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3

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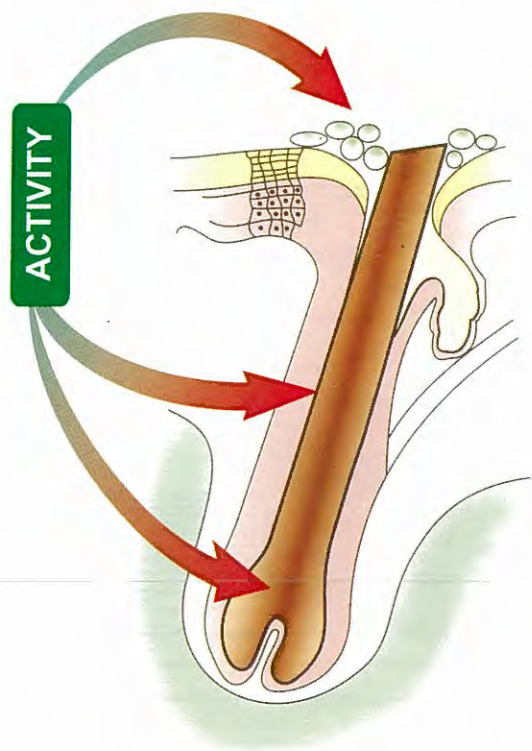
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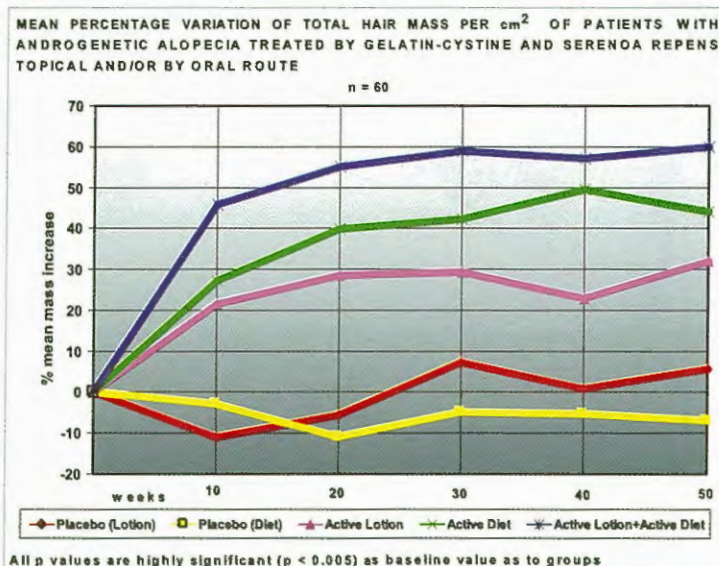


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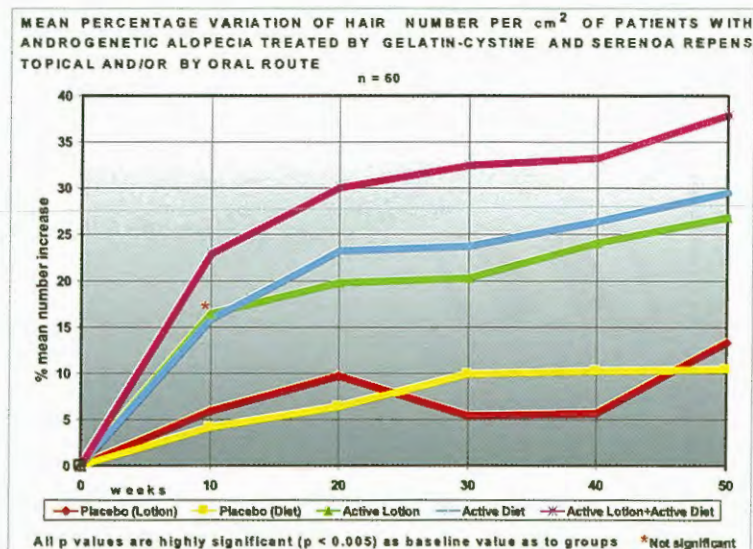
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) Morganti P, Fabrizi G, James B, Bruno C, *J. Appl. Cosmetol.* **16**,57,1998

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

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AN "USE TEST" TO EVALUATE THE EFFICACY OF ORAL FISH CARTILAGE POLYSACCHARIDES IN THE TREATMENT OF PHOTOAGING

Rigano L, Rona C*, Bonfigli A, Scalise F, Distante F, Berardesca E*.

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Received: July 2001.

Key words: Skin ageing, Wrinkles, Fish cartilage, Use test, Clinical pictures

Summary

Topically applied cosmetic products can be helpful in improving aged skin condition. The study shows how oral fish cartilage food supplementation can be helpful in improving the treatment of aging skin. 30 healthy women with signs of skin ageing entered the study. 15 were treated with a food supplement based on polysaccharides derived from fish cartilage and a natural mix of antioxidants for 2 months and 15 with a placebo. Clinical evaluation as well as macrophotographic documentation was taken in order to monitor product effects. Parameters considered were: dryness, skin tone, age spots, fine lines. The results showed statistically significant improvement in the active treated group as compared to the placebo. In particular, fine lines ($p < 0.002$), skin dryness ($p < 0.003$), skin tone ($p < 0.02$), showed considerable improvement. No significant changes were detected for age spots. Most of these parameters are related to changes occurring within the dermal matrix, which is improved after treatment, while most of the topically applied cosmetic products have a short term effect on superficial structures. A combination treatment (oral and topical) can be more effective in reducing the signs of skin aging.

Riassunto

I prodotti topici cosmetici possono essere utili nel migliorare le caratteristiche della cute nel corso dell'invecchiamento cutaneo. Lo studio mostra come i polisaccaridi della cartilagine di pesce integrati oralmente nella dieta possono essere utili nel combattere i danni del photoaging. 30 soggetti di sesso femminile hanno partecipato allo studio. 15 sono stati trattati con placebo e 15 con un prodotto a base di antiossidanti, ginkgo biloba, e cartilagine di pesce (Reviface, Medestea Internazionale, Torino). E' stata effettuata una valutazione clinica mediante macrofotografia. I parametri considerati sono stati: secchezza cutanea, tono cutaneo, macchie senili e rughe sottili. I risultati mostrano un significativo miglioramento di alcuni dei parametri osservati rispetto al placebo. In modo particolare le rughe sottili ($p < 0,002$), la secchezza cutanea ($p < 0,003$) ed il tono cutaneo ($p < 0.02$) hanno mostrato un significativo miglioramento. Nessun cambiamento e' stato rilevato per le macchie cutanee senili. La maggior parte di questi risultati sono collegati ad un miglioramento della qualita' della matrice dermica ottenuta dai principi attivi del trattamento. Mentre i prodotti topici sono agiscono a

livello delle strutture cutanee superficiali, l'integrazione orale permette di creare sinergismi efficaci nello sviluppo di prodotti più efficaci nel ridurre i segni dell'invecchiamento cutaneo.

INTRODUCTION

The appearance of wrinkles is one of the most visible signs of skin ageing. This results from both intrinsic and extrinsic ageing which affect dermal properties. Cosmetic products based on different active principles are very active in treating and preventing skin ageing after topical application: therefore, they may affect mainly epidermal structures 1. Dermal effects of such treatments may be visible after several months. On the contrary, cosmetic treatments as food supplements or dietary products have been proved of little effect in the short term 2. The aim of this study is to evaluate the efficacy of oral fish cartilage in the prevention and treatment of the cutaneous changes associated with skin aging such as wrinkles.

MATERIALS AND METHODS

STUDY DESIGN

A double-blind vs. placebo conducted on 30 healthy female volunteers for 8 weeks. During this period, subjects were treated with 3 capsules (product or placebo) a day, each capsule containing 250 mg of marine fish cartilage associated with an antioxidant mix (gingko biloba, flavonoids, centella asiatica). The placebo contained only soybean oil.

SUBJECTS

30 healthy female volunteers (age range 35-60) participated to the study. They were visited at baseline (week 0) and week 8. They were selected according to the following inclusion criteria:

- caucasian race (II or III phototype according to the Fitzpatrick scale)
- signs of photoageing and skin wrinkling ranging from 3 to 5 according to Larnier's scale 3;
- discontinuation of treatments containing reti-

noids and derivatives at least 6 months before the beginning of the study

- discontinuation of treatments containing alphahydroxiacids and derivatives at least 45 days before,
- discontinuation of other antiageing treatments at least 2 months before;
- signing of an informed consent;
- avoidance of sun exposure
- avoidance of any oral medication (hormone replacement therapy, birth control pills, etc..) which could interfere with the results of the study.

At the baseline visit volunteers were instructed not to apply or get orally other anti-wrinkle products/food supplements, and not to apply, at each visit, any product on the face over the previous hours. Subjects rested for a 20 minutes acclimatation period before undergoing the visual assessment performed by a trained dermatologists.

