

Journal of applied cosmetology

OFFICIAL JOURNAL OF

international society of cosmetic dermatology

THE 8th ISCD INTERNATIONAL CONGRESS
"announcement inside" 3

www.iscd.it

INTERNATIONAL
EDIMME



Volume 24 - Number 3
July/September 2006

ISSN 0392-8543 Spediz. Abb. Postale 70% Filiale di Roma

BETAEFFE *plus*

■ ■ ■ **Antiage** ■ ■ ■

**Tutti i giorni
Tutto l'anno**

**Every day
All year**



1 capsule in the evening



Mavi Sud srl - V.le dell'Industria, 1 - 04011 Aprilia (LT)
Tel. 06.9286261 - Fax. 06.9281523 - info@mavicosmetics.it

LINEA BETAEFFE

MAVIGRAPHICS by G. FORNITI



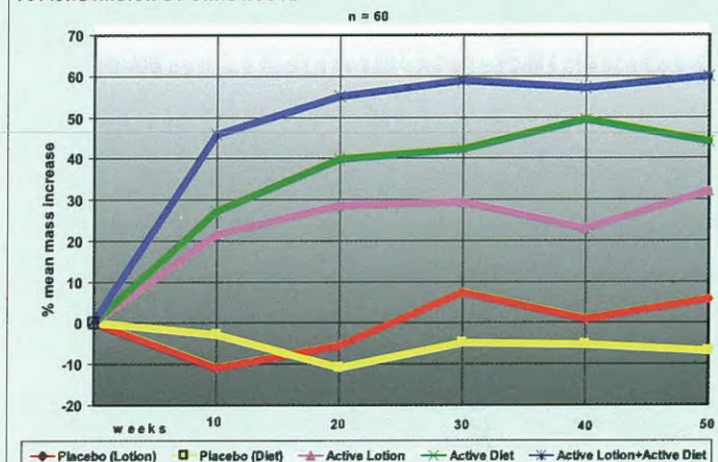
Clinical studies demonstrated Bioesse efficacy to treat mild to moderate hair loss in the frontal parietal scalp ^{(1),(2),(3)}

Increases hair mass and number in a short period ^{(1),(2),(3)}

BIOESSE

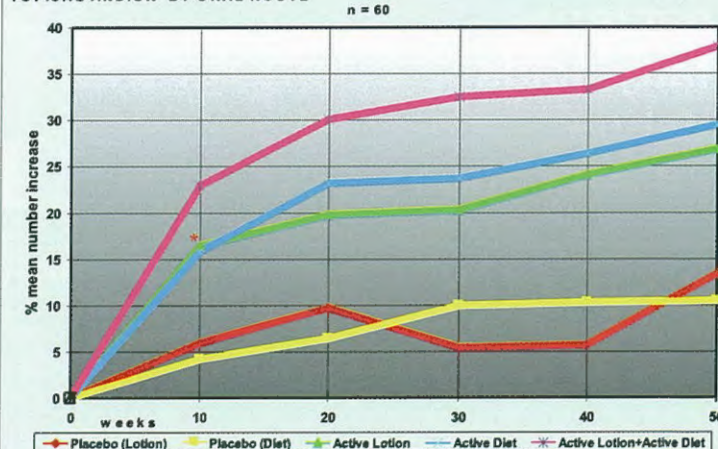
60%
increase in
hair mass

MEAN PERCENTAGE VARIATION OF TOTAL HAIR MASS PER cm^2 OF PATIENTS WITH ANDROGENETIC ALOPECIA TREATED BY GELATIN-CYSTINE AND SERENOA REPENS TOPICAL AND/OR BY ORAL ROUTE



38%
increase in
hair number

MEAN PERCENTAGE VARIATION OF HAIR NUMBER PER cm^2 OF PATIENTS WITH ANDROGENETIC ALOPECIA TREATED BY GELATIN-CYSTINE AND SERENOA REPENS TOPICAL AND/OR BY ORAL ROUTE



NO SIDE EFFECT WAS RECORDED

1) Morganti P, Fabrizi G, James B, Bruno C, *J. Appl. Cosmetol.* 16,57,1998

2) Fabrizi G, Morganti P, (1999), *SÖFW-Journal*, 125, 2/3 :10-13

3) Morganti P, (1999), *Eurocosmetics* 9: 30-32

MAVI sud srl
V.le dell'Industria, 1 - 04011 Aprilia (LT) Italy
Tel. 06/9286261 - Fax 06/9281523
E-mail: info@mavicosmetics.it
URL: http://www.mavicosmetics.it

THE DAILY TREATMENT TO

the most efficacious nano-structured combination

ZEROAC[®]

nano-CREMA[®]

a topical short/ long term
therapy to treat acne lesions

Lifts from the follicle

- sebum
- bacteria
- fatty acids
- debris
- inflammation



proven efficacy with high

**fragrances - colours -
NON PHOTO-**

- 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



Applied science in skin care

Mavi sud srl - V.le dell'Industria, 1 - Aprilia (LT)



COMPLEMENT ACNE THERAPY

azelaic acid and phosphatidylcholine

ZEROAC[®] AQUASFERA

a daily foaming wash to
control sebum and acne



Proven activities

- gentle cleansing
- exfoliating
- sebum balancing
- hydrating
- softening

tolerability in acne therapy

preservatives - and oil free
SENSITIZING

ne therapy, In print on SOFW - P. Morganti, G. Fabrizi, X. Z. Feng (2002), A new delivery system to improve acne therapy, *Microcosmetics*, 10 (5): 33-37 - D. Raskovic (2002) The Soluble Azelaic Acid To Increase The Anti-Acne Activity Of Phosphatidylcholine, Presented at the 2nd World Conference on Medical Esthetics & Cosmetology, Beijing, July 19-22, 2002 - E. Berardesca, P. Morganti (2002) Topical Phospholids In The Treatment Of Acne, Presented at the 2nd World Conference on Medical Esthetics & Cosmetology, Beijing, July

Applied science in skin care

- e-mail: info@mavicosmetics.it - www.mavicosmetics.it

mavi

MAVI
Unica Azienda Vincitrice tra
le Industrie Cosmetiche Italiane

the Only Winner among
the Italian Cosmetic Industries

BEST INNOVATOR 2005



BIOLOGICAL AND
CLINICAL STUDIES



INNOVATIVE
FORMULATION



PACKAGING STUDY



LABELLING
REQUIREMENTS



MAVI
Clinically Correct Cosmetics

FOR MORE INFORMATION:
MAVI sud - V.le dell'Industria, 1 - 04011 Aprilia (LT) Italy
Fax. 39 06 9281523 - e-mail: info@mavicosmetics.it

www.mavicosmetics.it

IGIENE INTIMA

INTIMATE CARE

ELAGENO[®] donna MICOSPUMA

ANCHE IN PRESENZA DI FLOGOSI

ACTIVE ON PHLOGOSIS ALSO

SAFETY AND
EFFICACY

with or without rinsing

SICUREZZA
ED EFFICACIA

con o senza risciacquo



RESEARCH
&
INNOVATION



epitelio vulvare con flogosi
vulvar epithelium with phlogosis

www.mavicosmetics.it
info@mavicosmetics.it

REFERENCES:

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: results of post operative treatment with vitamin A+E gelatin mixture *J. Appl. Cosmetol.*, 9, 73-76 - 3) - Morganti P, Lanzone A, Veri L. (1998) A new diffusion system through the mucous membranes, skin and hair, *J. Appl. Cosmetol.*, 16, 45-50 - 4) - Lanzone A, 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 trends in Cosmetic Science, Conference Proceedings, Verlag, H. Ziolkowsky GmbH, pp. 44-50 - 6) - Beyer N, Driller H, Büniger (2000) Ectoin an innovative, multifunctional active substance for the cosmetic industry, *SOFW- Journal*, 126, 26-29 - 7) - Glana F, Dionisi B, Lipka P, Ronca S. (2003) Fisiologia e Omeostasi del distretto vulvo-vestibolo-vaginale, In *Trattato di Ginecologia Vulvare*, vol. I, pag.47-60, SEE- Firenze



innovaLazio2005
mavi
clinically correct cosmetics

AVI sud - V.le dell'Industria, 1 - 04011 Aprilia (LT) Italy - Tel. 06.9286261 - Fax. 06.9281523

BETAEFFE

BETAEFFE *plus*

per contrastare il crono e il fotoinvecchiamento cutaneo
to fight chrono and photo aging

BETAEFFE *complex*

per modulare le discromie cutanee di qualsiasi origine
to attenuate hyper and hypo pigmentation

BETAEFFE *fast*

per accelerare l'abbronzatura fotoproteggiendo occhi e seno
to accelerate suntan protecting eyes and breast

■ **Effective**
■ **Synergic**
■ **Safe**



■ **THE INNOVATIVE COMBINED** ■
SKIN PROTECTION BY ORAL ROUTE



MAVI sud - V.le dell'Industria, 1 - 04011 Aprilia (LT)
Tel. 06.9286261 - Fax. 06.9281523
www.mavicosmetics.it - info@mavicosmetics.it

EDITOR-IN-CHIEF

P. MORGANTI, Ph.D.
Secretary General
International Society of Cosmetic Dermatology
Via Innocenzo XI, 41 - 00165 Roma (Italy)
E-mail=info@iscd.it

EDITING ASSISTANT

M.L. NUNZIATA
Via Innocenzo XI, 41 - 00165 Roma (Italy)
Fax +39-06-92.81.523
E-mail=info@iscd.it

ASSOCIATE EDITORS

HONG-DUO CHEN, MD
Professor of Dermatology
No.1 Hospital of China Medical University
Shenyang 110001, China
E-mail : chenhd@cae.cn

C. JACOBSON, M.D.
Past President - International Society of Cosmetic Dermatology
3600 Gaston Ave. Suite 1051 Dallas
TX 75246 USA
Fax +1-214-8241900

SCIENTIFIC SECTIONS AND EDITORIAL BOARD

Cell and Tissue Culture

G. Biagini (I)
L. Di Silvio (UK)
N. Stark (USA)

Molecular Biology

L. Bruckner-Tuderman (D)
V. Calabrese (I)
T. Krieg (D)
J. Uitto (USA)

Skin Biology

B. Berra (I)
M. Ponc (NL)

Photobiology

H. Honigsmann (A)
F.P.Noonan (USA)
Y.K.Park (Korea)

Skin Immunology

A. Giannetti (I)

Skin Permeation

J.P. Marty (F)
G. Puglisi (I)

Skin Pharmacology

F.H. Kemper (D)
R. Paoletti (I)

Skin Toxicology

S. Paglialunga (I)
M.G. Rozen (USA)

Skin Ageing

S. Jablonska (PL)
M. Noszczyk (PL)
M. Verschoore (F)

Natural Cosmesis and Balneology

G. Agostini (I)
B.R. Balda (D)

Non-Invasive Methods and Biotechnologies

H. Tronnier (D)
W. Gehring (D)
U. Heinrich (D)
E. Berardesca (I)
P. Elsner (D)

Skin and Cosmetic Microbiology

J. Kabara (USA)
D.Orth (USA)
D. Steinberg (USA)

Skin Bioengineering

L. Andreassi (I)
L. Rodrigues (P)
P. Elsner (D)

Allergy Testing

F.K.E. Andersen (NL)
A. Sertoli (I)
Chundi He (CHINA)

Cosmetic Manufacture and Control

L. Nieta (SA)
A. Parsons (SA)
H.C. Roos (SA)

Cosmetics and Fragrances

G. Angelini (I)

Cosmetics and Environment

Retno I.S. Tranggono (Indonesia)
P. Suvanprakorn (Thailand)

Aromatherapy and Natural Raw Materials

G. Salvatore (I)

Cosmetics' Safety Evaluation

E. Chiaccherini (I)

**Clinical Investigations
in Cosmetic Dermatology**

H. Maibach (USA)
Xing-Hua Gao (CHINA)
Hong-Duo Chen (CHINA)

Oral Mucosa and Dental Care Problems

E. Benagiano (I)

Nail Care Cosmetics

R. Baran (F)
B. Richert (B)
A. Tosti (I)

Hair Care Cosmetics

S. Calvieri (I)
W.A.D. Griffiths (UK)
C.E. Orfanos (D)

Cosmetics and Skin Disorders

V. Mordovstev (R)
W. Raab (A)
T. Ruzicka (D)

Plastic and Aesthetic Surgery

P. Palombo (I)

Cosmetic Pediatrics

G. Fabrizi (I)
Y. Kazuya (J)
A. Taieb (F)

Cosmetic Gynaecology

A. Lanzzone (I)
S. Mancuso (I)
M. Massobrio (I)

Trimestrale di Dermatologia Cosmetologica

Quarterly Review of Cosmetic Dermatology

Contents

General Articles

- 105** Multifunctional use of innovative chitin derivatives for skin care
P. Morganti, R.A.A. Muzzarelli and C. Muzzarelli

Original Laboratory Studies

- 115** Bioresonance As A Tool To Predict Contact Dermatitis To Cosmetic Preservatives
L. Montenegro, C. Carbone, M. Millisenda, G. Manuele, E. Giannazzo, G. Puglisi
- 123** Topical application of lignans and phytosterols in seborrheic skin
M. Vettor, P. Perugini, S. Gagliardi, B. Conti, I. Genta, T. Modena, F. Pavanetto

Book Reviews

- 131** Injectable Fillers In Aesthetic Medicine
- 134** Skin Aging

XIII Announcements

MULTIFUNCTIONAL USE OF INNOVATIVE CHITIN NANOFIBRILS FOR SKIN CARE

Pierfrancesco Morganti*, Riccardo A.A. Muzzarelli and Corrado Muzzarelli

*R&D, Director, Mavi Sud Srl, Aprilia, Italy

Institute of Biochemistry, University of Ancona, Italy

Received: July, 2006. *Presented at The VI Health and Textile International Meeting, May 4-5, 2006, Biella, Italy*

Key words: Chitin Nanofibrils; Wound Dressing; Dermal Fillers; Textiles;

Summary

Chitin nanofibrils occur in biological tissues, mainly crustacean and insect exoskeletons, according to structural hierarchies together with proteins and inorganic compounds. They have helped elucidate the chitin structure, considering their high degree of crystallization. Isolated nanofibrils are useful to impart strength to several materials such as soy proteins, natural rubber, poly(caprolactone) and poly(vinyl alcohol). They also find applications in the cosmetic and biomedical areas, particularly for the ordered regeneration of wound tissues and as dermal fillers. The main advantage over the usual chitin powders is their enormous surface area, that enables them to interact effectively with cells, factors, proteins and other compounds.

