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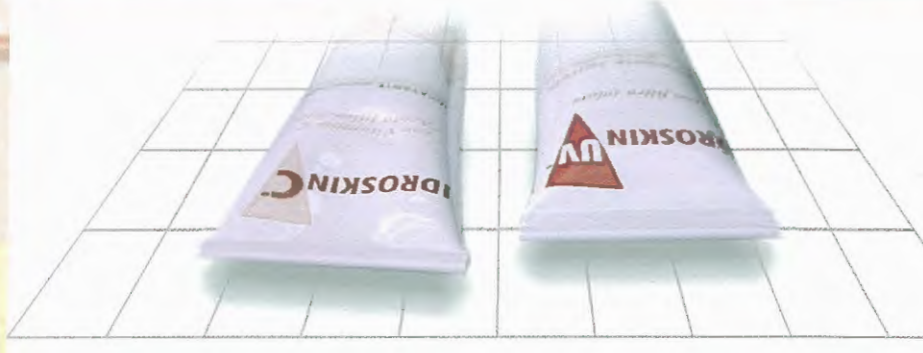
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after



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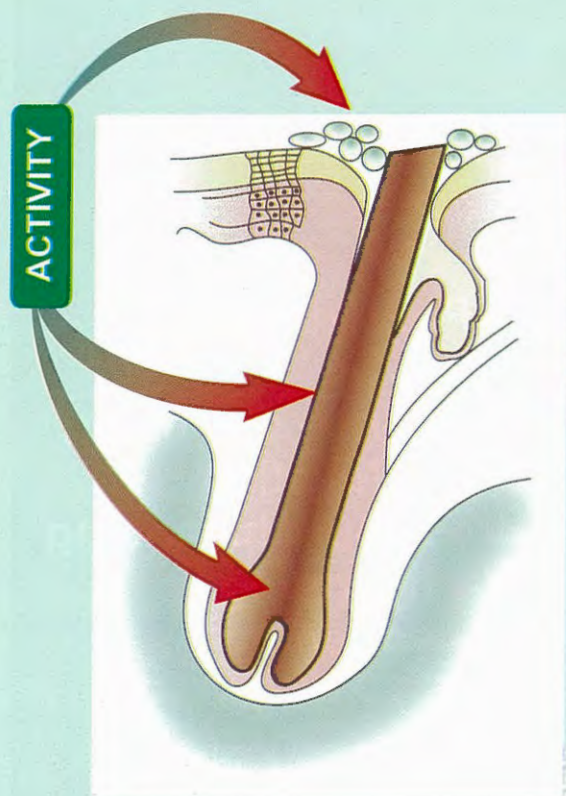
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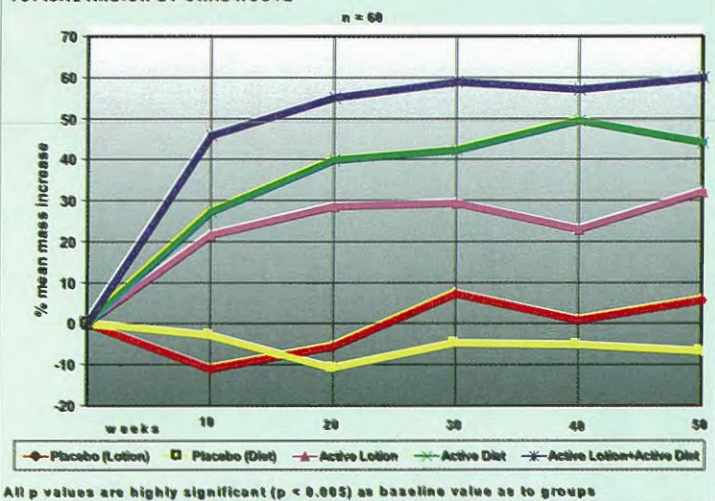
increases hair mass and number in a short period ^{(1),(2),(3)}