CLINICAL ASSESSMENT

Clinical assessment was performed by a trained investigator evaluating the following parameters:

- skin dryness, with particular regard to the changes induced in microrelief
- age spots, or mottled discoloration consequent to photoageing of the face
- skin tone, i.e. the firmness of facial skin, with particular regard to the cheek and crow's feet area
- the presence of fine lines in the crow's feet area

The scoring system was based on a scale ranging from 0 to 9 according to the following ranks:

0 = no signs

1-3= smooth
4-6= moderate
7-9= severe

Statistical analysis of the data was performed by Wilcoxon test using Statistica (Statsoft, Inc, USA) software package.

MACROPHOTOGRAPHY

Pictures of the crow's feet area were taken before and after the study in standardized conditions using a macrocamera (Asahi Pentax Co, Japan) with a macrolens (macro 50, Asahi Pentax Co, Japan).

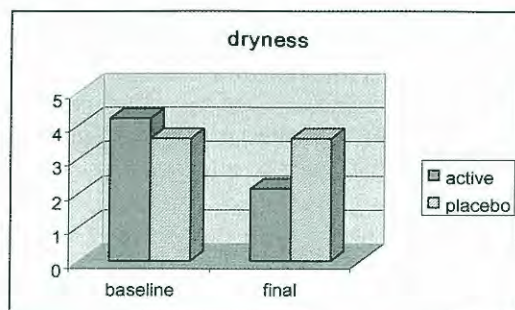


Figure 1. Significant improvement of skin dryness ($p<0.003$) in the group treated with the active product. No changes in the placebo treated group.

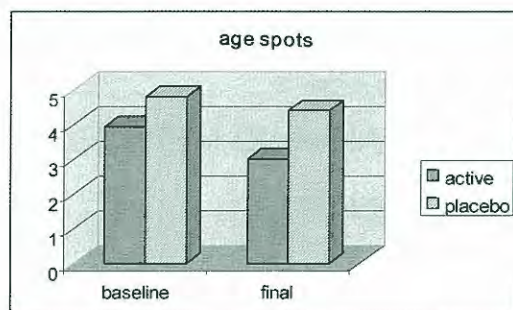


Figure 2. Mottled hyperpigmentation before and after treatment. No significant changes.

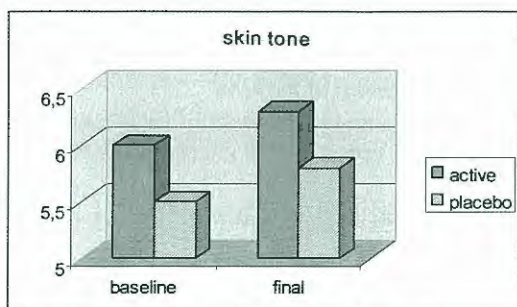


Figure 3. Statistically significant improvement in skin tone ($p<0.02$) in the group treated with the active product consistent with increased firmness of facial skin. No significant changes in the placebo group.

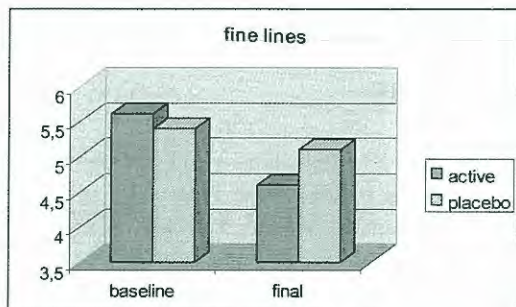


Figure 4. Significant reduction of fine lines in crow's feet area ($p<0.002$) in the group treated with the active product. No significant changes in the placebo group.



Figure 5. Baseline before the study



Figure 6. Same subject of Fig. 5 after 8 weeks of the active product.



Figure 7. Baseline before the study.



Figure 8. Same subject as in Fig. 7 after 8 weeks of treatment with the active product

DISCUSSION

Solar exposure and intrinsic ageing lead to a deterioration of dermal and epidermal properties causing several changes on skin surface such as wrinkling, changes in skin colour, and loss of skin mechanical properties⁴. From a structural viewpoint the major damage is located at the dermal level with the formation of the so called solar elastosis, i.e. the accumulation of degenerated collagen and elastic fibers in the upper dermis⁵. Cosmetic treatments or cosmoceutical products, when topically applied, can be effective in improving epidermal components of skin ageing and after long term application help in removing solar elastosis by promoting new collagen synthesis.

Fish polysaccharides, when taken orally, are reported to be effective in treating photoageing (with particular regard to elastosis, and wrinkles) and chronological ageing (especially as to the thinning of the skin) ^{2,6,7,8}.

Their mechanism of action is still basically unknown: previously reported data suggest a possible role in promoting collagen synthesis and in particular procollagen and Type III collagen which stimulate repair processes within the dermis ⁷. The high content of mucopolysaccharides can improve dermal ground substance thus improving skin tone and thickness ² thus reducing skin wrinkling. Indeed, the improved quality and amount of dermal structures results in a decreased depth and volume of wrinkles. Our "in use" study shows a significant improvement of some clinical cosmetic features related to skin ageing such as fine lines, skin tone and perceived skin hydration which can be explained with the functional and biochemical changes occurring in the dermis. The improvement of the perceived skin hydration can be related both to an increased level of glycosaminoglycans in the dermis, but also to a thicker stratum corneum as hypothesized by some authors after observing an increased thickness of the epidermal en-

try echo detected by C-scan ultrasound. Other groups reported improvement of the clinical signs of photoaging such as teleangectasias and hyperpigmentation ^{2, 9} these findings were reported on the basis of a randomized evaluation of clinical pictures; in our study, the mottled discoloration was improved but not significantly. This effect can be consequent to the light reflection changes occurring in the dermis after the replacing of elastotic material. No side effects, besides some sense of epigastric discomfort after the first days of treatment have been reported during the study. At the moment no data are available on the duration of the clinical and instrumental improvements recorded in the present work. Further studies to elucidate in deep the mode of action and the dynamics in relation to length of treatment are needed.

The study confirms the importance of fish polysaccharides in the cosmetic treatment of skin ageing when taken orally. This can be a new strategic approach in combination with topically applied active principles exerting a synergistic and simultaneous effect on epidermis and stratum corneum.

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A NEW COSMETIC-CARRIER CHITOSAN-BASED

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Summary

The polysaccharide chitin/chitosans considered environmentally friendly raw materials, play a pivotal role in many industrial fields cosmetics included. They seem also to be hydrating and immunopotentiating considering their similarities with hyaluronic acid through the common N-acetylglucosamine sugar, and have been shown to be able to scavenge reactive oxygen species.

In the light of above consideration, the aim of this study was to evaluate the property of a PCA-chitosan and a Glycochitosan gel to restore the skin lipid content and to eventually decrease inflammation and skin hydroperoxides alleviating the symptoms of dry skin.

The treatment by the used creams, in a double blind placebo controlled study evaluated by 3C System, induced a significant and progressive improvement in skin hydration (+72%, $p < 0.05$), and skin surface lipids (+38%, $p < 0.05$), and a contemporary decrease in TEWL (-37%, $p < 0.05$) compared to the placebo. The most interesting property obtained is also the intense anti-inflammatory and scavenging activity detected by the SDS challenge test.

The improvement, evident from the first week of treatment, shows how these creams could be considered as useful means to improve skin hydration, and cutaneous elasticity ameliorating also the inflammatory status of subjects affected by skin xerosis.

Riassunto

I polisaccaridi, derivati dalla chitina e dal chitosano, considerati prodotti biocompatibili, svolgono un ruolo chiave in molti settori industriali tra cui quello cosmetico. Hanno infatti dimostrato di possedere un'azione reidratante, immunostimolante e antiossidante, grazie soprattutto alle caratteristiche delle loro molecole molto simili alla struttura dell'acido ialuronico.

Scopo di questo lavoro è stato quello di valutare le proprietà reidratanti ed antinfiammatorie di due gel basati sull'uso di due derivati chitosanici, il PCA ed il Glico-chitosano.

Il trattamento effettuato a doppio cieco nei confronti del solo veicolo mediante l'uso del 3C System ha posto in evidenza un incremento dell'idratazione cutanea (+72%, $p < 0.05$) e dei lipidi di superficie (+38% $p < 0.05$) a fronte di un decremento della TEWL (-37% $p < 0.05$). Quel che è interessante rilevare è l'intensa attività antinfiammatoria rivelata da questo tipo di formulazione che sembra essere molto utile nel trattamento delle xerosi anche di natura patologica.

INTRODUCTION

The polysaccharide chitin/chitosan derived from animal world of crustacean shells obtained from fungi, play a pivotal role in many industrial fields, cosmetic included (1-11).