Riassunto

Le nanofibrille di chitina sono presenti nelle strutture biologiche, principalmente esoscheletri di crostacei e insetti, dove ricorrono secondo gerarchie strutturali con proteine e composti inorganici. Esse sono servite per elucidare la struttura della chitina, essendo altamente cristalline. Una volta isolate, esse possono servire per irrobustire materiali a base di proteine di soia, gomma naturale, poli(caprolattone), e poli(vinil alcol), oltre a trovare applicazioni nel campo cosmetico-biomedico, in particolare per la rigenerazione ordinata dei tessuti delle ferite e come fillers per l'estetica della pelle. Il principale vantaggio rispetto alle polveri di chitina è la loro enorme superficie che consente alle nanofibrille di interagire efficacemente con cellule, fattori, proteine e altri composti.

INTRODUCTION

The presence of crystalline fibrils in the chitinous integuments of arthropods has been elucidated several decades ago: early reports are those by Richards (1951), by Weis-Fogh (1970) and by Rudall (1967). The subject has been dealt with in a chapter of the first book devoted to chitin (Muzzarelli, 1977), in two concomitant books by Hepburn (1976) and by Neville (1975), and in the recent book by Jollès and Muzzarelli (1999).

Let us say that chitin, the most abundant nitrogen compound on Earth, is present in countless forms of life including the yeast *Saccharomyces cerevisiae* commonly used for bread baking, thus we currently eat some small quantity of chitin every day. Certain African and South American populations prepare traditional dishes based on insects, thus they eat large quantities of chitin. Ten Gigatons (1×10^{13} Kg) of chitin are constantly present in the biosphere.

The chitin biosynthetic pathway is the same for all organisms. In chitin, a copolymer of N-acetylglucosamine and glucosamine, the structural unit is chitobiose, that, interestingly is the bridge between the carbohydrate and the protein components of mammalian glycoproteins.

Chitin is highly crystalline, with two main polymorphs, alpha and beta. All chitins are made of chitin nanofibrils (crystallites) embedded into a less crystalline chitin (Muzzarelli and Muzzarelli, 2005).

The present chapter intends to direct attention to the chitin crystallites otherwise called whiskers i.e. highly crystalline chitin nanofibrils. While they have been isolated by various research teams years ago, their purpose has always been the analytical study of their structure, and the behaviour of their suspensions. It is felt that this subject represents at this time an opportunity for a better exploitation of chitin, as well as for innovation in the textile and cosmetic fields.

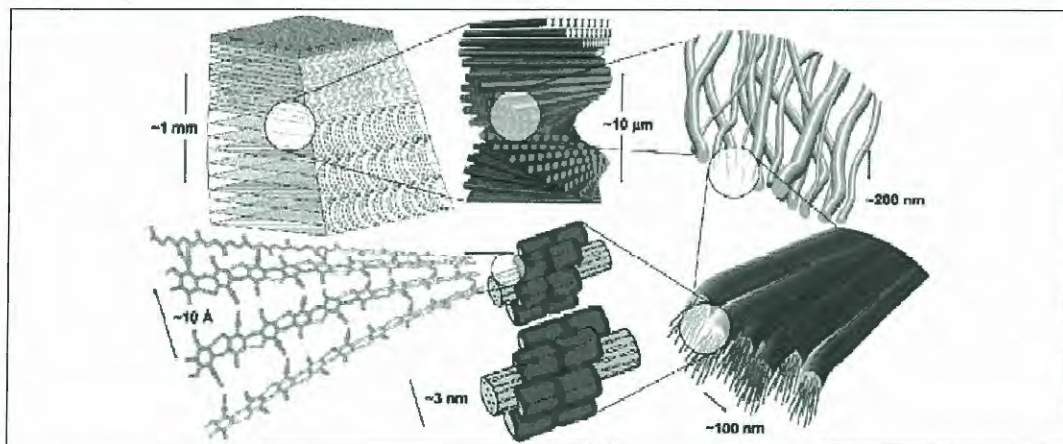


Fig. 1 Structural elements of the exoskeleton material (exocuticle, endocuticle) of *Homarus americanus*. From lower left corner counter-clockwise: The most characteristic feature of the material is its hierarchical organization which reveals six main different structural levels. The first level is the polysaccharide molecule (chitin). The antiparallel alignment of these molecules forms α -chitin crystals. The second structural level is the arrangement of 18–25 polysaccharide molecular chains in the form of narrow and long crystalline units, which are wrapped by proteins, forming nanofibrils of about 2–5 nm diameter and about 300 nm length. The third level is the clustering of some of these nanofibrils into long chitin–protein fibers of about 50–300 nm diameter. The fourth level in the hierarchy is the formation of a planar woven and systematically branched network of such chitin–protein fibers. The spacing between these strands is filled by a variety of proteins and biominerals (crystalline and amorphous calcite in the case of lobster). The fifth level, visible already in an optical microscope, is usually referred to as a twisted plywood or Bouligand pattern. This level is created from the woven chitin–protein planes. Their gradual rotation from one plane to the next creates complex structures (sixth level) which appear as fibril arches when viewed in cross sections (Raabe et al., 2006).

FIBRILS IN CRUSTACEAN TISSUES

Chitin fibers in crustacean shells (and collagen fibers in bones) are associated with calcium phosphate and/or carbonate that diffuse and precipitate after the fibrous component has been excreted and stabilized. In these tissues, the supporting organic component is made of preformed nanometer to micrometer-size elongated particles arranged into supramolecular structures with geometries analogous to those of some liquid crystals. In compact bones, arthropod cuticles, and plant cell walls, these structures have the macroscopic features of a cholesteric phase, except fluidity. In most cases, collagen, chitin, and cellulose, can be extracted from the biological tissues and dispersed in aqueous media to form colloidal suspensions. At appropriate concentrations, liquid crystalline phases can be identified, indicating that rod-like or spindle-like particles tend to align cooperatively in these systems. The particles are rigid and their shape is constant throughout the phase diagram. This makes it easier to understand the influence of different parameters, such as concentration, pH, and ionic strength, on the behavior of the suspensions (Giraud-Guille et al., 2004).

NANOFIBRIL ISOLATION FOR ANALYTICAL PURPOSES

Suspensions of chitin crystallites form cholesteric phases. Revol et al. (1993, 1996) investigated the effects of pH and ionic strength on the proportion of nematic phase in biphasic samples and found few changes because the contribution of the crystallites themselves is large. Comparisons of experimental data to theoretical predictions were also made using Onsager's treatment and showed a reasonable qualitative agreement.

Suspensions of chitin crystallites were prepared by acid hydrolysis of technical grade crab chitin. Similar results could also be obtained with shrimp as well as a variety of other chitin sources. Typically, 5 g of dry chitin powder were treated with 100 ml 3 M HCl at the boil (104°C) for one hour. The sample was then washed with distilled water by successive low-speed centrifugation-dilution cycles until the supernatant reached a pH of about 2. At this pH, the coarse dispersion from the residue of the shell fragments begins to convert spontaneously into a colloidal suspension. Due to acid hydrolysis of the sample, a 30–40% mass loss occurs after one hour of HCl treatment.

Chitin nanofibrils possess enormous surface area per gram (180 m²/g), and their physical form enhances the known chitin performances. The chitin suspensions (20–40 g/l) are biphasic (disordered + cholesteric); at higher concentrations they are chiral nematic. The nanofibrils are slightly cationic (1 g is titrated with 0.16 mmol NaOH). In water, the protonated amino groups and their counter-ions form an electrical double layer around the crystallites; perturbation of the layer by solvents or electrolytes promotes reversible aggregation.

For HCl concentrations between 0.01 and 0.5 mM and chitin concentrations below 2.5 wt %, the samples remain completely isotropic and show no birefringence. Beyond 2.5 and up to 4 wt % chitin, the liquid appears bright in polarized light, and within a few days, a birefringent phase settles at the bottom of the tubes, separated from an upper isotropic one by a sharp interface. When the chitin concentration is further increased, the samples are entirely anisotropic. The boundaries of the biphasic domain only slightly change in this HCl concentration range. Samples of HCl molarity larger than 2.5 mM but lower than 0.01 M only showed complete phase separation in the biphasic gap at low values of C_{tot} . Samples prepared with 0.01 M HCl and

beyond never exhibited bulk phase separation, in test tubes, in the whole range of chitin concentration investigated. Instead, the birefringence increased continuously from dark to very bright samples when viewed between crossed polaroids.

In water, the protonated amino groups and their counterions form an electrical double layer around the crystallites, which prevents flocculation thus yielding a stable colloidal suspension. The electron diffractogram of the preparation corresponds to the α -chitin crystal structure. Thus, the acid hydrolysis treatment has not changed the original crystalline structure of the sample. Unlike that of cellulose the HCl hydrolysis of chitin does not encourage crystallite aggregation into spindle-like bundles. Instead, as the hydrolysis proceeds, just a few free amino groups are uncovered and in their protonated state they provide the electrostatic repulsion which stabilizes the suspension. After standing overnight at room temperature, a two-phase system formed from a 5% suspension: a lower anisotropic phase and an upper isotropic phase (Paillet, Dufresne, 2001).

PREPARATION OF CHITIN NANOFIBRILS

Previous studies on the isolation of chitin nanofibrils failed to provide convincing evidence of the industrial process feasibility because of low yield (ca 20 %), troublesome handling of huge water masses, problems with the drying of final product and aggregation of resuspended nanofibrils. These problems have recently been overcome (Morganti and Muzzarelli).

Chitin and derivatives are approved as functional food ingredients by the Japan's Health Department and are generally recognized as safe by the US Food and Drug Administration.

COMPOSITES WITH POLY(ACRYLIC ACID)

An extension of the above mentioned studies on the incorporation of nanofibrils in poly(acrylic acid) were those aimed at the preparation of composites based on chitin nanofibrils. The orientation of the nanofibrils was obtained by shearing or by magnetic alignment. The X-ray diffraction data for the composites showed uniplanar orientation of the chitin crystallites, with the molecular long axes perpendicular to the direction of the magnetic field (Nge et al., 2003a,b,c).

COMPOSITES WITH SOY PROTEIN ISOLATES

Soy protein isolates (SPI) of desired weight and various content of chitin were mixed and stirred to obtain homogeneous dispersions. The dispersion was freeze-dried, and 30% glycerol was added. The resulting mixture was hot-pressed at 20 MPa for 10 min at 140 °C and then slowly cooled to room temperature. The SPI/chitin nanofibril composites (thickness about 0.4 mm) were thus obtained (Lu et al., 2004).

Compared with a glycerol plasticized SPI sheet, the chitin filled SPI composites increase in Young's modulus and tensile strength from 26 to 158 MPa and 3.3 to 8.4 MPa with increasing chitin content from 0 to 20 wt %. As the chitin nanofibrils increase in the SPI matrix, the composites show greater water-resistance. The improvement in all of the properties of these novel SPI/chitin nanofibril composites may be ascribed to three-dimensional networks of intermolecular hydrogen bonding interactions between filler and filler and between filler and SPI matrix.

