations* are reported together with all ultraviolet filters permitted to be used in USA, EU, Japan, Australia, Canada, China, New Zeland, Mercosur, South Africa, South Korea and Asia. As shown in the different 15 tables reported, numerous are the regulations on sunscreens from one country to another. Therefore the armonization of sunscreen international regulations is an important and thorny problem to be solved.

Chapter 4 reports and discusses a briefly review of the techniques used for the quality control procedures for ultraviolet filters, as well as for finished sun care cosmetics containing UV filters.

All quality parameters and products specifications are based on the use and maintenance of acceptable product standards. Quality parameters can be objective or subjective and the training of the staff is pivotal to consistently evaluate products certain to meet the customer's expectations.

The key subjective criteria used for finished sunscreen products are color, odor, and appearance. Key physical parameters include specific gravity, refractive index and viscosity; among the key chemical parameters are water, pH, and actives (UV filters) content.

Depending on the UV filters used in a product, they can be determined by spectroscopy for inorganic and liquid chromatography for organic materials. Other specific analysis such as *Mass Spectrometry, Atomic emission Detector, X-ray Photoelectron Spectroscopy,* and *X-ray Fluorescence Spectroscopy* are reported.

However QC procedures must ensure that the sunscreen product is produced consistently throughout the life cycle of the product, to have the quality definition that relates to consumer acceptance.

Since the cosmetic industry began formulating a myriad of new sunscreen active agents into an array of functional products, it has become necessary for the cosmetic chemist to know more about the chemical structure and reactivity of UV filters and their potential interaction with other ingredients in the sun care cosmetic formulations.

Chapter 5 reports a compendium data on *Ultraviolet Filters in Commerce Worldwide*.

Two compilations for all ultraviolet filters are included. The first is comprised of tables 17-19. Table 17 lists the regulatory data for the UV filters used worldwide. Table 18 contains the chemical and optical properties. Table 19 lists the manufacturers and distributors of these compounds.

The second compilation is the *Compendium of Global Ultraviolet Filters* alphabetically listed by their (INCI) name. Each of the 55 UV filters reported has a product identification listing its molecular formula, molecular weight, chemical abstract service (CAS) number, the EINECS number, its

USAN name (United States Adopted Name), other common names as well as trade names and suppliers. The final **Chapter 6** reports the major manufacturers and distributors worldwide listed by their web sites (tables 20 and 21).

The sunscreen market is expanding dramatically in response to the alarming increase in the incidence of skin cancer worldwide. Excessive exposure to sunlight can lead, in fact, to increase risk of basal and squamous cell carcinomas as well as decreasing the elasticity of the skin. However the intensity and duration of exposure to visible light plays also an important role for mental wellbeing and regular circadian rhythms.

Thus, we should not forget that the correct exposure to sunlight has many beneficial effects when used in an intelligent fashion. At this purpose the correct use of topical sunscreens and well formulated diet supplements may be the best way to take the positive part of the sunrays addressing the concerns of the medical community.

This new *Encyclopedia of Ultraviolet Filters* will be useful to formulate or reformulate more and specialized active sunscreen products for children, sport or for the elderly population.

Moreover by topical application of these well-formulated products is now possible to improve the rate of repair of DNA, to stimulate melanin production and to boost the immune response just to improve the quality of life. Thus all the data reported on this book should be useful as guidelines to formulate different oral and topical treatments appropriate to stimulate endogenous and exogenous protective and reparative sunscreens before and after the solar insult. Therefore this interesting UV-filter- Encyclopedia will be also an useful guide in sun care formulations for both the chemical and medical community who want to know all the sun-filters used today to prevent and treat both healthy people or people affected by dermatological diseases. Nevertheless, written for cosmetic chemists, this book could be useful also for people involved in marketing, and for university researchers whishing to share their technical knowledge with the industries in order to better understand all the aspects connected with the setting up of the sunscreen products.

P. Morganti
Editor-in-Chief

In copertina / Front cover

Cristalli di Luteina al microscopio elettronico a scansione (SEM). *Su gentile concessione* dell'Istituto di Morfologia Umana Normale, Università Politecnica delle Marche, Ancona, Italia.
Chrystals of Lutein, scanning electron microscopy (SEM). *On kind permission of* the Institute of Human Normal Morphology, Università Politecnica delle Marche, Ancona- Italy

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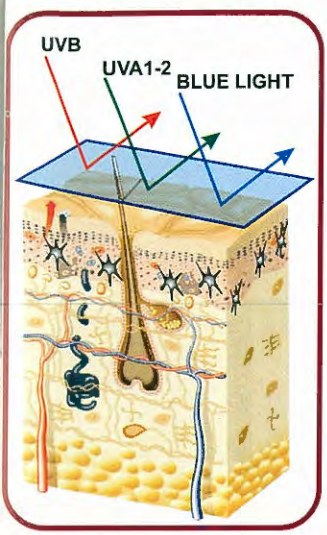

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