Moreover, chemically modified chitins, easily recycled by biodegradation operated by microbial genera, are considered environmentally friendly raw materials. They seem also to be hydrating and immuno-potentiating considering their similarities with hyaluronic acid through the common N-acetylglucosamine sugar, and have been shown to be able to scavenge reactive oxygen species. (12-15)

AIM

In the light of above consideration, the aim of this study was to evaluate the property of a PCA-chitosan and/or a glycochitosan gel (glycolic acid and chitosan) to increase hydration, to restore the skin lipid content and skin hydration and to eventually decrease inflammation and skin hydroperoxides, alleviating the symptoms of dry skin.

MATERIALS AND METHODS

MATERIALS

For this study was used a glycochitosan and a PCA-chitosan gel neutralized or not with gelatin-glycine, according with our preliminary studies. (16)

PATIENTS

The two month trial was a randomized double-blind-placebo-controlled study carried out for 8 weeks on 60 dry skinned female volunteers, aged 29-48 with a moderate xerosis of grade 5 according to Dahl (17). Surface lipids, skin hydration and TEWL were detected by the 3C Sy-

stem (Dermotech Italy) (18,19) meanwhile irritation potential and anti-inflammatory activity were investigated both by SDS challenge test and Pyrexal erythema test (20) using also the Chromameter® C 200.

TEST PROCEDURE

The subjects were randomly divided into four groups of 15 patients each and received respectively:

- I gel A (active A): glyco-chitosan neutralized with gelatin-glycine at pH 4.8
- II gel B (active B): PCA glyco-chitosan neutralized with gelatin-glycine at pH 4.8
- III gel C (carrier A): glyco-chitosan neutralized with sodium hydroxide at pH 4.8
- IV gel D (carrier B): PCA-chitosan neutralized with sodium hydroxide at pH 4.8.

Each patient, supplied with two identical tubes containing the testing cream (A and C or B and D) was instructed to apply them on their face twice a day for all the study period, and was not allowed to use any other skin care product. Each subject was used as her own control, the testing creams (A and C or B and D) being applied on a randomized basis, on the right or left area of the face.

Moreover they were instructed to apply the same cream always to the designed site after washing, first in the morning and just before retiring in the evening. Subjects were also instructed that only the cleansing cream supplied to each at the beginning of the study should be used to cleanse the test area.

Other instructions included not to apply the testing creams the day of evaluation and to wash their face at least 4 hours before the control.

CLINICAL EVALUATION

Clinical and biophysical examinations were performed on the first day (baseline), and at week 2, 4, 6, 8 and 10 (end of the treatment). Clinical evaluations included the efficacy for reduction of symptoms of dry skin as well as the skin tolerance after the application phase. The obtained clinical results are reported on Tab. I

OVERALL SKIN TOLERANCE ON NEW CHITOSAN-BASED GELS

		DAY 15	DAY 30	DAY 45	DAY 30
VERY GOOD	ACTIVE A	32	38	39	39
	ACTIVE B	31	31	35	35
	CARRIER A	30	30	30	30
	CARRIER B	30	30	30	30
GOOD	ACTIVE A	25	21	20	20
	ACTIVE B	27	27	24	24
	CARRIER A	28	28	28	28
	CARRIER B	28	28	28	28
SATISFACTORY	ACTIVE A	2	0	1	1
	ACTIVE B	2	2	1	1
	CARRIER A	1	2	0	0
	CARRIER B	2	2	0	0
POOR	ACTIVE A	0	0	0	0
	ACTIVE B	0	0	0	0
	CARRIER A	1	0	0	0
	CARRIER B	0	0	0	0

Tab. I Patients involved

BIOPHYSICAL NON-INVASIVE MEASUREMENTS

The determinations were always carried out on four areas of right or left areas (forehead, cheek, chin and nose) and the results are expressed as mean values of the measurements performed. To achieve an higher degree of assurance all evaluations were performed after a 30 minutes acclimatization period in a room at 21°C to 22°C and 45% to 50% humidity, even if the 3C System automatically adjusts environmental conditions to 22°C and 50% relative humidity.

Skin surface lipids.

Determination is based on photometric measurement of light transmission through a skin surface imprint obtained applying to the designed

skin area a frosted plastic foil. It allows adherence of skin lipids in a 1 cm² area. The obtained readings are automatically converted into (g/cm²) and reported in Fig. 1

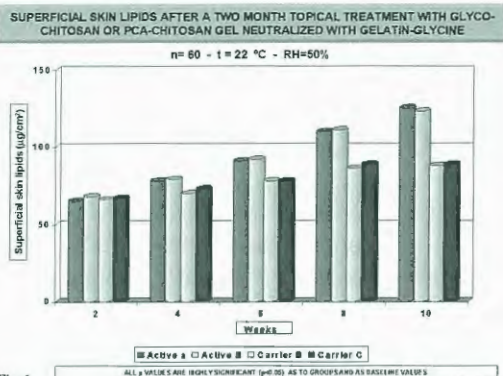


Fig. 1

Skin Hydration

The hydration of the horny layer was assessed by measuring electrical capacitance of the skin surface by means of the 3C System. When the probe was applied to the skin (recording time 0.5 s), the capacitance is displayed digitally in arbitrary 3C units. The obtained results are reported in Fig. 2.

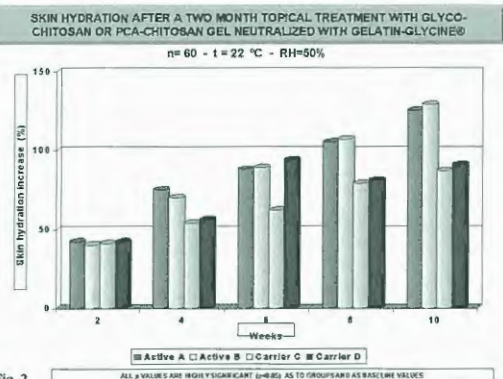


Fig. 2

Transepidermal Water Loss (TEWL)

The 3C System probe consists of a cylindrical open chamber measuring system, diameter 14 mm, height 10 mm and skin area 0,95 cm², two sensor units, containing thin capacitive film transducer, are placed at 3 and 7 mm distance from the skin surface. TEWL is calculated digitally in g/m² h.

SDS CHALLENGE TEST

This test is generally assessed to evaluate protective and anti-irritation potential efficacy of the studied products. Thereafter, one occlusive patch with 20% sodium dodecyl sulfate (SDS) solution were applied in a randomized way to left or right forearm of each subject for 3 days and different hours before and after 8 weeks of twice daily treatment with the different kind of gels used.

Two SDS treated and untreated sites were left as control.

There was no further application of the studied gels after removal of the last patch. Patches were applied to the pre-marked forearm areas, removed after 4 hours and reapplied to the same sites for two consecutive days for 4 hours each. After 1 hour and 24 hours of removal of the last set of patches, the skin reaction was graded and evaluated by TEWL and Chromameter® 200 measurements.

TEWL after repeated SDS application on the forearm skin pre-treated with Glyco-Chitosan or PCA-Chitosan

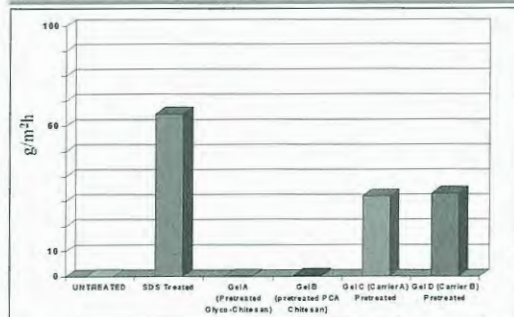


Fig. 3 All p values are highly significant ($p < 0.05$) as baseline values.

The intensity of the irritation was assessed by visual scoring/chromametry and TEWL measurements 5 hours and 24 hours after application of the patches.

(0 = no-erythema; 0.5 = equivocal reaction; 1 = slight erythema; 2 = moderate, uniform erythema; 3 = intense; 4 = fiery redness with edema.) The obtained results are reported on fig. 3 and 4.

ERYTHEMA TEST

This inflammation is obtained injecting intracutaneously 0.1 ml. Pyrexal (lipo-polysaccharide from salmonella abortus equi) into dorsal skin of the volunteer subjects, according to Heilmeyer and Hiemejer (20)

These bacterial pyrogens induce an inflammation. The morphologic signs take the form of a sharply defined erythema whose surface area is measured over time. Simultaneous application of an anti-inflammatory cream inhibits its spread. By this injection 5 vials were induced in each subject. These were then topically treated in randomized succession with 0.2 ml. of the 4 different preparations and covered with transparent film.