More than 75% of the nanofibrils have a length below 300 nm. The average length and width

were estimated to be around 240 and 15 nm, respectively. The average aspect ratio (L/d , L being the length and d the diameter) of these nanofibrils is therefore around 16. These dimensions are close to those reported for chitin nanofibrils obtained from squid pen ($L = 50\text{--}300$ nm, $d = 10$ nm, $L/d = 15$). Sharp and well-defined diffraction rings indicated the crystalline nature (amorphous protein part and amorphous chitin domains had been removed during acid hydrolysis) of chitin nanofibrils present in the suspension. For composite materials filled with crab chitin nanofibrils, interfacial phenomena are important owing to the high specific area of the filler ($\text{ca. } 180 \text{ m}^2 \cdot \text{g}^{-1}$). For example, for a 20 wt % chitin nanofibril filled composite, there are $\text{ca. } 40 \text{ m}^2$ of filler surfaces in 1 cm^3 of the material. An infrared spectrum was taken for a film of chitin nanofibrils obtained by evaporating the suspension in order to display the absence of residual proteins on the chitin fragments. In the carbonyl region, the spectrum presents three strong absorption peaks at 1658, 1622, and 1556 cm^{-1} characteristic of anhydrous chitin. The absence of the peak at 1540 cm^{-1} corresponding to the proteins proves that the successive treatments were strong enough to eliminate all the proteins and to obtain pure chitin (Lu et al., 2004; Nair et al., 2003a,b,c).

COMPOSITES WITH NATURAL RUBBER

Reinforced natural rubber nanocomposites were developed from colloidal suspension of chitin nanofibrils and latex of unvulcanized and pre-vulcanized natural rubber. The chitin nanofibrils, prepared by acid hydrolysis of chitin from crab shells, consisted of slender parallelepiped rods with an average length around 240 nm and an aspect ratio close to 16. After the aqueous suspensions of chitin nanofibrils and rubber were mixed and stirred, solid composite films

were obtained by casting and evaporating methods. For unvulcanized systems a freeze-drying and subsequent hot-pressing processing technique was also used. All the results lead to the conclusion that the processing technique plays a major role in the properties of final composites developed. The chitin nanofibrils form a three-dimensional rigid network only in the evaporated samples, and it is assumed to be governed by a percolation mechanism.

The preparation of the latex requires the use of poloxamer 407 BASF Lutrol F127, a surfactant, in order to obtain a stable suspension (Morin et al., 2002). It is a bloc copolymer of $\text{ca. } 70 \text{ wt } \%$ poly(ethylene oxide) and $30 \text{ wt } \%$ poly(propylene oxide) with number-average molecular weight $\text{ca. } 13000 \text{ g mol}^{-1}$.

COMPOSITES WITH POLY(CAPROLACTONE)

Poly (caprolactone) is a biodegradable, semi-crystalline and thermoplastic polymer used for instance to manufacture suture threads; there is much interest in improving its mechanical properties and biochemical significance. Chitin nanofibrils were obtained from tubes secreted by *Riftia*, a vestimentiferan worm (much longer than those of animal origin: $L = 0.5\text{--}10 \text{ }\mu\text{m}$, $d = 18 \text{ nm}$, $L/d = 120$). The results showed that at high temperature and above 5 % nanofibrils, the chitin network is allowed to restore thus stabilising the mechanical properties of the composite (Morin et al., 2002).

COMPOSITES WITH CHITOSAN OR WITH POLY(VINYL ALCOHOL)

Alpha-Chitin nanofibril-reinforced poly(vinyl alcohol) composite films were prepared by solution-casting technique. The as-prepared nanofi-

brils exhibited the length in the range of 150–800 nm and the width in the range of 5–70 nm, with the average length and width being about 417 and 33 nm, respectively. Thermal stability of the as-cast nanocomposite films was improved from those of the pure PVA film with increasing nanofibril content. The presence of the nanofibrils did not have any effect on the crystallinity of the PVA matrix. The tensile strength of alpha-chitin nanofibril-reinforced PVA films increased, at the expense of the percentage of elongation at break, from that of the pure PVA film with initial increase in the nanofibril content and leveled off when the nanofibril content was greater than or equal to 2.96 wt %. Sriupayo, et al., 2005a. Similar preparations were made with alpha-chitin nanofibrils dispersed in chitosan by solution-casting, thanks to the high filmogenicity of chitosan. The length of the as-prepared nanofibrils ranged between 150 and 800 nm, while the width ranged between 5 and 70 nm, with the average values being about 417 and 33 nm, respectively. The addition of alpha-chitin nanofibrils did not affect much the thermal stability and the apparent degree of crystallinity of the chitosan matrix. The tensile strength of alpha-chitin nanofibril-reinforced chitosan films increased from that of the pure chitosan film with initial increase in the nanofibril content to reach a maximum at the nanofibril content of 2.96 wt% and decreased gradually with further increase in the nanofibril content, while the percentage of elongation at break decreased from that of the pure chitosan with initial increase in the nanofibril content and leveled off when the nanofibril content was greater than or equal to 2.96 wt %. As in the case of chitin nanofibril composites with PVA, both the addition of alpha-chitin nanofibrils and heat treatment helped improve water resistance, leading to decreased percentage of weight loss and percentage degree of swelling of the nanocomposite films (Sriupayo et al., 2005b).

ELECTROSPUN NANOFIBERS MADE OF CHITOSAN WITH PEO OR PVA

Subramanian (2005) evaluated a novel electrospun chitosan mat composed of oriented sub-micron fibers for its tensile property and biocompatibility with chondrocytes (cell attachment, proliferation and viability). Scanning electronic microscope images showed that the nanofibers in the electrospun chitosan mats were indeed aligned and there was a slight cross-linking between the parent fibers. The electrospun mats have significantly higher elastic modulus (2.25 MPa) than the cast films (1.19 MPa). Viability of cells on the mat was 69% of the cells on tissue-culture polystyrene (TCP control) after three days in culture, which was slightly higher than that on the cast films (63% of the TCP control). Cells on the mat grew slowly the first week but the growth rate increased after that. By day 10, cell number on the mat was almost 82% that of TCP control, which was higher than that of cast films (56% of TCP). The electrospun chitosan mats have a higher Young's modulus than cast films and provide good chondrocyte biocompatibility. The electrospun chitosan mats, thus, have the potential to be further processed into three-dimensional scaffolds for cartilage tissue repair (Subramanian et al., 2005). Electrospun nanofibers with average diameters between 20 and 100 nm have been prepared by electrospinning of 82.5% deacetylated chitosan (M-v 1600 kDa) mixed with poly(vinyl alcohol) (PVA, M-w 124–186 kDa) in 2% (v/v) aqueous acetic acid. The formation of bicomponent fibers was feasible with 3% concentration of solution containing up to an equal mass of chitosan. Finer fibers, fewer beaded structures and more efficient fiber formation were observed with increasing PVA contents. Nanoporous fibers could be generated by removing the PVA

component in the 17/83 chitosan/PVA fibers with 1 M NaOH (12 h). Fiber formation efficiency and composition uniformity improved when the molecular weight of chitosan was halved by alkaline hydrolysis. The improved uniform distribution of chitosan and PVA in the fibers was attributed to better mixing mostly due to the reduced molecular weight and to the increased deacetylation of the chitosan (Li and Hsieh, 2005).

APPLICATIONS IN THE BIOMEDICAL FIELD

There are several commercial hemostatic patches and gels available such as:

- Chitin-based: Clo-Sur[®] (Scion), Chitoseal[®] (Abbott), Syvek Patch[®] (Marine Polymer Technologies),
- Chitosan-based: Hemcon[®]
- Collagen-based: Actifoam[®]
- Fibrin-based: Bolheal[®]
- Cellulose-based: Surgicel[®]

The Syvek Patch[®] is made of chitin microfibrils from the centric diatom *Thalassiosira fluviatilis* grown under aseptic conditions. It is seven times faster in achieving hemostasis than fibrin glue, because it agglutinates red blood cells; activates platelets whose pseudopodia make a robust contact with chitin, promotes fibrin gel formation within the patch;

platelets generate force through the clot retraction process and vasoconstriction takes place very soon; and a platelet + chitin + red cells + fibrin plug is formed.

The *T. fluviatilis* microfibrils have been tested in the most demanding and crucial conditions requiring hemostasis, such as splenic hemorrhage, cardiac catheterization, and bleeding esophageal varices, and found superior to all competing products. While the *T. fluviatilis* microfibrils are longer (60 x 01 micron) than crustacean nanofibrils, both chitins used in these instances have

the same molecular weight (2×10^6 Dalton) and acetylation degree (> 0.90).

It is therefore reasonable to expect that the crustacean nanofibrils will be of at least comparable efficacy, while being less expensive because their production technology is much simpler (Kulling et al., 1999. Chan et al., 2000. Fischer et al., 2005).

WOUND DRESSINGS FOR SCAR-LESS HEALING

Early demonstrations of the efficacy of chitin/chitosan in wound healing by Malette et al. (1986) were based on irregularly shaped, high mesh powders. Later freeze-dried layers were adopted that permitted scar-less restoration of vascularized tissue, and complete healing even in aged patients. Microspheres are under study. Chitin nanofibrils are expected to conform to the wound geometry, to have immediate contact with cells in all the usual presentations. Moreover they can be suspended in gels, including chitosan gels, prepared to solidify upon application as a consequence of photocrosslinking reactions, enzymatic reactions, and spontaneous rapid drying.

IMMUNO-STIMULATING PRODUCTS

The intravenous administration of chitin particles has been found to promote macrophage priming in mice. The particles become bound to macrophage plasma membrane mannose / fucose receptors that mediate internalization. They are then degraded by lysozyme. Within 3 days macrophages give a large oxidative burst when elicited with phorbol myristate acetate. The mechanism involves the production of endogenous interferon-gamma by natural killer cells NK1.1 due to macrophage / NK1.1 interaction.

These responses are similar to those generated by microbial particulate components. It is expected that chitin nanofibrils will be more effective in view of their larger surface, and easier to administer (Shibata et al., 1997).

ACTIVATION OF MACROPHAGES

Chitin is phagocytosed and is a potent macrophage stimulator. Oral administration of chitin micro- / nano-particles is effective in down-regulating serum IgE and lung eosinophilia in a mouse model of ragweed allergy. The intranasal application of microgram doses of chitin micro-particles is an effective treatment for reducing serum IgE and peripheral blood eosinophilia, airway hyper-responsiveness and lung inflammation in allergy models. It results in elevation of cytokines, IL-12, interferon-gamma and TNF-alpha and reduction of IL-4 production during allergen challenge (Han et al., 2005).

COSMETIC FILLERS FOR AESTHETIC MEDICINE

Work in progress indicates that chitin nanofibrils suspended in saline can be injected under the wrinkled skin to restore its normal look, with the aid of a G30 needle. The nanofibrils last longer than hyaluronic acid, and do not give rise to any adverse effect. Facial masks can also be manufactured with dibutylryl chitin incorporating chitin nanofibrils (Morganti, Muzzarelli).

The chitin nanofibrils may be incorporated in a number of biological agents capable of different combined functions. The ability of nanofibrils to travel through the intercellular spaces of *stratum corneum* is probably due to their diffusion along the polar head-groups of the intercellular lipids. The intercellular lipid pathway provides, in fact, the primary barrier to the passive diffusion of

water-soluble and lipid-soluble molecules across the *stratum corneum*, whose porous / polar organization may favour their permeation. Advantages are expected from this emerging technology, based on chitin nanofibrils, useful for the development of advanced functional products needed to improve the quality of life.

DRUG DELIVERY

Chitin nanofibrils may be used as drug carriers thanks to their ability to deliver active compounds across the skin. They may be useful as injectable systems in plastic surgery to restore the mechanical stability of the skin, or for the regeneration of any other tissue. The injectable systems have great potential for applications in interactive tissue engineering approaches as they can be designed with a wide range of properties and configurations.

CONCLUSION

The ample evidence of the biocompatibility of chitin, and in particular the impressive range of its favorable effects on human tissues and cells, supports the applicability of chitin nanofibrils in the textile industry, as well as in the cosmeticeutical areas. Chitin nanofibrils exist in nature in regularly arranged structures, having the highest degree of crystallinity. We have developed proprietary technology for the preparation of chitin nanofibrils on a conveniently large scale. The combination of a natural compound such as chitin with our innovative biotechnology will surely increase the production of new medical devices, health textiles and cosmetics in the near future, thus improving the field of regenerative / substitution medicine.