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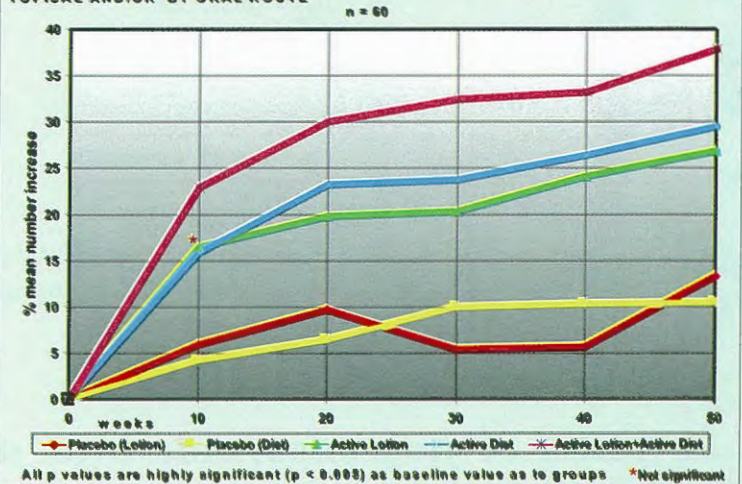
60%
increase in
hair mass

38%
increase in
hair number

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



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

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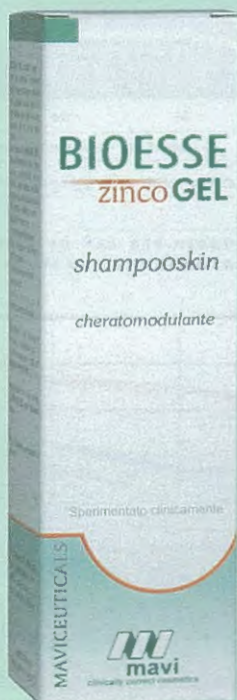
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XIII Announcements



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HOLISTIC COSMETICS

Ewa Farjon

Gems & Herbs Inc. - USA

Received: June, 2008. Presented at the "All For Cosmetics Conference", Warsaw, Poland, November, 20-21, 2007.

Key words: Holistic cosmetics; Natural cosmetics; Gemstones in cosmetics; Essential oils;

Summary

One of the fastest growing areas in the cosmetics industry is that of natural cosmetics. This is a direct result of people becoming aware of the difference between the use of *harmful* and *harmless* ingredients and the impact that these ingredients can have on us and on our environment.

But it is not enough to use natural ingredients; these ingredients must also be used in the right amounts in order to be effective.

Although the word "natural" has been much more popular than "holistic" when referring to cosmetics, recently the term "holistic cosmetics" has come to be used, too. In 2003, the Society of Cosmetic Chemists began presenting its biennial Holistic Cosmetics Symposium and Suppliers Show in the Twin Cities in Minnesota, USA.

We will be hearing the term, "holistic cosmetics" more frequently. The same is true of holistic medicine, holistic lifestyle and holistic treatments. As a society, we are now more aware that our future lies in a holistic approach to life, and to the environment.

Riassunto

Una delle aree di maggiore crescita dell'industria cosmetica è quella dei prodotti naturali.

Tale crescita è direttamente collegata alla crescente consapevolezza che i consumatori riservano agli ingredienti ritenuti più sicuri per gli esseri umani e per l'ambiente che ci circonda. Non è però sufficiente utilizzare ingredienti naturali, ma è importante soprattutto che essi vengano utilizzati nella giusta quantità affinché siano veramente efficaci.

Anche se il termine "naturale" per caratterizzare alcuni cosmetici è certamente più conosciuto del termine "olistico", quest'ultimo comincia ad essere usato sempre più spesso.

Infatti, nel 2003 l'associazione dei Chimici Cosmetologi Americani ha organizzato nel Minnesota un meeting su questo specifico tema.

Pertanto, a breve, comincerà ad essere utilizzato anche il termine cosmetica olistica, allo stesso modo e con la stessa frequenza con cui si parla sempre più spesso di medicina olistica o di stile di vita e trattamenti olistici.

E' per questa crescente tendenza che la nostra società, nel futuro, avrà certamente un approccio più olistico sia nei confronti della vita che dell'ambiente.

INTRODUCTION

Cosmetics are as old as vanity. They were very prized in ancient Egypt. Fragrant body oils with essential oils were very popular in Biblical times. The Bible contains 1,035 references to essential oils, aromatic plants, and their use.

One of the fastest growing areas in the cosmetics industry is that of natural cosmetics. This interest in natural personal care products is a direct result of people becoming aware of the difference between the use of *harmful* and *harmless* ingredients, and the impact that these ingredients can have on us and on our environment.

We have heard many times that the choice of food you eat is very important, and that the junk food can accelerate aging process. But how many times have we heard that cosmetics could accelerate aging process as well? Our skin absorbs the ingredients of the preparations that are applied to it through our pores. If the *harmful* chemical additives in food can hasten the aging process, how would these chemicals perform in cosmetics? They would perform exactly the same way; they would accelerate the aging process. And they do. Therefore, it is vital to view cosmetics in a holistic way, to be aware of the influence - both positive and negative - that different chemicals have on our whole body.