Two of these areas were treated with the carriers to serve as a control and another was treated by 0.2 ml. of betamethasone valerate 0.1% and co-

CHROMAMETER® values a* of the forearm skin after repeated SDS application on site pre-treated with Glycochitosan or PCA Chitosan gel

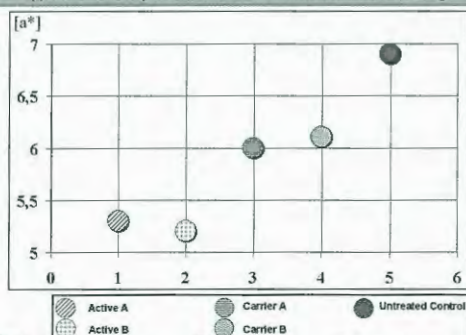


Fig. 4

All p values are highly significant ($p < 0.05$) as to groups and as baseline values.

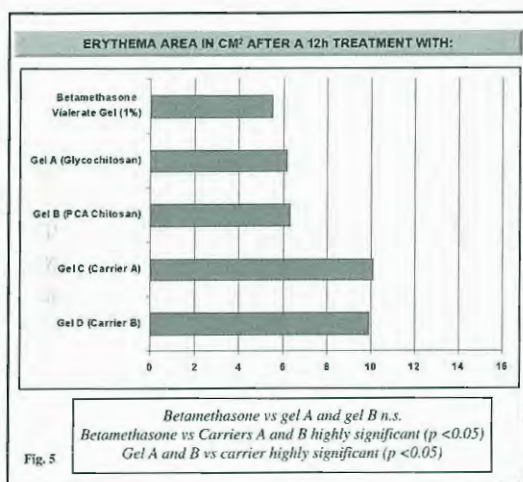
vered also with transparent film.

In this way the erythema is visible at all times and can be measured through the film. The extent of the erythema was determined 6,8,10 and 12 hours after application, the maximum (a) and minimum (b) diameter being measured and the surface area calculated by the elliptic formula:

$$F = \frac{a \times b \times \pi}{4}$$

The efficacy was classified by determining the sum of the erythema surface areas from the 4th to the 12th hour. A small area means that the preparation is highly effective. The comparative evaluation was performed with the aid of bifactorial analysis of variance

The obtained results are reported on Fig. 5



STATISTICAL ANALYSIS

Results are the mean $\pm$ S.E.M. of at least 5 measurements. Data were analyzed by one way *Anova*, followed by inspection of all difference by Duncan's new multiple-range test.

Differences were considered significant at ($p < 0.05$).

RESULTS AND COMMENTS

The treatment by used creams induced a significant and progressive improvement in skin hydration (+72%, $p < 0.05$), and skin surface lipids (+38%, $p < 0.05$), and a contemporary decrease in TEWL (-37%, $p < 0.05$), and on skin redness compared to the placebo. The first and the most interesting property obtained is undoubtedly the intense anti-inflammatory activity comparable and close to beta-methasone valerate, used as parameter. As clearly see on Fig. 4 and 5.

The improvement, evident from the first week of treatment, shows how these creams could be considered as useful means to improve skin hydration, to rebalance skin lipids tone and elasticity ameliorating also the inflammatory status of subjects affected by skin xerosis caused by environmental or pathological reasons.

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POTENTIAL APPLICATIONS OF THE AFM (ATOMIC FORCE MICROSCOPY) IN COSMETOLOGY.

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Key words: Atomic Force Microscopy, hair morphology.

Summary

The Atomic Force Microscope (AFM) is able to image the surface properties of all types of materials (conductors, insulators, biological and vegetal samples) with a very high resolution and in the native state.

In particular, AFM is used to study a number of biologic system such as DNA-replication and protein/ protein, DNA/protein interactions; furthermore, in cosmetology, it's suitable to study the fundamental characteristics of the skin, like elasticity and wrinkledness, and to compare for example morphological properties of human virgin hair and chemical or polymer treated hair.

The most important characteristics of this instrument in cosmetological analyses are:

- the samples can be directly examined without previous treatment, i.e. in native conditions,
- data are collect in a digital form,
- 3-D image rendering with variable magnification and shading,
- view of the recorded image from any point of the space.

In our laboratory we analyse numerous samples of cosmetic interest and in particular human hair and replica of human skin. In this short paper we present preliminary data on human vergin hairs.

Riassunto

Il Microscopio a Forza Atomica (AFM) è particolarmente indicato nello studio delle proprietà superficiali di materiali di ogni tipo (conduttori, isolanti, materiali biologici, vegetali) con risoluzioni mai raggiunte fino ad ora e senza ricorrere alla necessità di effettuare una complicata preparazione del campione.

In biologia l'AFM può misurare le forze intermolecolari che governano processi biologici quali la replicazione del DNA, le interazioni tra proteine e proteine e tra DNA e proteine; in cosmetica in particolare è in grado di sondare caratteristiche fondamentali della pelle, come l'elasticità e la rugosità, e di mettere a confronto la morfologia di un capello umano vergine e di un campione trattato chimicamente o con polimeri.

Tra le caratteristiche fondamentali che rendono l'AFM uno strumento all'avanguardia per rilevare l'efficacia di un prodotto cosmetico, possiamo enumerare:

- la superficie dei campioni può essere esaminata direttamente senza la necessità di preparare il

campione

- i dati possono essere immagazzinati in forma digitale
- l'immagine può essere ricostruita in tre dimensioni
- l'immagine tridimensionale può essere osservata da differenti angolazioni.

Nel nostro laboratorio è stata messa a punto l'analisi di molti campioni di interesse cosmetico, tra i quali i capelli o la replica di una zona ben individuata della pelle. In questo lavoro breve riportiamo i dati preliminari ottenuti analizzando campioni di capelli non trattati.

INTRODUCTION

An Atomic Force Microscope (AFM) (1,2) is able to image biological molecules and living cells in native conditions at very high resolution. Until recently, biologists have had to use SEM and TEM vacuum technologies to image surface of samples and this prevented *in vivo* analysis since in general biological materials cannot withstand prolonged vacuum. In addition since SEM and TEM operate only on conducting samples, the surfaces of the samples had to be coated with metals prior to imaging. Unlike traditional microscopes, AFM forms topographic images by detecting the deflection of a sharp-tipped cantilever as it is scanned across the sample surface. Biological samples can be imaged (3-6) fixed and mounted on mica or glass or unfixed in a liquid or gaseous environment without additional preparation. The AFM collects the data in a digital form, in software allows to reconstruct the 3-D surface structure on a computer screen and to calculate and display the main parameters generally used to characterise any rough real surface.

AFM TECHNIQUES

In an AFM the tip is mounted on the end of a flexible cantilever. As the sample is scanned beneath the tip, small forces of interaction with the sample cause the cantilever to deflect, enabling to record the sample surface topography. The AFM can be operated in three different modes: Contact Mode, Non-Contact Mode and Tapping Mode.

In Contact Mode the tip is maintained in contact with the sample during measurements and slides over the surface as the sample is scanned.

In Non-Contact Mode the tip is forced to oscillate at high frequency (100 kilohertz to 1 megahertz) a few nanometers above the surface. Using this technique the sensitivity of the microscope results to be greatly increased so that

even weak, long-range forces as attractive van der Waals forces and electrostatic forces can be detected. During scanning, the topography of the surface is tracked by detecting the effect of these forces on the amplitude and/or the frequency of the cantilever oscillation. It is therefore possible to image even the softest sample without damage.

In Tapping Mode the cantilever is oscillated with larger amplitude as compared with the Non Contact Mode, and the tip is allowed to make transient contact with the sample at the bottom of its swing. The Tapping Mode is a compromise between the Contact and the Non Contact Mode so that the resolution is usually almost as good as in Contact Mode, but the damage caused by shear forces is almost completely eliminated since the contact tip-sample is extremely brief.

BIOLOGICAL APPLICATIONS

- DNA protein and chromatin structure;
- Enzyme/substrate interactions;
- Protein/protein, protein/DNA interactions;
- Adsorption properties of cells and molecules to biological or other surfaces;
- Recognition of cell surface antigens;
- Visualisation of antigen/antibody complexes;
- Studies on cell morphology and motility;
- Evaluation of dynamic variation in chemical and mechanical properties of biological materials;
- Synaptic release and signal transduction processes;
- Structural characterisation of planar membranes and liposomes;
- Surface analysis of virus and bacteria;
- Particle interactions;
- Evaluation of the performances of biomaterials (e.g., titanium implants, biomedical plastics).

CONTACT AFM

The Contact Mode usually produces stable, high-resolution images, but compression and shear forces generated between the tip and the surface may cause damage. This possibility can be especially troublesome when imaging biomolecules, which are almost always soft and only weakly attached to the substrate.