References

- 1) Chan MW, Schwaartzberg SD, Demcheva M, Vournakis J, Finkielstein S, Connolly RJ. (2000) Comparison of poly-N-acetyl glucosamine (P-GlcNAc) with absorbable collagen (Actifoam), and fibrin sealant (Bolheal) for achieving hemostasis in a swine model of splenic hemorrhage. *Journal of Trauma-Injury Infection and Critical Care*, **48**: 454-457
- 2) Fischer TH, Thatte HS, Nichols TC, Bender-Neal DE, Bellinger DA, Vournakis JN. (2005) Synergistic platelet integrin signaling and factor XII activation in poly-N-acetyl glucosamine fiber-mediated hemostasis. *Biomaterials*, **26**: 5433-5443
- 3) Giraud-Guille MM, Belamie E, Mosser G. (2004) Organic and mineral networks in carapaces, bones and biomimetic materials. *Comptes Rendus Palevol*, **3**: 503-513
- 4) Han YP, Zhao LH, Yu ZJ, Feng J, Yu QQ. (2005) Role of mannose receptor in oligochitosan-mediated stimulation of macrophage function. *International Immunopharmacology*, **5**: 1533-1542
- 5) Hepburn HR. (1976) *The Insect Integument*. Elsevier, Amsterdam
- 6) Jollès P, Muzzarelli RAA. (1999) *Chitin and Chitinases*. Birkhauser, Basel
- 7) Kulling D, Vournakis JN, Woo S, Demcheva MV, Tagge DU, Rios G, Finkielstein S, Hawes RH. (1999) Endoscopic injection of bleeding esophageal varices with a poly-N-acetyl glucosamine gel formulation in the canine portal hypertension model. *Gastrointestinal Endoscopy*, **49**: 764-771
- 8) Li L, Hsieh YL. (2006) Chitosan bicomponent nanofibers and nanoporous fibers. *Carbohydrate Research*, **341**: 374-381
- 9) Ln Y, Weng L, Zhang L. (2004) Morphology and properties of soy protein isolate thermoplastics reinforced with chitin whiskers. *Biomacromolecules*, **5**: 1046-1051
- 10) Malette WG, Quigley HJ, Adickes ED. (1986) Chitosan effects in vascular surgery, tissue culture and tissue regeneration. In R.A.A. Muzzarelli, C. Jeuniaux and G.W. Gooday eds., *Chitin in Nature and Technology*, p. 435-442. Plenum Press, New York
- 11) Morin A, Dufresne A. (2002) Nanocomposites of chitin whiskers from *Riftia* tubes and poly(caprolactone). *Macromolecules*, **35**: 2190-2199
- 12) Morganti P, Muzzarelli C. To be published.
- 13) Muzzarelli RAA and Muzzarelli C. (2005) Chitin nanofibrils, In *Chitin and Chitosan: Research Opportunities and Challenges*. P.K. Dutta, ed., New Age Intl., New Delhi, India
- 14) Muzzarelli RAA. (1977) *Chitin*, Pergamon. Oxford
- 15) Nair KG, Dufresne A. (2003a) Crab shell chitin whisker reinforced natural rubber nanocomposites. 1. Processing and swelling behavior. *Biomacromolecules*, **4**: 657-665
- 16) Nair KG, Dufresne A. (2003b) Crab shell chitin whiskers reinforced natural rubber nanocomposites. 2. Mechanical behavior. *Biomacromolecules*, **4**: 666-674
- 17) Nair KG, Dufresne A. (2003c) Crab shell chitin whiskers reinforced natural rubber nanocomposites. 3. Effect of chemical modification of chitin whiskers. *Biomacromolecules*, **4**: 1835-1842
- 18) Neville AC. (1975) *Biology of the arthropod cuticle*. Springer, Berlin
- 19) Nge TT, Hori N, Takemura A, Ono H, Kimura T.. (2003a) Synthesis and FTIR spectroscopic studies on shear induced oriented liquid crystalline chitin/poly(acrylic acid) composite. *Journal of Applied Polymer Science*, **90**: 1932-1940

- 20) Nge TT, Hori N, Takemura A, Ono H, Kimura T. (2003b) Synthesis and orientation study of a magnetically aligned liquid-crystalline chitin/poly (acrylic acid) composite. *Journal of Polymer Science, B: Polymer Physics*, **41**: 711-714
- 21) Nge TT, Hori N, Takemura A, Ono H. (2003c) Phase Behavior of liquid crystalline chitin/acrylic acid liquid mixture. *Langmuir*, **19**: 1390-1395
- 22) Paillet M, Dufresne A. (2001) chitin whisker reinforced thermoplastic nanocomposites. *Macromolecules*, **19**: 6527-6530
- 23) Raabe D, Romano P, Sachs C, Fabritius H, Al-Sawalmih A, Yi S, Servos G, Hartwig HG. (2006) Microstructure and crystallographic texture of the chitin-protein network in the biological composite material of the exoskeleton of the lobster *Homarus americanus*. *Material Science and Engineering*, **A-421**: 143-153
- 24) Revol JF, Li J, Godbout L, Orts WJ, Marchessault RH. (1996) Chitin crystallite suspension in water: phase separation and chiral nematic ordering. In *Advances in Chitin Sciences*, A. Domard, C. Jeuniaux, R.A.A. Muzzarelli and G. Roberts, eds., Jacques André, Lyon; p. 355-360
- 25) Revol JF, Marchessault RH. (1993) *In vitro* chiral nematic ordering of chitin crystallites. *International Journal of Biological Macromolecules*, **15**: 329-335
- 26) Richards AG. (1951) *The Integument of Arthropods*. Univ. Minnesota Press, Minneapolis
- 27) Rudall KM. (1967) Conformation of chitin-protein complexes. In *Conformation of biopolymers*. Ramachandran GN, ed. Academic Press. London
- 28) Shibata Y, Foster LA, Metzger WJ, Myrvik QN. (1997) Alveolar macrophage priming by intravenous administration of chitin particles, polymers of N-acetyl-D-glucosamine, in mice. *Infection and Immunity*, **65**: 1734-1741
- 29) Sriupayo J, Supaphol P, Blackwell J, Rujiravanit R. (2005a) Preparation and characterization of alpha-chitin whisker-reinforced poly(vinyl alcohol) nanocomposite films with or without heat treatment. *Polymer*, **46**: 5637-5644
- 30) Sriupayo J, Supaphol P, Blackwell J, Rujiravanit R. (2005b) Preparation and characterization of alpha-chitin whisker-reinforced chitosan nanocomposite films with or without heat treatment. *Carbohydrate Polymers*, **62**: 130-136
- 31) Subramanian A, Vu D, Larsen GF, Lin HY. (2005) Preparation and evaluation of the electrospun chitosan / PEO fibers for potential applications in cartilage tissue engineering. *Journal of Biomaterials Science-Polymer Edition*, **16**: 861-873
- 32) Weis-Fogh T. (1970) Structure and formation of insect cuticle. *Symposia of the Royal Entomological Society*, **5**: 165-183

Author Address:

Riccardo A.A. Muzzarelli
 Institute of Biochemistry
 University of Ancona
 Via Ranieri 67, Ancona
 60100, Italy
 Email: muzzarelli@univpm.it

BIORESONANCE AS A TOOL TO PREDICT CONTACT DERMATITIS TO COSMETIC PRESERVATIVES

Lucia Montenegro¹, Claudia Carbone¹, Miriam Milisenda¹, Giuseppina Manuele², Edilio Giannazzo², Giovanni Puglisi¹

¹ Department of Pharmaceutical Sciences, University of Catania, Italy

² Department of Physiological Sciences, University of Catania, Italy

Received: July, 2006

Key words: *Bioresonance; Cosmetic preservatives; Allergic contact dermatitis; Patch test; EAV test;*

Summary

Preservatives could be regarded as one of the main causes of cosmetic-induced contact dermatitis. In this work we evaluated the feasibility of using an unconventional diagnostic procedure based on bioelectrical skin responses referred to as Electro-Acupuncture Diagnostics according to Voll (EAV) to predict contact dermatitis to cosmetic preservatives. This technique could provide a fast, easy, non invasive and sensitive evaluation of the irritating potential of cosmetic ingredients, in addition to patch test. Five of the most commonly used preservatives in cosmetic products (methyl paraben, propyl paraben, imidazolidinyl urea, benzalkonium chloride, methylchloroisothiazolinone/methyltioisothiazolinone) were assessed on 46 healthy human volunteers. The results obtained by the EAV bioresonance method were compared to conventional patch tests performed on the same subjects. The percentages of subjects who showed a matching response for the same preservative using both the patch test and the EAV bioresonance method ranged from 74% (methyl paraben) to 85% (benzalkonium chloride). These findings suggest that the EAV bioresonance method could represent a valuable and sensitive tool to evaluate potential contact dermatitis arising from the use of preservatives in cosmetics.

Riassunto

I conservanti possono essere considerati una delle principali cause di dermatiti da contatto causate da prodotti cosmetici. In questo lavoro è stata valutata la possibilità di utilizzare una procedura diagnostica non convenzionale basata sulle risposte bioelettriche della cute, l'elettro-agopuntura secondo Voll (EAV), per prevedere l'insorgenza di forme allergiche da contatto indotte da conservanti di uso cosmetico. Questa tecnica potrebbe consentire di ottenere una valutazione rapida, semplice, non invasiva e sensibile del potenziale irritante di ingredienti cosmetici, in alternativa al patch test. Sono stati, quindi, testati sperimentalmente cinque dei più comuni conservanti cosmetici (metilparaben,

propilparaben, imidazolidinilurea, benzalconio cloruro e metilcloroisotiazolinone/metiltioisotiazolinone) in 46 soggetti volontari sani. I risultati ottenuti mediante il test EAV sono stati confrontati con quelli determinati mediante il patch test. Le percentuali di soggetti che hanno mostrato la stessa risposta con entrambe le tecniche variava dal 74% (metilparaben) all'85% (benzalconio cloruro). Questi risultati suggeriscono che il test EAV potrebbe essere utilizzato con successo per la valutazione delle dermatiti da contatto indotte dai conservanti impiegati nei prodotti cosmetici.

INTRODUCTION

Cosmetics and toiletries may induce several adverse effects among which irritation, contact dermatitis, photosensitivity and pigmentary changes are the most commonly reported (1,2). Cosmetic products contain many kinds of preservatives whose type, concentration and ratio vary among products, manufacturers and countries. Preservatives are regarded as a group of important contact allergens (3). Their activity depends on their chemical reactivity since the low molecular weight of these substances allows their penetration into the skin and their reaction with endogenous proteins (4). The most frequently used preservatives in cosmetic products are the parabens, followed by imidazolidinyl urea, quaternium, formaldehyde releasing preservatives and isothiazolinones (5-7). Their ability to elicit cutaneous adverse effects is routinely evaluated in humans by patch testing.

Many unconventional diagnostic procedures based on bioelectrical skin responses are widely used for allergic diseases. The perturbation of the skin electrical response is evoked by a process of bioresonance (8). According to Voll (9) the electromagnetic frequencies typical of test substances can be sent to the patient via cables and their effects on the organism can be determined by means of electro-acupuncture measurements (EAV). Previous studies showed that the EAV acupuncture technique could be regarded as useful test to evaluate food allergy (8,10). Since this diagnostic technique is fast, easy to perform and non-invasive, it could provide an alternative method to patch test to evaluate the toxicological potential of several harmful substances to which the skin could be exposed.

In this paper we assessed the feasibility of using electro-acupuncture tests according to Voll (EAV) to predict contact dermatitis to some of the most frequently used cosmetic preservatives such as methyl paraben, propyl paraben, imida-

zolidinyl urea, benzalkonium chloride, isothiazolinones. The results obtained by EAV measurements were compared to those obtained by means of conventional patch tests performed on healthy human volunteers.

MATERIALS AND METHODS

Materials

Imidazolidinyl urea (Gram 1) and methylchlorisothiazolinone/methyltio-isothiazolinone (Kathon CG) were a kind gift of Sinerga (Italy). Methyl paraben (MP), propyl paraben (PP) and benzalkonium chloride (BC) were bought from Galeno (Italy). Deionised water was prepared in our laboratories. Hill Top Chambers were supplied by Hill Top Research Inc. (Cincinnati, OH).

Methods

Subjects

Patch tests and EAV tests were performed on 46 healthy volunteers (both sexes, 33 women and 23 men) in the age range 18-60. The participants did not suffer from any ailment and were not on any medication at the time of the study. The volunteers were fully informed of the nature of the study and the procedure involved. They were rested for 15 min prior to the tests and room conditions were set at $22 \pm 2^\circ\text{C}$ and 40-50% relative humidity.

Patch tests

Six sites on the ventral surface of the forearm of each volunteer were demarcated with permanent ink using a circular template (1 cm^2). One site was used as control applying one Hill Top chamber whose cotton pad was saturated with $100\text{ }\mu\text{l}$ of deionised water.

On the other five sites, Hill Top chambers containing $100\text{ }\mu\text{l}$ of an aqueous solution of each preservative under investigation were applied. The following concentrations were used for

each preservative: MP 0,2% w/v; PP 0,2% w/v; Gram 1 0,2% w/v; Kathon CG 0,1% w/v; BC 0,1% w/v. After 24 h, the chambers were removed, the skin surfaces were gently washed with water and the induced erythema was visually scored by an observer using a 0-4 arbitrary scale codified as follows: 0, no variation; 1, slight, diffuse erythema with indistinct outline; 2, more intense erythema with half of the treated site perimeter outlined; 3, marked erythema with a distinct outline of the treated site; 4, severe erythema with a distinct outline of the treated site.

EAV tests

EAV bioresonance tests were performed using a Bicom 2000 (Brugermann GmbH, Germany) equipped with an electrodermal screening devi-

ce (EDS) (Fig. 1). This instrument is basically a galvanometer that measures current variations of acupuncture points and has been used to indicate the energetic state of meridians for the diagnosis and the treatment of body disorders.

The device Bicom measures the skin conductivity at acupuncture points via two electrodes. One electrode is a brass cylinder with a large surface and has to be kept by the subject in his hand; the other electrode (stylus) has a small contact area and delivers a direct current (approximately 1 V), by applying a slight pressure at chosen skin meridian points. The body impedance between the skin point and the hand electrode is then measured: the value is shown on an analogical device with an arbitrary scale reading from 0 to 100 units.

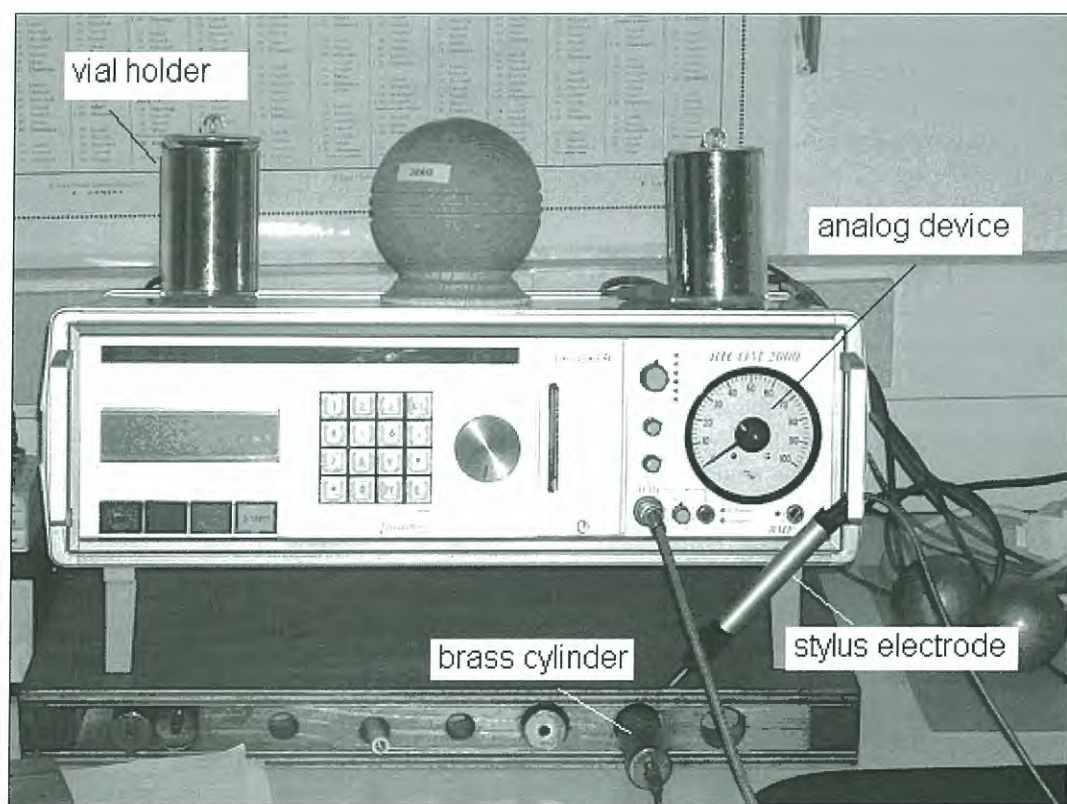


Fig. 1 The instrument Bicom 2000 used to perform EAV tests.

The operator was an experienced acupuncturist physician. As there is a great variability of skin conductivity among individuals due to a number of factors (skin thickness, humidity, blood flux), a baseline level of resistance was determined for each subject by placing a brass electrode in each hand. The subject was considered "in balance" if the instrument readings were at approximately 80% of the full scale. Subjects with readings below 70% and above 90% were disqualified from the study. Then, the level of conductivity of the terminal point of the meridian selected was measured in the absence of the test compounds, and the value was taken as the reference baseline value. A vial containing an aqueous solution of the preservative to be tested was put in a metal vial-holder electrically connected to the hand electrode via Bicom device. Skin conductivity was then measured for each of the substances placed in the vial-holder. A positive reaction to the compound being tested was recorded as a decrease from the baseline value. The same concentrations of preservatives used for the patch test were analysed.

RESULTS AND DISCUSSION

The results of patch tests performed using the cosmetic preservatives under investigation are shown in Fig. 2. Each preservative was tested at the concentration generally used in cosmetic products. The maximum score observed in our patch test was 1 for all the preservatives assayed. The induced erythema was regarded as an index of the irritation potential of the preservatives tested due to the development of irritant (nonimmunologically mediated inflammation of the skin) or allergic contact dermatitis.

Methyl- and propylparaben caused a slight skin erythema in 21,7% and 10,9% of the subjects, respectively. Although the use of paraben preservatives is very popular in cosmetics due to their broad spectrum of activity, low toxicity, regulatory acceptance, biodegradability and low cost, parabens have been reported to cause contact dermatitis in some individuals after skin exposure (11). Furthermore, parabens have been involved in several cases of cutaneous sensitisation although the mechanism of this sensitivity is still unclear (11).

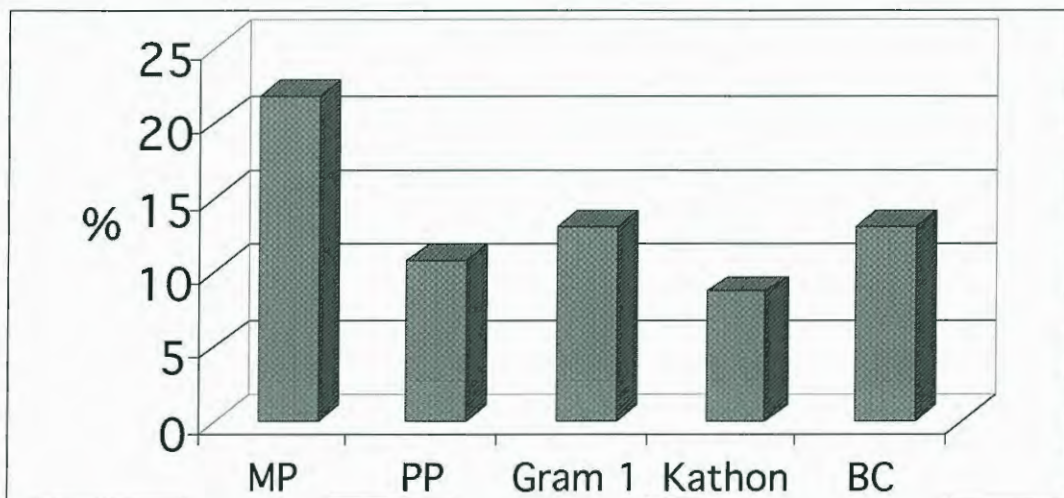


Fig. 2 Percentages of subjects showing a light erythema (score 1) after topical application of cosmetic preservatives (patch test).

In our study, propylparaben proved less irritant compared to methylparaben likely due to its higher molecular weight (MW 180,2) and hence the lower number of millimoles (1,11) applied on the skin surface with respect to methylparaben (MW 151,1; mmol 1,31).

As shown in Fig. 2, using the patch test, Kathon CG resulted the less irritant preservative since only 2,2% of the subjects showed a slight skin erythema after its topical application for 24 h. Kathon is regarded as an allergen with a prevalence of positive reactions of 3% - 8% among patients with contact dermatitis (12). However, studies on humans showed that the induction of allergic contact dermatitis was strongly dependent on Kathon concentration in the product and concentrations as high as 15 ppm in rinse-off products and 7,5 ppm in leave-on products were unlikely to elicit any sensitisation (13-14).

As regards imidazolinyl urea, our patch test results were in agreement with previous works reporting that this preservative is the safest among formaldehyde releasers (15).

Benzalkonium chloride is a cationic surfactant used as preservative in cosmetic and pharma-

ceutical products. Harvell et al. (16) observed that its irritation potential after topical application of a 0,5% solution is higher than that observed for a 0,5% solution of sodium lauryl sulphate. In our study BC proved as irritating as Gram 1 using the patch test likely due to the low concentration used.

As shown in Fig. 3, the percentages of subjects showing a positive response using the EAV test were similar for all the assayed preservatives and ranged between 17% and 24%.

The comparison between the individual reactions observed using the patch test and the EAV technique showed that at least 70% of the responses matched for all the preservatives tested (Fig. 4). Similar findings have been reported by Tsuei et al. (10) comparing the EAV technique results with those obtained by routinely laboratory tests for the diagnosis of food allergy. As shown in Fig. 4, some individuals gave a positive response to the patch test but not to the EAV test. However, these subjects showed a slight erythema also at the control site where a patch without preservative was applied on the skin surface.

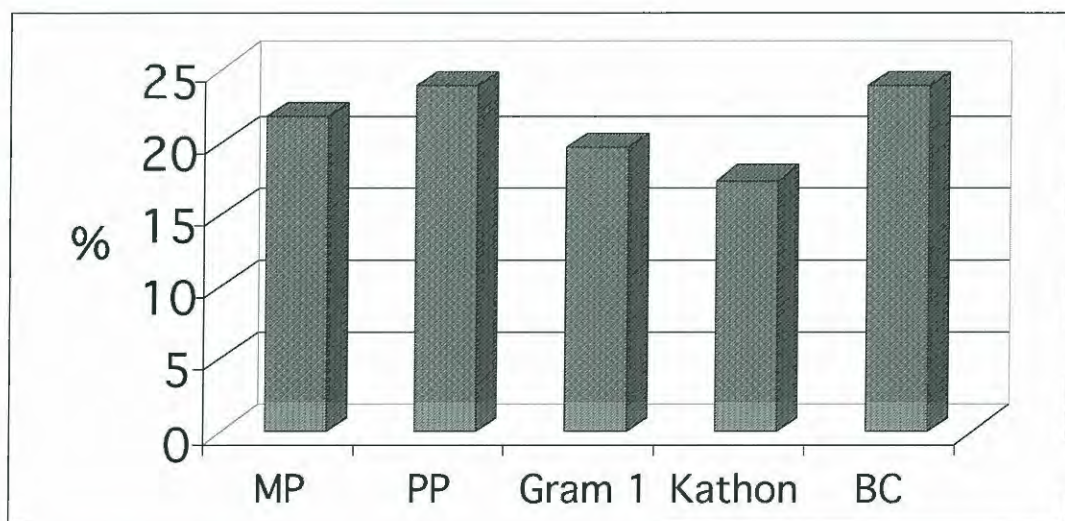


Fig. 3 Percentages of subjects showing a positive response to cosmetic preservatives using the EAV technique.

Therefore, this slight erythema could be attributed to a skin irritation caused by the patch itself rather than to the cosmetic ingredient tested.

Furthermore, some of the subjects who did not show any reaction to a given preservative using the patch test, gave a positive response using the EAV technique. These data suggest that measurements of electrical alterations of the skin may provide more sensitive evaluations of the irritation potential a topically applied substances compared to patch tests.

However, several factors such as the environmental influences, the healthy conditions of the volunteers and the diagnostician skill, need to be better investigated in order to improve EAV test's sensitivity and specificity.

CONCLUSIONS

The ability of identifying the factors that may elicit skin irritation and of performing an exact evaluation of the irritation potential of cosmetic

products and their ingredients are major concerns of cosmetic manufacturers. Therefore, many tests have been developed to assess skin irritation both *in vivo* and *in vitro*.

In this paper, we evaluated the feasibility of using the EAV electro-acupuncture technique for the diagnosis of contact dermatitis to cosmetic preservatives. This technique is safe, non-invasive, time and cost saving. Furthermore, it avoids the actual contact between the patient and the substance being tested, thus eliminating the risk of adverse reactions during the test.

The results of our study suggest that the EAV technique may offer an interesting alternative to other diagnostic methods such as the patch test since the EAV data obtained showed a high degree of compatibility with the patch test. However, further studies are needed for a better comprehension of the factors affecting EAV data in order to obtain close correlations for other cosmetic ingredients so as to investigate their irritation potential by the EAV technique.