NON-CONTACT AFM

Non-contact AFM imaging produces high resolution characterisation of surface topography on soft samples using only the lightest interaction forces. Non Contact AFM can even avoid sample and tip damages on surfaces such as protein or nucleic acid complexes adsorbed to a flat substrate or sample. In biological applications NC-AFM is desirable because it provides a means for measuring sample topography with no contact between the tip and the sample. The total force between the tip and the sample in this regime is very low, generally about 10^{-12} N. A further advantage is that both biological samples and tips are not contaminated or degraded due to the contact with the tip.

AFM SOFTWARE CHARACTERISTICS

AFM instruments offer a complete imaging processing package and a control language for automated data acquisition and very complete image processing. It includes:

- Line and region analysis of the data;
- Direct measurement of the distances between relevant points of the image;
- Processing and analysis of the data to get the surface parameters;
- Exportation of the data files to allow further processing;
- Zoom software;
- Multi image presentation;

- 3-D image rendering with variable magnification and shading;
- View of the recorded image from any point of the space.

REGION ANALYSIS WITH AFM

In Region Analysis only the data pertaining to selected region of the recorded image are taken into consideration for processing. This allows to include/exclude selected regions of the recorded image (possibly even multiple inclusions/exclusions) in the processed data. Therefore all the parameters characterising the wanted involved regions can be evaluated.

SINGLE LINE ANALYSIS WITH AFM

In Single Line Analysis only the data pertaining to a selected cross section of the image (height profile given in different colours) are taken into consideration for processing.

Quantitative measurements of surface features by moving cursors along the line and collection of the surface parameters are possible.

RESULTS, DISCUSSION AND CONCLUSION

In this short paper preliminary data obtained with AFM on vergin hairs and some examples of the images and analyses of the samples produced in our laboratory are presented.

To our knowledge few and scattered data are present in the literature on the use of AFM in cosmetic and in particular on hair analysis (7,8). We believe that from our preliminary results it appears very clear the potential of this technique to test cosmetic products and to verify their efficacy.

We are presently analysing more samples and we hope in the near future to present data for comparison between vergin and treated hairs.

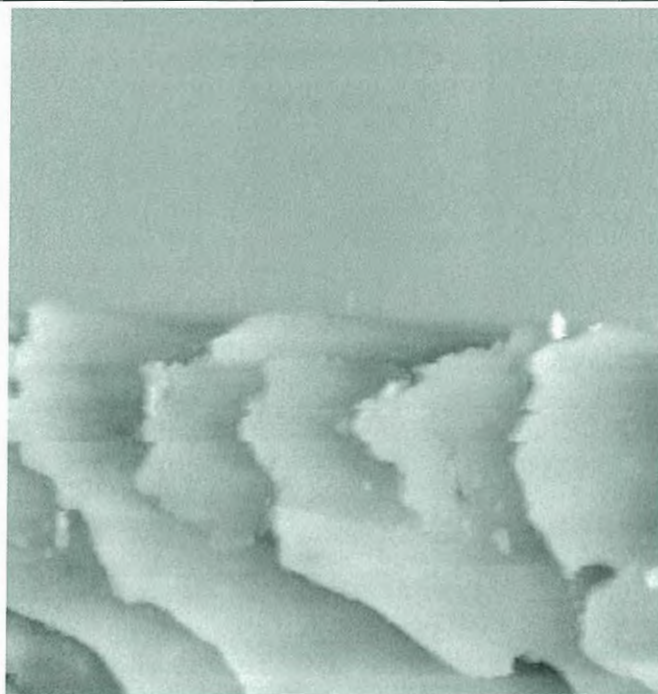


Figure 1: Non-contact AFM image of an human hair (30 μm)

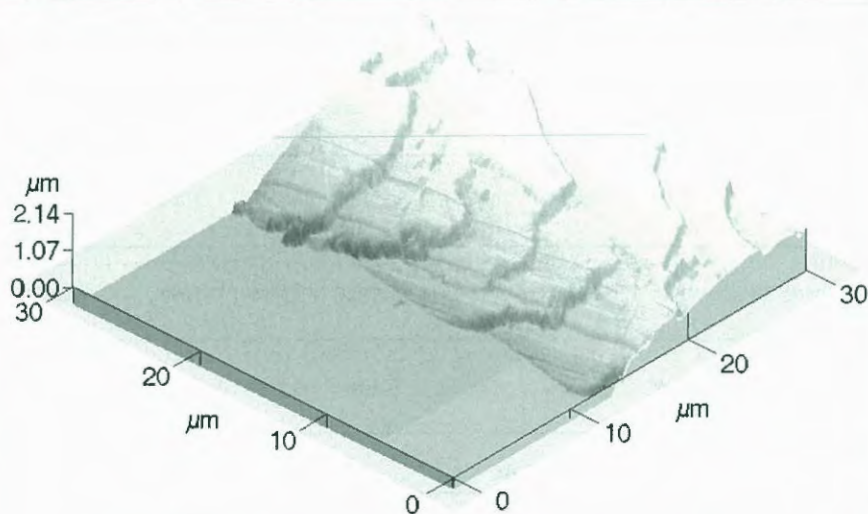


Figure 2: 3-D Non-contact AFM image of an human hair (30 μm)

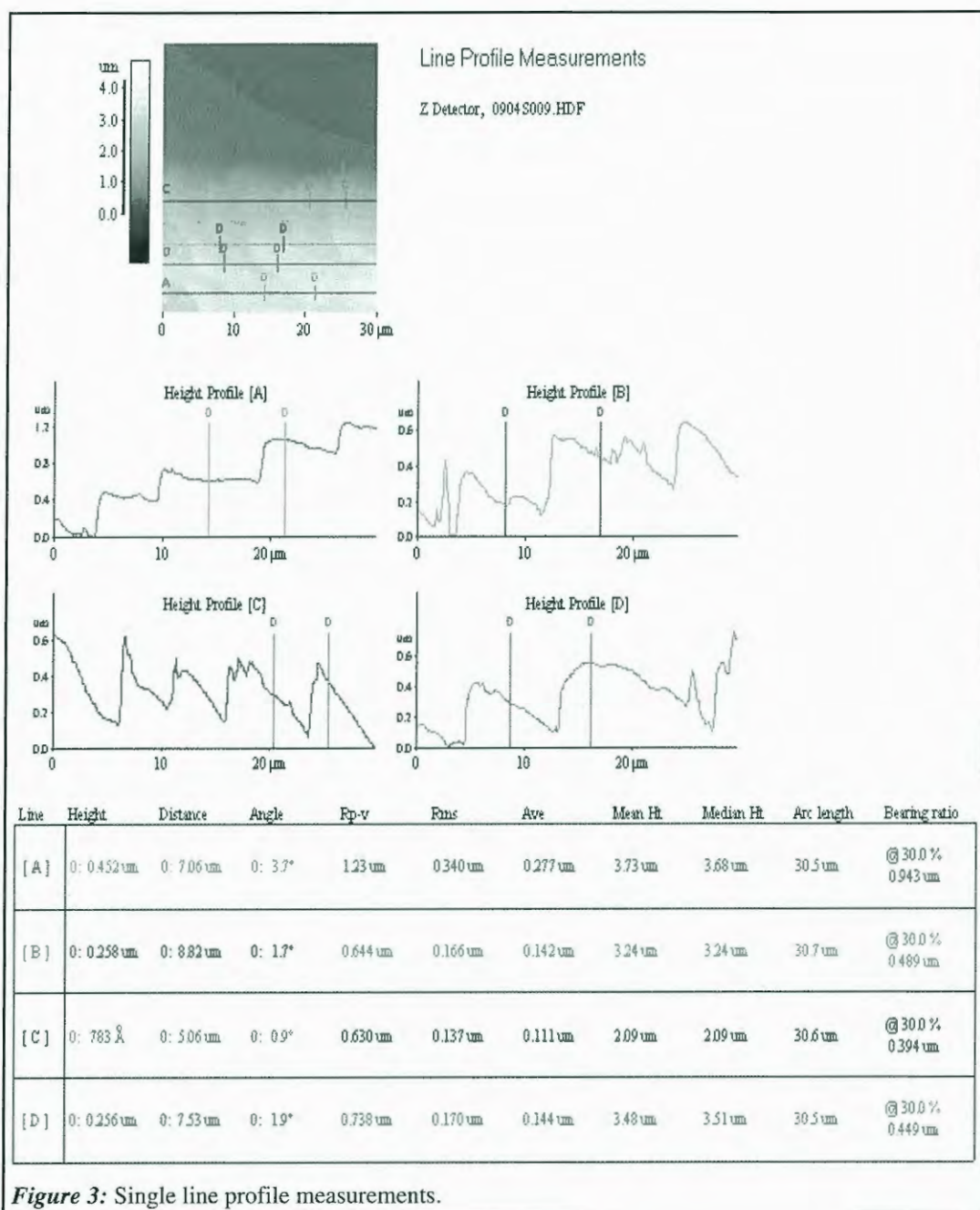




Figure 4: Non-contact AFM image of an hair's detail (10 mm).