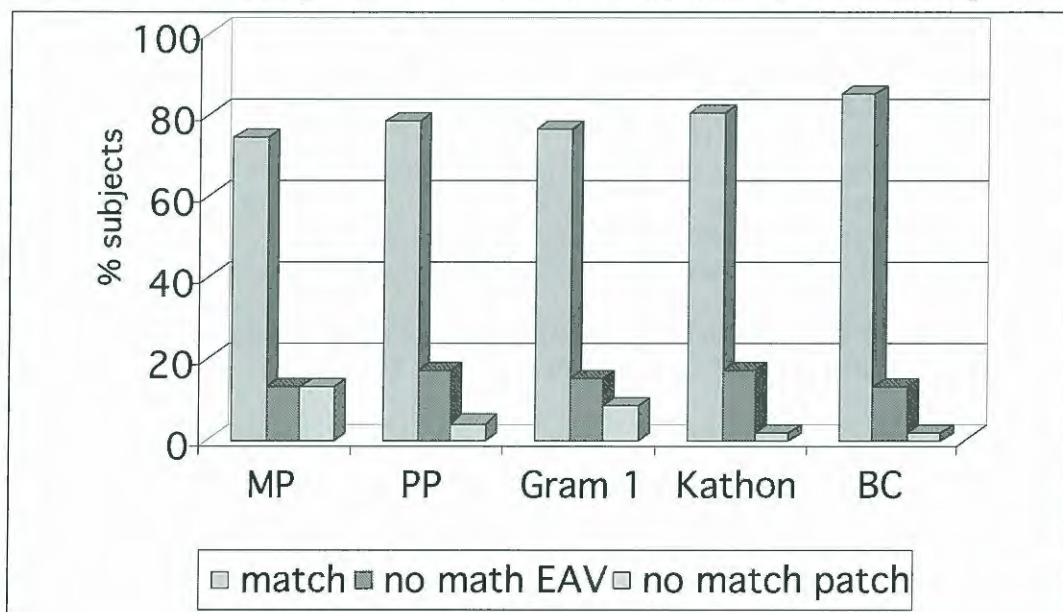


Fig. 4 Percentages of subjects showing the same results using patch and EAV tests (match) or a positive response only to patch test (no match patch) or a positive response only to EAV test (no match EAV) for each cosmetic preservative assayed.

References

- 1) De Groot AC. (1985) Unwanted effects of cosmetics. *J. Drug Ther. Res.* **10**: 793-797
- 2) De Groot AC, De Nater JP, Van der Lende T, Rijcken B. (1987) Adverse effects of cosmetics and toiletries: a retrospective study in the general population. *Int. J. Cosm. Sci.* **9**: 255-259
- 3) Schnuch A, Geier J, Uter W, Frosch PJ. (1998) Patch testing with preservatives, antimicrobials and industrial biocides. Results from a multicentre study. *Br. J. Dermatol.* **138**: 467-476
- 4) Basketter DA, Rodford R, Kimber I, Smith I, Walberg JE. (1999) Skin sensitization risk assessment. A comparative evaluation of 3 isothiazolinone biocides. *Contact dermatitis* **40**: 150-154
- 5) Decker R.jr. (1985) Frequency of preservative use in cosmetic formulas as disclosed to FDA-1984. *Cosmetic Toiletries* **100**: 65-68
- 6) Gruvberger B, Bruze M, Tammela M. (1998) Preservatives in moisturizers on the Swedish market. *Acta Derm. Venereol* **78**: 52-56
- 7) Rastogi S, Schouten A, De Kruijf N, Weijland J. (1995) Contents of methyl-, ethyl-, propyl-, butyl and benzylparaben in cosmetic products. *Contact dermatitis* **32**: 28-30
- 8) Puzzo D, Giannazzo E, Valenti S, Palmeri A. (2001) Measurements of electrical skin parameters by electro-acupuncture techniques and correlation with healthy state. Atti 52° Congr. Naz. S.I.F., Ancona 25-28 settembre
- 9) Voll R. (1975) Twenty years of electroacupuncture therapy using low frequency current pulses. *Amer. J. Acupuncture* **3**: 291-314
- 10) Tsuei JJ, Lehman CW, Lam FMK jr, Zhu DAH. (1999) A food allergy study utilizing the EAV acupuncture technique. *J. Adv. Med.* **12**: 49-68
- 11) Soni MG, Carabin IG, Burdock GA. (2005) Safety assessment of esters of p-hydroxybenzoic acid (parabens). *Food Chem. Toxicol.* **43**: 985-1015
- 12) De Groot AC, Liem DH, Weyland JW. (1985) Kathon CG: cosmetic allergy and patch test sensitization. *Contact dermatitis* **12**: 76-80
- 13) Rastogi S. (1990) Kathon CG and cosmetic products. *Contact dermatitis* **22**: 155-160
- 14) Cardin C, Weaver J, Bailey P. (1986) Dose response assessments of Kathon biocide. (I). Threshold prophetic patch testing. *Contact Dermatitis* **15**: 10-16
- 15) Wolf R, Wolf D, Tuzun B, Tuzun Y. (2001) Contact dermatitis to cosmetics. *Clinics Dermatol.* **19**: 505-515
- 16) Harvell J, Tsai YC, Maibach HI. (1994) An *in vitro* correlation with three *in vitro* assays to assess skin irritation potential. *J. Cut. Ocular Toxicol.* **13**: 171-183

Author Address:

Lucia Montenegro
Department of Pharmaceutical Sciences
University of Catania
V.le A. Doria 6
95125 Catania, Italy
Tel. + 39 095 738 4010
Fax. + 39 095 738 4211
Email: lmontene@unict.it

TOPICAL APPLICATION OF LIGNANS AND PHYTOSTEROLS IN SEBORRHOIC SKIN

Manuela Vettor, Paola Perugini, Simona Gagliardi, Bice Confi, Ida Genta, Tiziana Modena, Franca Pavanetto

Department of Pharmaceutical Chemistry, University of Pavia, Pavia, Italy

Received: August, 2006

Key words: *Lignans; Phytosterols; Seborrheic skin; Clinical evaluation;*

Summary

Topical application of cosmetic products can be helpful in improving seborrheic skin condition. The aim of this work is to evaluate the sebum regulation efficacy of a cosmetic formulation containing REGU®-SEB a compound rich in lignans and phytosterols showing strong 5 α -reductase inhibition "in vitro" activity. Thirty healthy female volunteers with seborrhoea disease in their skin face were involved in the study. Clinical evaluation as well as biophysical non-invasive measurements were taken in order to monitor product effects. The parameters considered were sebum skin content (Sebumeter® SM 815) and skin hydration (Corneometer® CM 825). The results showed improvement of skin conditions with statistically significant reduction of clinical score ($p < 0.001$) and sebum skin content ($p < 0.05$) and with increase of skin hydration ($p < 0.01$).

The formulation was in general well accepted and tolerated even if some subjects judged the formulation greasy and difficult to be absorbed by the skin. Further studies could be carried out on formulations containing lower amounts of lipophilic phase (gels for example) more suitable to seborrheic skins.

Riassunto

L'applicazione topica di prodotti cosmetici può rappresentare un valido aiuto nel migliorare le condizioni della cute affetta da seborrea.

Lo scopo di questo lavoro è la valutazione dell'efficacia seboregolatrice di una formulazione contenente REGU®-SEB un composto ricco in lignani e fitosteroli che presenta "in vitro" elevata attività di inibizione nei confronti della 5 α -reduttasi.

Lo valutazione è stata condotta su trenta volontari sani di sesso femminile con problemi di seborrea cutanea al viso. Al fine di studiare l'efficacia della formulazione sono state utilizzate sia la valutazione clinica che l'applicazione di misure biofisiche non invasive. I parametri indagati sono il contenuto cutaneo di sebo (Sebumeter® SM 815) e l'idratazione cutanea (Corneometer® CM 825). I risultati hanno mostrato un miglioramento delle condizioni cutanee con significativa riduzione dello score clinico ($p < 0.001$) e del contenuto di sebo ($p < 0.05$) e con aumento dell'idratazione cutanea

($p < 0,01$).

La formulazione è stata in generale ben accettata e tollerata anche se alcuni soggetti in studio hanno giudicato la formulazione grassa e di difficile assorbimento. Ulteriori studi dovrebbero essere condotti su formulazioni contenenti minori quantità di fase liofila (per esempio gel), maggiormente adatti alle pelli affette da seborrea.

INTRODUCTION

Seborrheic skin is perceived by many people to be a serious cosmetic problem and it often provokes much concern for people who suffer from it. The skin appears greasy and shiny and is often accompanied by large pores on the cheeks, nose, chin and forehead. Oily skin results from large quantities of sebum being produced by the sebaceous glands, filling the follicular reservoir, and excretion onto the skin surface (1).

Sebum is an oily mixture of lipids, keratin and cellular membrane structures excreted by the sebaceous glands (2). These glands form part of the pilosebaceous unit, i.e. they are always found in connection with a hair follicle. They are found mainly on the face and on the back, and they are the anatomical substrate of *acne vulgaris*. Moreover increased sebum production stimulated by androgens is nearly always the first listed pathogenic factor promoting acne (3).

Sebaceous gland activity depends largely on endocrine stimulation by the androgen hormones. Testosterone is transformed to the active metabolite dihydrotestosterone (DHT) by the enzyme 5 α -reductase, which is present in sebaceous glands.

Acne and its variants from comedones to the cystic nodules of acne conglobata, is the most important and an extremely frequent disease of the pilosebaceous unit. The development of acne is closely correlated to seborrhea and the suppression of sebum production is a powerful therapeutic principle for acne management (4).

The aim of this work is the "*in vivo*" evaluation of the sebum regulation efficacy of a cosmetic formulation containing as active a solution of Argania Spinosa Kernel Oil, Serenoa Serrulata Fruit Extract and Sesamum Indicum Seed Extract (REGU®-SEB). This solution shows "*in vitro*" strong 5 α -reductase inhibition as determined by decreasing DHT levels in reconstructed human epidermis SkinEthic® (5,6) due to its

excellent balance of natural polyphenols and lignans.

MATERIALS AND METHODS

Materials

Argania Spinosa Kernel Oil/Serenoa Serrulata Fruit Extract/Sesamum Indicum (Sesame) Seed Extract (REGU®-SEB) was a gift by Pentapharm Ltd (Switzerland). Sweet Almond Oil was purchased by Balestrini Chimica (Italy), Cetearyl alcohol/Cetearyl/Glucoside, Polyacrylamide/C13-14 Isoparaffin/Laureth-7 were supplied by Seppic Inc (Italy). Methyl/Ethyl/Butyl-parabens, tocopherol, lecyrin, ascorbic acid and citric acid were purchased by Biochim Srl (Italy). Finally Glycerine, Imidazolidinyl urea, Disodium EDTA were supplied by Acef (Italy).

Preparation and characterization of the cosmetic formulation

A formulation containing 5% of REGU®-SEB was prepared mixing lipophilic and aqueous phases warmed at 70°C using a turboemulsifier Silverson SL2T (Silverson machine Ltd, England) at 3300 rpm for 40 minutes to obtain a O/W emulsion. Thermosensitive components were added after slowly cooling of the emulsion at 40°C.

The formulation was characterized for density weighing exact volumes (measured with a syringe) of cream collected in different parts of the container and for viscosity using a Brookfield apparatus RVT 230V (Brookfield Engineering Labs. Inc., USA). Tests were performed to evaluate the stability of the cosmetic preparation to light conserving a portion of the formulation for three months in a transparent glass box and to temperature increase submitting a portion of the formulation to a thermic treatment at 45°C for a period of two weeks.

Sebum regulation efficacy

Thirty healthy volunteers (female subjects, age range 20 to 30) participated in this study. Each woman had at least a moderate degree of seborrhoea in her skin face. The subjects were observed before the treatment and after two weeks always by the same investigator. All were instructed to apply the test cream on their face (T zone) twice a day (morning and evening) for two weeks and they were not allowed to use any other skin care product during the study period. Volunteers were selected according to the following inclusion criteria:

- ◆ female subject aged between 20 to 30 with seborrheic skin as defined by an overall score between 2 and 2.5 on a visual analogue scale 0 (none) to 3 (severe);
- ◆ absence of hypersensitivity against any ingredient of the test cream;
- ◆ discontinuation of systemic/topical treatments (cosmetics, drug products, nutraceuticals) which could interfere with the results of the study at least three weeks before;
- ◆ absence of lesions in investigation anatomical sites;
- ◆ signing of an informed consent.

Pregnant women and nursing-mothers were excluded from the study.

Clinical evaluation

Clinical evaluations were performed on the day one (baseline) and after two weeks (end of the treatment). Control of seborrhoea was performed by a trained investigator using a visual scoring system based on a scale ranging from 0 to 3 according to the following ranks:

- 0 = no clinically relevant seborrheic skin;
- 1 = slightly seborrheic skin;
- 2 = moderate seborrheic skin;
- 3 = obviously seborrheic skin.

Biophysical measurement

The skin surface sebum was controlled by

means of the Sebumeter® SM 815 (CK electronic GmbH, Germany) on the 1st day (baseline) and after two weeks. Determination is based on grease-spot photometric measurement of light transmission through a special tape that becomes transparent in contact with the sebum of the skin surface. A microprocessor calculates the result, which is shown on the display in units from 0 to 350.

Skin hydration of the horny layer was assessed by measuring electrical capacitance of the skin surface by the Corneometer® CM 825 (CK electronic GmbH, Germany). When the probe is applied to the skin (recording time 1 s) the capacitance is displayed in arbitrary units (0-130). The parametric t-test was used to analyze differences between pre- and post-treatment values. A p-value of less than 0.05 was taken as a significant difference, a p-value less of 0.001 was taken as a very significant difference.

RESULTS

Emulsion preparation and characterization

The optical microscopy analysis of the formulation by using methylene blue as colorant highlights that we are in presence of a O/W homogeneous emulsion. The density of the formulation is 1.0052 ± 0.02 mg/ml, close to the density value of water as expected because of the large amount of water present in it. Rheologic analysis shows that the formulation has a pseudo plastic behaviour, with a decrease of viscosity as the applied force increases.

Stability tests show that the formulation is stable to light and to temperature increase, in fact observing the emulsion with optical microscope there are no differences in samples before and after treatment.

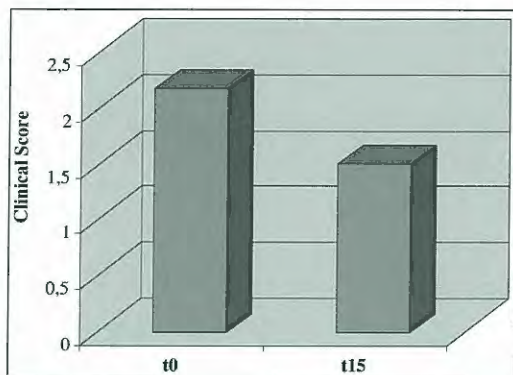


Fig. 1 Results of clinical evaluation. Highly statistically significant ($p = 2.41 \times 10^{-7}$) decrease of seborrhoea clinical score comparing basal (t_0) and after treatment (t_{15}) data.

Sebum regulation efficacy

Results of sebum regulation efficacy are showed in Figures 1 and 2.

The results of clinical evaluation, reported as mean of scores evaluated for each subject before and after treatment, show a significant decrease of the score from 2.167 to 1.500. This reduction suggests an improvement in skin condition. A reduction in sebum skin content is also supported by biophysical non-invasive measurements carried out with Sebumeter® SM 815. Results obtained comparing the average of basal results with those obtained after 2 weeks treatment show a reduction in sebum content from 224.625 to 196.438.

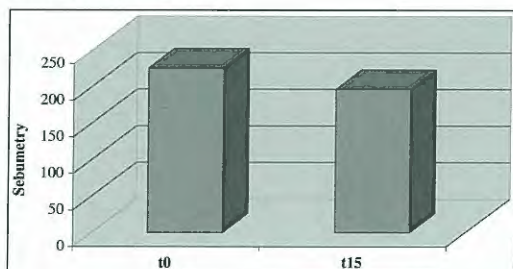


Fig. 2 Results of Sebumeter. Statistically significant ($p = 0.049126$) reduction in sebum skin content comparing basal (t_0) and after treatment (t_{15}) data.

Finally the evaluation of the moisture content of epidermis (Fig. 3) measured by the Corneometer® CM 825 show that the application of the emulsion test cause an improvement in skin hydration.

Hydration increase is probably due not only to the application of an emollient formulation (pseudo-occlusive effect) but also to the humectant function of glycerol that is able to retain the water of the formulation in the horny layer.

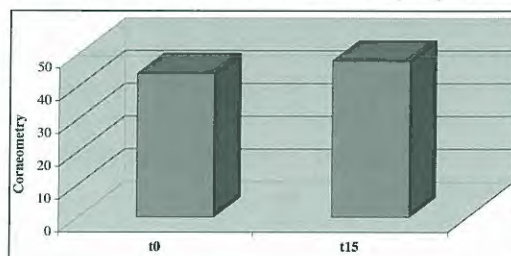


Fig. 3 Results of Corneometer. Statistically significant ($p = 0.002551$) improvement in skin hydration comparing basal (t_0) and after treatment (t_{15}) capacitance values.

DISCUSSION

REGU®-SEB is a compound showing strong “in vitro” activity on 5 α -reductase inhibition.

An “in vivo” protocol was carried out joining clinical evaluation and biophysical non-invasive measurements to evaluate its effective sebum regulation efficacy.

The physician’s visual assessment performed highlight a significant decrease in severity of seborrhoea (30.77% decrease in mean score) after two week of application.

Sebumeter data support this result showing a sebum secretion decrease in the application sites after the treatment. It’s also important that the application of the formulation doesn’t cause cutaneous dryness or other skin disease and Corneometer data show that after the treatment the skin hydration degree results increased.

The product is generally well accepted by the

majority of the subjects in study, even if some subjects judge the formulation greasy and difficult to be absorbed by the skin. There are no adverse events related to the test product during this study.

Results obtained in this pilot study demonstrate the efficacy of the tested formulation in improving skin condition. In conclusion REGU®-SEB show good efficacy in decreasing the severity of seborrhoea and can be considered a helpful compound in the treatment of seborrheic skins.

Further studies could be carried out to optimize the vehiculation of the compound by studying formulations containing less quantities of lipophilic phase (gels for example), more suitable to seborrheic skins.

References

- 1) **Rizer RL (1999)** Oily skin: Claim Support Strategies. In: *Cosmetics: Controlled efficacy studies and regulation*, Elsner P., editor, p.81-91
- 2) **Caputo R., Monti M. (1995)**, La deodorazione. In: *Manuale di Dermocosmetologia Medica*, Raffaello Cortina, editor, p.121-133
- 3) **Youn SW, Park ES, Lee DH, Huh CH, Park KC (2005)**, Does facial sebum excretion really affect the development of acne?, *Brit. J. Dermatol*, **153**:919-924
- 4) **Elsner P (1995)** Sebum. In: *Bioengineering of the skin: Method and Instrumentation*. Berardesca E, Elsner P, Whielm KP, Mailhach HI editors, CRC Press, p.81-89
- 5) **Bernard FX, Barrault C, Deguerce A (2000)** Expression of type 1 5 α -reductase and metabolism of testosterone in reconstructed human epidermis (SkinEthic®): a new model for screening skin-targeted androgen modulators, *Int. J. Cosm. Sci.*, **22**:397-407
- 6) **Pentapharm Ltd. (2001)** Bioalternatives study n° AD010818, Effect of the compound "REGU-SEB" on testosterone metabolism in reconstructed human epidermis

Author Address:

Manuela Vettor
Department of Pharmaceutical Chemistry,
University of Pavia,
via Taramelli 12,
27100 Pavia, Italy
Phone: +39 0382 987363
Fax: +39 0382 422975
e-mail: manuela_vettor@yahoo.it

Injectable Fillers In Aesthetic Medicine

By De Maio M. and Rzany B.

2006. 106 pages. Hardcover

US\$ 139,00 - Euro 99,95

ISBN 3-540-23941-3

Springer-Verlag, Heidelberg

Berlin - Germany

Fax: +49 6221-487-8141

e-mail: Gerda.dietz-jung@springer.com

website: www.springer.com

Over the years, in contrast to the United States, in most countries in Europe and South America many injectable fillers agents have been promoted to cosmetically improve soft-tissue defects and deficiencies.

Some of these agents, such as adulterated silicones and impure paraffines, frequently resulted in cosmetic disasters.

Doing to the great variety and availability of distributed product for novices it can be quite difficult to decide which filler to use for which indications. However for a substance or device to be amenable for soft-tissue augmentation by the general medical community, it must meet certain criteria.

incorrect or indiscriminate It has to have both a high use potential, producing pleasing cosmetic results with a minimum of undesirable reactions, and a low abuse potential, in that widespread and possibly use would not result in significant morbidity.

It must be non-teratogenic, non-carcinogenic, and non-migratory. In addition, the material must provide predictable, persistent correction through reproducible implantation techniques.

Finally, if not autologous, the substance, agent, or device must be EU and/or FDA approved.

To clarify the use of all the fillers sold worldwide this book, divided in 7 chapters, gives an overview on the most common biodegradable and non- biodegradable fillers, their way of using and the most common indications in aesthetic medicine.

Injectable fillers may be grouped according to the degree of degradability as: biodegradable and non-biodegradable.

Their relative advantages and disadvantage are described and discussed on **chapter 1**.

What is important to underline is that no clinical data on efficacy and safety exist for most of the new injectable fillers. Marketing approaches tend to include claims of durability and safety for biodegradable and non- biodegradable products that may not stand the scrutiny of a clinical trial or post-marketing safety studies.

This is because the CE certification doesn't require clinical trials for new filler when comparable substances are already on the market.

Technical ability is fundamental for good medical practice, but the correct selection of patients is mandatory. Thus the risk of dissatisfaction in the cosmetic dermatology patient can be minimized with the correct approach during the initial evaluation.

Patients are prone to ask for procedures that they have heard or read about in **lay** magazines. During the consultation, the physician should be careful not to downplay the extent of risks of the procedu-

re, nor the patient's responsibilities after surgery.

Thus the first consultation is very important, as it gives the physician the opportunity to establish the kind of patient he will be treating. *How can I help you?* Is a good initial question.

The selection of patients is focused on **chapter 2**.

To ensure a safe and efficient procedure, several requirements have to be met. Thus a thorough documentation of all treatment-related data is highly recommended. This will help to improve one's own performance, the patient's satisfaction and however, will be advisable for legal and billing reasons. At this purpose all the general and technical requirements are reported and described on **chapter 3**, by some photos also.

Pain is one of the most fearful experience for human being. Topical anesthetics, infiltration, and nerve blocking have been found helpful in making cosmetic procedures more pleasant and tolerable for the patient. Local anesthesia is defined as the pharmacologic induction of sensory loss in a regional anatomic site, which may be achieved by injectable or topical indications.

It has, of course, many advantages over general anesthesia and is especially desirable in dermatologic and cosmetic surgery, where procedures are typically minimally invasive. Local anesthesia enables cosmetic procedures to occur in an outpatient setting with not only less morbidity and first recovery but also substantially less expense.

However, for a variety of reasons, the vast majority of dermal filler injections are undertaken under insufficient topical or no anesthesia at all.

The preoperative evaluation and the different anesthetic procedures and relative techniques are focused on **chapter 4**.

Soft-tissue augmentation has become increasingly important because more individuals seek aesthetic improvement without major surgical procedures. Naturally, the most common reasons for patients to seek cosmetic surgery include the aging face and treatment of facial rhytides. It is the part of the body most visible to others and typically, next to the hands, it is the most reliable way to determine a person's age.

The difficulty of treatments comes in choosing the proper treatments techniques and meeting patient expectations. The patient evaluation, the relative selection and the treatments of choice are reported on **chapter 5** together with many explicative photographic examples of different procedures.

Although most injectable fillers are usually considered to be safe, adverse reactions do occur.

These reactions vary from persistent erythema and edema to granulomatous reactions on even ulcerations.

Thus the goal should be to reduce the visible impact of the adverse reaction, as amply reported in **chapter 6**.

Efforts to develop the perfect soft-tissue augmentation material and methodologies continue. This is way the today tendency to use combined therapies. As a matter of fact, single therapies have proven to be inefficient in dealing with all the signs that appear with time.

Therefore, as focused on the final **chapter 7**, patients should be educated to understand that the most natural appearance obtainable involves the use of multiple treatments.

With this chapter ends this interesting and agile book, reporting all the last, truthful and practical information for all the clinicians involved in aesthetic medicine.

Its easy reading and the reported material: patient selection, preparations, anesthesia, regional injec-

tions including techniques, combination therapy and complications with treatment, makes it an indispensable tool book for the novice clinicians beginning a filler practice, as well as for those with long experience.

P. Morganti
Editor-in-Chief

Therefore today, aging is a pressing medical and socioeconomic problem, and prevention of premature aging is constantly increasing importance to the general population. Combinations of synergistic antioxidants such as vitamins E, C, melatonin, carotenoids and polyphenols may help to avoid pro-oxidative free radical damage that occur upon UVR, infrared and pollutants exposure. Thus the main strategies to save our skin is photoprotection by UV filters used not only by sunscreens, but in many cosmetic products which are meant for daily use, such as foundations, make-ups, and cream and lotions. Moreover modern sunscreen products combine also UV filters with one or more biologically active molecules as, antioxidants and immuno-suppressants, capable to prevent some of the biochemical and molecular consequences which occur in the skin after UV irradiation has been absorbed.