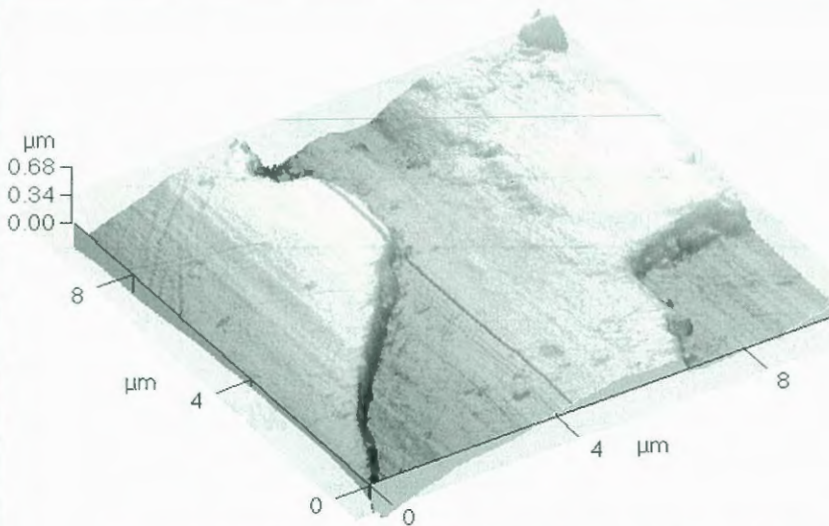
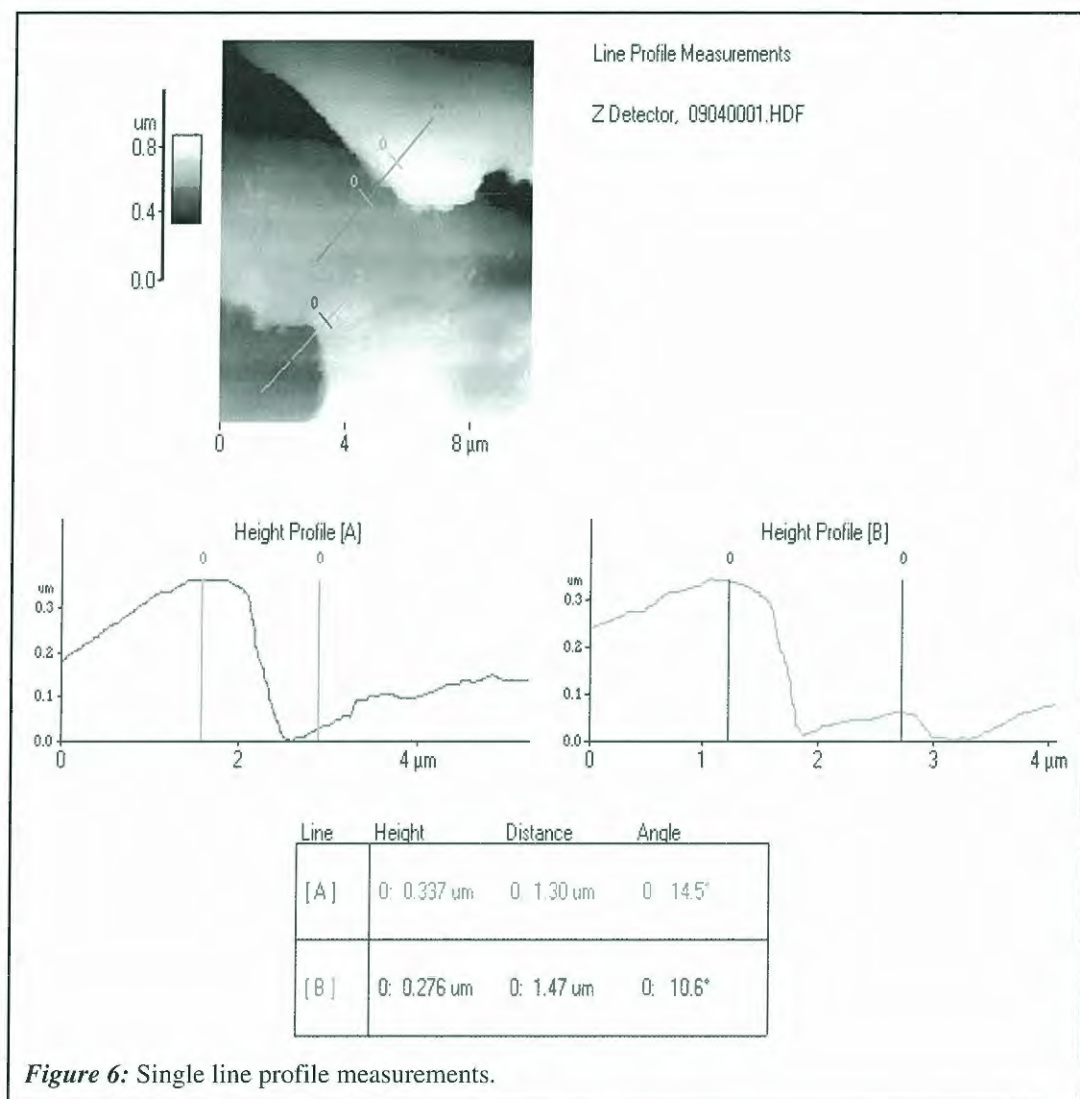


Figure 5: 3-D Non-contact AFM image of an hair's detail (10 mm).



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COSMETIC SKIN CARE

By A. Shai, H.I. Maibach, R. Baran

2000. 354 pages. Hardback

£39.95

ISBN 1-85317-752-0

MARTIN DUNITZ Ltd.

E-mail: info.dunitz@tandf.co.uk

This interesting handbook, in 15 chapters offers a complete and purely scientific overview of all the topics and data on cosmetic of great interest for dermatologists, medical doctors, chemists and biologists whose aim is to get into the vast field of the Cosmetic Dermatology.

Many of the chapters discuss common skin problems and conventional skin treatments to broaden the reader's knowledge regarding the wide range of available regimens.

The 1st chapter highlights the grey area existing between a drug and a cosmetic product, while the 2nd describes the structure and the functions of the skin, the main protective layer of our body towards environment and its chemicals.

The 3rd chapter reviews the principle preparations of cosmetic or medical product for use on the base, water, powder, or various combinations thereof. Both cosmetic and medical preparations for application to the skin are called preparation for external use.

In every case, the cosmetic or medical product is a combination of a base, the carrier, and one or more supplementary substances, the active ingredients.

This combination ensures that the actives penetrate the skin.

Chapter 4 treats moisture and moisturizers and the different causes provoking skin dryness, such as dry environment wind or changes in the skin's ability to retain its moisture. As matter of fact, skin loses its suppleness in proportion to the decrease in its water content. In this chapter the beneficial effects of moisturizers are reported together with its correct use.

An overview of skin cleansing is reported in chapters 5th, 6th and 7th which describes also the different types of surfactants, their activities, and the different solid products or emulsions used to cleanse thoroughly the skin respecting its surface lipid film.

Among the detergents big room is given to masks, often used as supplement in acne treatment.

Chapter 9 offers an overview of acne as inflammatory disease supported by a complete photo session underling the difference existing between open and closed comedones papules, pustules and nodules of acne-affected skin.

The main anti acne preparations and medications topically and orally administered are also reported. Meanwhile chapter 8 describes both chronological and photo aging of the skin, chapter 10 is dedicated to the long-term effects caused by sun exposure.

Cumulative solar radiation is, in fact, leading to the appearance of wrinkles, and an uneven distribution of pigment in the skin, known as sun spots.

Moreover the main damage to dermis, following cumulative exposure to the sun, is the destruction of elastin and collagen fibers, and the damage to the blood vessels and to the lens of the eye with the developing cataract.

The best protection for the skin is to avoid sun exposure, even if a correct use of sunscreens and carotenoids is certainly helpful.

Chapter 11 reports telangiectasia, which is a manifestation of cumulative damage to superficial blood vessels of the skin. Causes prevention and different treatments of telangiectasia are also well documented and described.

Chapter 12 gives some ideas on the so-called cellulite, composed mainly of fat distributed under certain areas of the body such as thighs and buttocks. Cellulite that has not scientific basis and is not

a pathology is generally treated by cosmetic preparations based on methylantrines or by a surgical method such as liposuction.

The entire chapter 13 is focused onto causes and types of skin inflammation and dermatitis. Cosmetics that can cause contact dermatitis are also reported and diagnosing. Due to the sensitivity to a particular substance there is no cosmetic preparation that can never cause an allergic reaction in someone. Therefore, an offending agent may be present in many different cosmetic preparation and, if known, by more patch testing, has to be avoided and no more used.