But a decrease in serum estrogen accompanies aging and may contribute to age-related skin changes such as atrophy, wrinkling, dryness, and poor wound healing.

Studies investigating the supplemental use of estrogen and phytoestrogens compounds in the later years have demonstrated some beneficial effects on skin aging parameters.

Thus the quest to find or develop a systemic photo protectant that would avoid the eliminations associated with topical application of photoprotective agents has been a compelling endeavour. Recent attempts at dietary manipulation show some promise for protection against certain solar-induced effects.

Specific strategies, namely reduction in total dietary fat and, increased supplementation of polyphenols, omega-3 fatty acids and carotenoids, have resulted in protection against some skin cancer and other markers of UV-induced tissues damage.

Beside acting as accessory pigments, carotenoids are associated with photoprotection, being involved in the dissipation of excess light energy through the xanthophyll cycle, quenching excited triple state molecules and single oxygen.

And clinical interventions to combat and correct the destructive effects of solar radiation and pollutants is another important theme focused on this book.

Volume and contour changes are as important or more important than two-dimensional surface characteristics when perceiving a person's age.

In the face, however, the distribution of subcutaneous fat and the unique cutaneous musculature create a third dimension that strongly influences the patient's apparent age. Thus the cutaneous fat and the superficial muscular-aponeurotic system (SMAS) have an enormous impact on apparent age, and can be manipulated by derma-surgeons and other cosmetic surgeons with great benefit. Therefore, lifting and re-suspension, volume reduction and volume addition are employed to restore a youthful appearance in all three dimensions.

At this purpose different techniques are used and combined for face contouring and skin rejuvenation, such as topical photodynamic therapy (PDT), botulinum neurotoxin, different kind of dermal fillers (collagen, hyaluronic acid and some alloplastic materials), chemical peels (alpha and beta hydroxyl-acids) and finally the laser skin resurfacing methodology.

Owing to multiple technological developments and to the resulting range of treatment options, laser applications in dermatology have been expanding rapidly within the past decade. As a consequence, therapists must now select from among several modern laser technologies as they seek aesthetically to improve many of the age-related skin disorders.

Some laser systems are used in attempts either to ablate or vaporize larger surface areas or to thermally influence the dermal tissue while sparing the epidermal surface by sub-ablative energy delivery.

In addition, non-ablative skin rejuvenation techniques such as intense pulsed light (IPL) or radiofrequency (RF) device for non-optical energy delivery as well as photodynamic photo-rejuvenation have remarkably influenced the use of light sources in the treatment of skin aging.

The rapid aging of the populations in the industrial world has created an enormous need for so-called anti-aging remedies.

Because of greatly improved medical science and understanding of our dietary needs it is in many cases possible to reduce, but not eliminate, many of the signs of ageing.

In the 1950s an individual of 50 was considered old. Today an individual in their 60s will frequently still be playing tennis, going to gym or playing golf, imaging playing polo on her/him 80th birthday!

For better understanding the direct relationship between age and wrinkles and the existing possibilities to correct the signs of photodamaged skin, the reading of this interesting book will be of great help to Dermatologists, Plastic Surgeons, Cosmetic Chemists and to all people involved in the new scientific field of Cosmetic Dermatology.

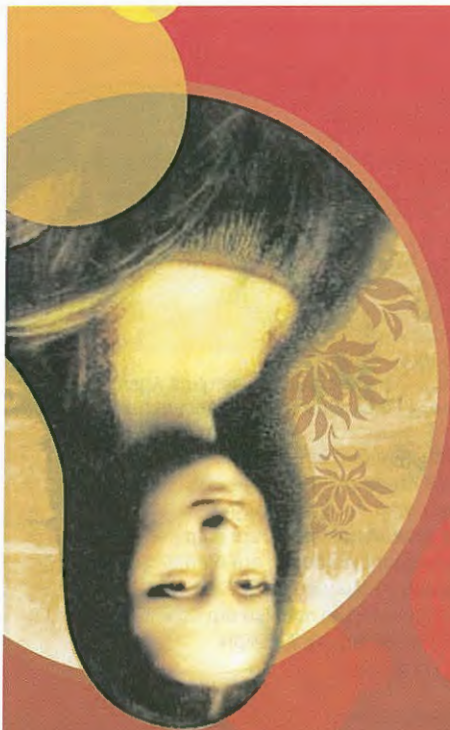
P. Morganti
Editor-in-Chief

The 8th International Congress of International Society of Cosmetic Dermatology

October 20-23, 2007 Beijing China

First Announcement

*Cosmetic Dermatology from West to East:
The Combined Reply for a Global Wellness*



Under the auspices of:

International Society of Cosmetic Dermatology

Organized by:

Chinese Medical Association

Chinese Society of Dermatology

Chinese Society of Medical Esthetics and Cosmetology

For more information please contact the SCS Secretariat
 GT House, 24-26 Rothessay Road, Luton, Beds LU1 1QX
 Telephone: +44 (0)1582 726661 E-Mail: fscsc.scs@btconnect.com



Marketing view of Naturals
 Functional Ingredients
 Natural Actives
 Natural Fragrances
 Supply of Naturals
 Extraction Techniques
 Plants in the Spotlight
 Marketing Banners

EARLY BOOKING DISCOUNT
 When you register before the 15 MARCH 2007



Staverton Park Conference Centre
 Near Daventry, Northants, UK
13-15 May 2007
 The solutions to marketing, formulating and selling natural products. All you need to know about natural products and green trends.



NATURAL
 CONFERENCE

Honorary Organiser
 Anthony C. Dwyer
 FLS FRSH FRSC

100%

THE TECHNICAL CONFERENCE



THE 100% NATURAL CONFERENCE Society of Cosmetic Scientists

Sunday 13th –15th May 2007

*The solutions to marketing, formulating and selling natural products
All you need to know about natural products and green trends*

Staverton Park Conference Centre, Daventry, Northants, UK

Hon Organiser: Anthony C. Dweck FLS FRSH FRSC

The naturals market is complex and growing. The terminology used in this industry is confusing and often misquoted. Comparative shopping is fun, but it only shows you last year's ideas. This conference is a chance to learn all about natural products and how to tailor them to meet the needs of both marketing and technical departments. Organised by Anthony C. Dweck who has 35 years experience in the natural's market and who fully understands the needs for successful natural brands. It will be a technical conference without any jargon and in a form that easily understood and digested.

Marketing view of naturals

Leading companies give their philosophies

Functional Ingredients

All the parts that go to make up the base

Natural Actives

The ingredients that give the claims and benefits

Natural Fragrances

Fragrance that gives aroma and psychological, physical benefit

Supply of Naturals

Searching for the new and exotic sources with marketing excitement

Extraction techniques

A graphic explanation of all the terms used in plant extraction

Plants in the spotlight

A look at the plants that are under investigation for the future

Marketing Banners

Organically grown, fair traded and wild crafted

This is a chance to come and learn how to make the 100% natural product, to learn the truth about the nonsense that is fostered and falsely propagated on the internet and other media sources. The opportunity to pick up samples and supplier literature of all the latest raw materials that will be in next year's leading ranges. It will be a fusion of marketing and technical ideas in simple, easily understood and readily digestible form. A free CD ROM with all the information will be given as a part of the conference package.

PROGRAMME

Sunday afternoon and evening

Tired of travelling all the way to the conference only to be stuck in the bar or hotel room waiting for Monday - then this is a new idea. All late afternoon and most of the evening we will be holding a cabaret style series of presentations. Sit together in a dinner style ambience, chill out from your journey and listen while some of the country's top experts tell you how to formulate a natural product, debunk some of the misinformation on parabens and other vital issues and concepts.

You order a drink and a bar meal or even a club sandwich with cup of tea and join colleagues in a casual and relaxed atmosphere to set the scene for the forthcoming conference. No formality, just a great warm up and stimulation for the action packed days ahead.

An event that will be of huge importance to the new or inexperienced chemist who wants to get a feel for the natural formulation and an equally valuable event for the marketer who wants to understand the mysteries and difficulties experienced by their technical teams.

This is a "Science for Dummies" session and will be tailored to normal people! It will be a chance to meet some of your colleagues and do some networking - in fact we will try and encourage it.

Monday and Tuesday

This conference is designed to do the work for you. All talks will be supplied on the CD ROM and a hard copy will be available for those who like to make notes on the slides they see. We will not allow trade names to be used during the talk, but at the end of the paper, the supplier or speaker will give out a 'goodie' bag that contains samples and literature on the latest materials they are offering.

All supplier papers will be short (15 minutes) and deliver the ideas and concepts rather than the proof (which will be in the handout).

Our proposal is to deliver quality and quantity in a one stop shop and to showcase as many materials as we can in as short a space of time as possible.

In copertina / Front cover

Cellule Neuro 2 A trattate con H_2O_2 e BETAEFFE PLUS 0,5 $\mu g/ml$ (immunofluorescenza con phalloidin, x100). *Su gentile concessione* della Prof.ssa Graziella Biagini, Istituto di Morfologia Umana Normale, Università Politecnica delle Marche, Ancona, Italia

Neuro cells 2A treated with H_2O_2 30 μM and BETAEFFE PLUS 0,5 $\mu g/ml$ (immunofluorescence with phalloidin, x100). *On kind permission of* Prof. Graziella Biagini, Istituto di Morfologia Umana Normale, Università Politecnica delle Marche, Ancona- Italy

Chiuso in tipografia: Ottobre 2006

Journal of Applied Cosmetology published quarterly by INTERNATIONAL EDIEMME, Via Innocenzo XI, 41 00165 Roma, Italy. Direttore responsabile: P. Morganti. Direzione, Redazione ed Amministrazione: Via Innocenzo XI, 41 - 00165 Roma, Italy. Impaginazione e Stampa: Grafica Flaminia, Roma. Copertina: Dr P. Morganti - Roma Italy - Sped. abb. Postale Comma 34 art. 2 Legge 549/95 Roma. Aut. del Trib. di Roma n. 3173/83 del 8-7-83.

MAVI Oil

Nature and Technology in daily hair and skin care to

- **Cleanse**
- **Re-hydrate**
- **Protect**



- for oily skin and hair
- for dry skin and hair
- for sensitive skin and hair

www.mavicosmetics.it
info@mavicosmetics.it

innovaLazio2005

mavi

MAVI sud srl - V.le dell'Industria,1 - Aprilia (LT) - Italy - Fax. +39.06.9281523

clinically correct cosmetics

NEL TURNOVER CELLULARE ALTERATO
IN ABNORMAL CELLULAR TURNOVER

BIOESSE

zinco GEL

shampooskin



visible results

LESS

- SQUAMAE
- SEBUM
- ITCH

risultati visibili

MENO

- SQUAME
- SEBO
- PRURITO

proved results

- DANDRUFF
- SEBORROIC DERMATITIS
- LOCALIZED PSORIASIS

risultati sicuri

- FORFORA
- DERMATITE SEBORROICA
- PSORIASI LOCALIZZATA

FREE of fragrance, colorings, preservatives



References

Muscardin L, Morganti P, Tartarini S, Valenzano L. (1976) Valutazione clinica dell'azione antiforforale e sebotatica di tensidi cationici associati allo zinco piritione, *Chron. Derm.*, 7:659-666 -2- Morganti P., (1999) Uv and Hair Weathering, *Eurocosmetics* 9: 30-32 -3- Morganti P., Morganti G., (2001) Hair and Cosmesis, *Soap & Cosmetics*, 77 (n.9): 26-31 -4- Sunonen R.E., Dawber P.R. and Ellis D.H. (1999) Fungal infections in the skin, hair and nails, p.120 -5- Morganti P., Fabrizi G. (1999) Safety evaluation of phytosphingosine and ceramides of pharmaceutical grade, *J. Appl. Cosmetol.*, 17: 1-9 -6- Cassano N, Amoroso A, Vena GA (2002) Zinc nutritive and skin: an overview, *J. Appl. Cosmetol.*, 20: 183-194 -7- Frydrych A., Arct J, Kasiura K. (2004) Zinc. A critical importance element in Cosmetology, *J. Appl. Cosmetol.*, 22: 1-13 -8- COLIPA n. P81 (2002) Opinion Concerning Zinc Pyrithione, SCCNFP/0671/03/Final

www.mavicosmetics.it



Mavi Sud srl - V.le dell'Industria, 1 - 04011 Aprilia (LT) Tel. 06.9286261 - Fax. 06.9281523 info@mavicosmetics.it