The entire 14th chapter is dedicated to skin tumors. Every cell body, including skin cell, can potentially trigger an abnormal division, developing into a tumor.

This abnormal cell division may be limited, in which case a benign tumor results, or it may be uncontrolled, overwhelming the body's defense systems, and resulting in a malignant tumor. For these reasons a regular check-up by a doctor should be scheduled every few months to control the skin.

In any case the treatment of skin lesions suspected of being cancerous is based on its total removal by an expert physician.

Active ingredients often used in cosmetic preparations are reported in chapter 15.

It reviews also what is today known about the action and the effects of these active compounds. All the active ingredients used for the cosmetic preparations can be classified into four main groups: 1) animal-derived substances, such as collagen and elastin; 2) botanicals obtained from wide range of plants; 3) vitamins and food stuffs, such as milk and propolis.

Many active compounds present in these groups are described and critically commentated.

Some interesting comments are the ones about the "incorrect" way to define natural those cosmetics, which contains nothing natural. I perfectly agree with the authors in giving more room to the eventual side effects due to the use of some active principles such as propolis, a well-known allergenic agent.

Moreover, before claiming the effects of a cosmetic is of primary importance to test and prove its real efficacy. The only substances shown to have real beneficial effects on skin aging seems to be alpha-hydroxy-acids and retinoic acid.

These two topics are extensively treated in chapters 16 and 17.

Retinoic acid is a drug, therefore it must be purchased with a physician's prescription, and precise directions must be followed.

Alpha-hydroxy-acids (AHAs) may be sold freely, without a doctor's prescription. Preparations containing up to 30% AHAs are permitted in the USA for use by trained cosmetologists on condition that the substance is applied to the skin for only brief periods of time, and is then immediately rinsed off. Bleaching and bleaching preparations are treated in chapter 18 where are described also the methodologies used in the treatment of hyperpigmented skin lesion, such as freckles, sun spots, the pregnancy mask and so on.

What is important to remember is that whenever hyperpigmented lesion appears on the skin, the patient must minimize the exposure to the sun, and consult a dermatologist.

In order to avoid some disfiguring conditions, is reported the use of laser to erase, for example, tattoos and various possible ways of camouflaging skin lesions by the use of specific make up.

At this purpose different kind of colored creams may be used such as a rose one, to reproduce broken capillaries, a golden brown mixture to imitate freckles; while for a beard stubble it may be selected a brown, dark brown, grey or black cover cream.

Superficial, medium or chemical deep peeling is also useful to eliminate black spots and some kind wrinkling. From chapter 25 to 29 all the problems connected to the hair are described, hair life cycle included.

The process by which hair is formed results the way in which the keratinous layer of the skin forms. During this process cells accumulate large amounts of Keratin that represents the major component of hair. In Anagen phase, the hair cells at the base of the hair follicle are dividing repeatedly, and the

hair grows steadily. Catagen phase is the transition phase, and Telogen the resting phase. The duration of the various phases of the cycle differs in different parts of the body.

For the hair care are used shampoos designed to clean, or conditioners designed to prevent damage to the outer covering of the hair and to neutralize the electric charges on the surface and prevent knots.

The effect of a conditioner is only temporary and it does not have any medical benefits. All the benefits of the active used in hair care are described in these chapters. Methods for temporary or permanent hair removal, with the relevant risks and benefits, are also reported.

The last chapter is an overview on composition, structure and general care of the nails.

Common nail problems and all the common cosmetic treatment are also reported in a simple language in order to give to the chemist and also to the cosmetologist the opportunity to comprehend all the problems connected to the Cosmetic Dermatology.

It is therefore, suitable also as a course textbook for beauticians and for students of various biomedical, chemical and pharmaceutical science, and as a supporting text for medical, chemical and post-graduate students who want to know better the modern Cosmetic Dermatology.

P. MORGANTI
Editor-in-Chief

MUSCARINIC RECEPTORS IN AIRWAYS DISEASES

By Zaagsma J, Meurs H, Roffel A.F.

2001. 270 pages. Hardcover

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ISBN: 3-7643-5988-9

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What are the different muscarinic acetylcholine receptor subtypes and how are they distributed in the airways and molecular signalling mechanisms?

What is their function in inflammatory mechanisms and in neurogenic and non-neurogenic control of the airways?

How is their gene expression regulated?

The great importance of muscarinic acetylcholine receptors for the patho-physiology and treatment of chronic inflammatory obstructive airways diseases, calls for a comprehensive and integrated overview of the current knowledge to address such questions, to discuss the role of these receptor subtypes in diseases such as asthma and COPD, and to present novel perspectives on antimuscarinic drug development.

The present book is the worthy result of this need.

It points out innovative insights into the hitherto poorly understood role of postjunctional muscarinic M2 receptor in airway smooth muscle contraction, and sheds some light on the novel concepts of antimuscarinic drug development, with special reference to the long-acting M3 and M1 receptor-selective antagonist tiotropium bromide.

The modulation of muscarinic receptor function and cholinergic vagal nerve activity by neurogenic and non-neurogenic inflammatory pathways is dealt with and emphasis laid on the potential importance of these mechanisms to the pathophysiology of asthma and COPD.

This book is an important reference for pulmonologists and medical, physiological and pharmacological scientists involved in airways research, to whom it offers an update of recent developments in the physiology, pathophysiology, pharmacology and therapeutics of the cholinergic system in the airways, and a scientific basis for further research in this field.

HANDBOOK OF COSMETIC SCIENCE AND TECHNOLOGY

By A.O.Barel, M. Paye and H.I. Maibach

2001. 886 pages Hardcover

US\$ 165

Marcel Dekker Inc.

ISBN: 0-8247-0292-1

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<http://www.dekker.com>

This interesting handbook edited by authors, leading experts in cosmetic and dermatological field, covers many topics, from structure and function of skin to cosmetic ingredients, vehicles and finished products to new delivery systems and sensory testing, to end with the safety, and regulatory requirements for the production and marketing of cosmetics throughout the world.

It consists of 9 sections for a total of 71 chapters aimed to offer the reader a "useful guide and source of innovative ideas for the formulation of modern cosmetics".

The introduction, which covers the whole part I, tries to define the goal in using cosmetics. In fact, the aim of cosmetics is not only to improve the appearance, but also to give real benefits to skin hair, teeth, and mucous membranes.

In order to support these activities, raw materials became more efficacious and safe, and new instruments and new methods have been created to objectively evaluate and measure the skin turnover rate and its topography, hydration or elasticity; or even to see it directly in vivo through more evaluated microscope technology.

Thanks to these new technologies, today any claimed effect of a cosmetic on the skin should find appropriate and clear demonstrations.

At this purpose and for better protection of the consumer against misleading claims, the 6^o Amendment of the European Directive on Cosmetic Products has required manufacturers to have a dossier with the proof of the claims, controlled from a multidisciplinary expert in Cosmetic Chemistry, Dermatology and Pharmacology.

Part 2 presents the reader with a description of the anatomy and physiology of the body targets for cosmetics, the skin and its appendages.

As matter of fact, one of the skin's primary physiological function is to act as a body's first line of defense against exogenous agents. The outer structure of the skin, the Stratum Corneum, can resist abrasion from the outside world, serving also as a water barrier. When toxic agents permeate it, the resulting adverse effects may cause considerable discomfort, especially in the facial area, which has an extensive network of sensory nerves. The aim of cosmetics is to protect the skin maintaining this structure in good condition.

In this way begins the third part of the handbook, where all the biological processes involved with the percutaneous absorption are reported.

The major route of permeation across the skin is the tortuous, but continuous intercellular lipids, the so called lamellae of Stratum Corneum (SC).

As such, the rate at which permeation occurs is largely dependent on the physiochemical characteristics of the penetrant, the most important, being the relative ability to partition into the intercellular

lamellae.

Therefore the different components of the cosmetic and its rate of penetration is important to maintain the protective and homeostatic role of the skin.

A cosmetic has to ameliorate the skin hydration without altering the structure and function of SC impeding that a single ingredient or a mixture of chemicals used for the vehicle can reach the micro-circulation.

The integrity of this barrier, with resultant increase in percutaneous absorption, is in fact reduced by any inflammatory process of the skin, such as any form of dermatitis or damage by aggressive chemicals.

It should be remembered that the skin of different individuals differs in susceptibility to irritation in a remarkable way, and a number of individual factors influencing development of irritant dermatitis include age, genetic background, anatomical region exposed, and pre-existing skin disease. Differently from topical used for short terms and on small skin areas, a cosmetic is used from many years and on the whole body.

For these reasons, application of products to the skin may cause sometime, irritant, phototoxic, contact and photocontact urticaria.

In fact, daily or prolonged use of cosmetics may cause ingredients to accumulate in the skin and thus increase the risk of adverse reactions.

It is also generally agreed that most skin-adverse reactions to cosmetic products are irritant in nature and that people with sensitive skin, as indicated by conditions like atopic dermatitis, are particularly liable to develop such reactions.

Sensitivity to cosmetics can never be totally avoided, but its incidence can be reduced developing more fruitful communication between dermatologists, and cosmetic chemists and cosmetic manufacturers.

Monitoring the cosmetic product, adverse effects before and after its marketing should be useful such as the preventive development of a battery of skin irritation test to predict the skin irritation potential of chemicals and formulations.

The fourth part of the book is dedicated to the vehicles of cosmetic products.

The term vehicle implies differentiation between active and inactive principles, even if, in topical dermatological and cosmetic compositions, a sharp differentiation between active and inactive principle is generally not possible because of the so-called vehicle effect. But while the pharmaceutical preparations are effective because of a pharmacologically active compound delivered with the aid of a vehicle, the cosmetic preparation achieves its activity by a caring or preventing effect mainly on skin, mucous membranes, hair and nails.

This effect may be achieved either by cosmetically active ingredients with a proved mean of action or by the vehicle itself. Therefore, in contrast to pharmaceuticals, in cosmetics the vehicle is of great importance also because cosmetics are applied on wide skin areas and for long terms.

Different are the vehicles used, such as solutions, multiple emulsions, hydrogels, micro and nano emulsions but they have to be always determined by the product target profile. Finally the sensory assessment is another useful tool for product and concept development and for quality control in the cosmetic industry.

Many are the delivery systems designed to control and implement the efficacy of the 'active' to its site of action at an appropriate rate.

At this purpose, the cosmetic technology constantly has developed new raw materials and new controlled delivery systems, such as microencapsulation, by different and inert natural or synthetic

polymeric materials, nanospheres or liposomes for example.

All these new technologies are well described and reported on this part of the book.

Part 5 is entirely dedicated to the cosmetic ingredients generally used to formulate a cosmetic product from preservatives to protect it against microbial attack before and during consumer use, to skin-feel agents useful for conferring sensorial properties.

Naturally are well described all the surfactants that have been grouped into six categories: cleaning agents, emulsifying agents, foam boosters, hydrotropes, solubilizing agents and suspending agents to demonstrate the indispensability use of these compounds for the cosmetic formulation. But a big room has been given also to lipids and ceramides, important components in the permeability barrier of the skin and water-holding properties of the Stratum Corneum.

A special note has been reserved to the importance reached in these years by the UV-filters, whose introduction in skin-care and cosmetic products represents a great benefit that cosmetics can provide consumers.

As matter of fact, the regular use of sunscreens has been shown to reduce the number of actinic or precancerous keratosis and solar elastosis, preventing also immunosuppression. Among the various active principles reported, such as the alpha hydroxy acids, the moisturizing compounds or the always new vitamins, are mentioned those substances used as anti irritants for sensory irritation. These should reduce the sensitivity of type-C nociceptors.

With repeated daily use, this ideal anti-irritant should provide the same effective level of anti-irritant protection (no tachyphylaxis) and most importantly, it must be safe for broad, unsupervised use.

Part 6 focuses on skincare, hair care and other cosmetic products.

Cosmetic products are intended for interaction with the superficial epidermis, creating objective and visible changes, such as improvement of scaling, skin color, wrinkles elasticity and other beneficial effects also on the hair and nails.

Many are the cosmetic categories of products studied for that purpose and described within this part of the book. Among them, the skincare product prophylactic in the prevention of wrinkle formation the so-called antiwrinkle products; the anticellulite products, that should be active on the orange-peel look of the skin, known as lipodystrophy; and the barrier creams especially used to prevent the irritant contact dermatitis (ICD) by re-establishing the broken skin barrier.

The use of these products aims to restore the physical integrity of the barrier via application of missing basic components of the intracellular lipid matrix in combination with occlusive materials to stimulate the repair mechanisms of the skin, and prevent excessive water loss.

Among the hair-care products, are mentioned the special formulations studied by the cosmetic industry to treat the Ethnic Differences.

As matter of fact African, Asian and Caucasian hair are different related to diameter, geometry, and other physical parameters. Closely related to these parameters are biophysical factors, tensile strength and combing forces, which might be influenced by cosmetic formulations that are applied to hair.

Very interesting are also the chapters dedicated to baby and elderly care and cosmetic. The elevated skin permeability in newborn needs to be considered when establishing a routine skincare regime. At the same way the major changes in the morphology of the skin in the elderly include dryness (roughness and scaliness) wrinkling, and laxity.

Skin tolerability is fundamental for baby-care formulations for tenderly has to deliver excellent moisturizing and superfatting capabilities to counteract the signs of intrinsic and/or photoaging.

The last parts of the handbook, precisely part 7, 8 and 1a part are respectively dedicated to the Le-

gislation and Regulations in EEC, in USA and in Japan (3 chapters), to the problem of testing cosmetic products (2 chapters), and to the Cosmetic Claims (7 chapters) where are reported all the tests necessary to control for example subjects affected by cutaneous hyperactivity or to evaluate human facial wrinkles, or to avoid skin irritancy caused by the cosmetic products and so on.

All these tests are necessary for claiming also the activity and efficacy of the cosmetics.

The book ends highlighting the most advanced technologies used in testing the activity and the safety of the different cosmetic products.

It has to be appreciated the accuracy of the large news reported and the vast bibliography devoted to every single chapter, to demonstrate to the reader the complexity of the Cosmetic Dermatology as a proper new multidisciplinary science.

This handbook provides a comprehensive knowledge for all those scientists interested in cosmetic dermatology and/or concerned with the marketing of cosmetic products, such as cosmetic chemists, dermatologists or students of Medicine and Chemistry, and it will be useful to those involved in research and development in the pharmaceutical cosmetic, household and general chemical industries.

P. MORGANTI
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The 2nd World Conference on Medical Esthetics & Cosmetology

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PRESENT

NUTRI-COSME-CEUTICALS: A CHALLENGE FOR THE FUTURE

Rome – 6-7-8 February 2002

This international and multidisciplinary Symposium organized to celebrate the XX anniversary of the scientific activity of the International Society of Cosmetic Dermatology, intends to give a complete picture of the progress had over the last twenty years in skin physio-biological activity and in the manufacturing of innovative cosmetics and dietary supplements useful to improve people's health and appearance, and to prevent possible pathologies in the early age, in the middle age and in aged people.

For the first time participants could assist to scientific discussions presented by international experts in Physiology and Biology involved in the basic studies on skin and its appendages; by specialized technicians in Cosmetic Chemistry and Dietetics involved in the setting-up and production of cosmetics and dietary supplements; by Dermatologists, Gynecologists, Pediatrics and Dieticians who daily advice and prescribe to their patients these categories of products.

The Symposium will give proper room also to dietary and cosmetic aspects in Alternative and Complementary Medicine, such as Chinese Medicine and Indian Ayurvedic Medicine.

Aesthetic Medicine using medical-surgical devices, such as "filler", or methodologies, such as ionophoresis, ozone therapy or mesotherapy and natural products based on mineral waters and therapeutic muds of thermal origin, will be widely treated.

These topics will try to highlight all the problems concerning the activity performed by the different active principles and by the relevant carriers used for the setting-up of the finished product. The activity performed by both cosmetic and dietary products is, in fact, always in dependence of the chemical physical formulation of the active principles selected, which will be described by the formulators working for the raw materials industries, and, of course, in dependence of the carriers used in the setting-up of the finished product described by the chemists and technicians working for the cosmetics and dietetics industries.

Their real efficacy and the eventual undesirable side effects when coming into contact with the skin areas or the mucous membranes, will be investigated by a huge number of biologists, physiologists and pharmacologists involved in the absorption through the different biologic membranes.

The medical community, comparing their opinions with their colleagues chemists, biologists and physiologists, will discuss why to prescribe and how to use these products.

Because of these considerations, the Symposium will consists of five main sessions:

1. The current knowledge on skin, hair, nail and mucous membranes
2. Percutaneous and mucous absorption: the new control-release carriers
3. Functional food and cosme-ceuticals needs at different age: the-state-of-the-art
4. Botanicals, sea salt and mud in Alternative and Complementary Medicine
5. Innovative medical devices and nutri-cosme-ceuticals in Aesthetic Medicine: present and future market

We have the pleasure to invite you to participate at this happening, as attendee or as a speaker, giving your personal contribute to its success.

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Authors who wish to present a paper for poster or oral presentation according to the Symposium topics are requested to forward their one-page abstract to the Symposium Secretariat.

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Presenters of abstracts will be informed by a separate letter regarding the status of their abstract whether it was accepted as an oral or poster presentation. Accepted abstracts will be printed in the book of abstracts and distributed at the symposium.

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A trade exhibition will be held in conjunction with the meeting.

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