What does the term "holistic cosmetics" mean? Holistic cosmetics must be beneficial for the entire person; not only body, but mind and spirit as well. They shouldn't contain any *harmful* ingredients, but ingredients that are beneficial for the whole person, such as essential oils.

We will be hearing the term, "holistic cosmetics" more frequently. The same is true of holistic medicine or holistic lifestyle. As a society we are now more aware that our future lies in a holistic approach to life, and to the environment.

COSMETICS ARE AS OLD AS VANITY

Cosmetics were prized in ancient Egypt, since Egyptians were very concerned about their appearance. Archeologists discovered a stone tablet, which listed the formula for a remedy that could change "an old man into a young man"(1). For those interested in this discovery I can reveal that the main ingredient of this phenomenal preparation was fenugreek oil. As we can see, the main problem in cosmetics is still not solved - after thousands of years we are persistently looking for miracle, rejuvenating remedies.

In Biblical times, fragrant body oils with essential oils were very popular. The Bible contains 1,035 references to essential oils, aromatic plants, and their use (2).

Even though the Middle Age wasn't the best time for cosmetics, Saint Hildegard of Bingen left some formulas for cosmetic preparation; for example, she recommended barley water for rough, dry skin (3).



Fig. 1 Saint Hildegard of Bingen was a pionier in Europe in using gemstones in skincare.

NATURAL COSMETICS

One of the fastest growing areas in the cosmetics industry is that of natural cosmetics. In addition to "natural cosmetics," this category includes the terms "organic cosmetics" and "holistic cosmetics." This interest in natural personal care products is a direct result of people becoming aware of the difference between the use of *harmful* and *harmless* ingredients, and the impact that these ingredients can have on us and on our environment.

Today, many people are concerned with living a healthy, holistic lifestyle. They are aware of global warming, and are trying hard to do something to prevent it. Small steps like recycling, saving energy, using natural personal care products, and supporting "green" companies are just the beginning. It is estimated that approximately eighty percent of Americans consider ecology when making their purchasing decisions. The problem is that people are confused about which companies are really "green," and which products are really "holistic", or "natural". This is not surprising, since the Food and Drug Administration still does not give a strict definition for the term "natural cosmetic" and "the term organic means whatever manufacturer says it performs" (4). Because of this, many cosmetics companies advertise their products as natural, even though their cosmetics are just "dusted" with a few natural ingredients.

Why are customers looking for natural products? Are they really beneficial? We've heard many times that the choice of food you eat is very important, and that junk food can accelerate the aging process. But how many times do we hear that cosmetics can accelerate the aging process as well? I'd say not too many, if at all.

The skin is the body's largest organ. Skin absorbs the ingredients of the preparations that are applied to it through its pores. This is why prescription medication is effective when used in

the form of patches worn directly on the skin. This is why we must be very careful when we choose our cosmetics. If *harmful* chemical additives in junk food can accelerate aging, it's logical to expect the same results from cosmetics containing *harmful* chemical additives. Researchers at the Kyoto Prefectural University of Medicine in Japan found that cosmetics with methylparaben can cause skin to age when it is exposed to the sun. According to environmental writer Simon Pitman, Professor Toshikazu Yoshikawa told *The Asahi Shimbun*, Japan's leading newspaper, "I think women should avoid strong and direct sunshine when wearing cosmetics containing methylparaben"(5) also if the real exposure-response relationship of a chemical has to be seriously verified by the modern epidemiology also (6).

We know that some chemicals actually accumulate over time in the body and other studies should be necessary to demonstrate their real safety. Therefore, it is vital to view cosmetics in a holistic way, to be aware of the influence - both positive and negative - that different chemicals have on our whole body.



Fig. 2 Close contact with nature is an important element of holistic lifestyle.



Fig. 3 Taking care of natural resources is a vital part of holistic approach to life.

WHY HOLISTIC COSMETICS ?

Although the word “natural” has been much more popular than “holistic,” when referring to cosmetics, the term “holistic cosmetics” has recently come to be used, too. In 2003, the Society of Cosmetic Chemists began presenting its biennial Holistic Cosmetics Symposium and Suppliers Show in the Twin Cities in Minnesota, USA.

What does the term “holistic cosmetics” mean? What is the difference between “natural cosmetics” and “holistic cosmetics”? Holism refers to the idea that an entity is greater than the sum of its parts. So how can be this definition applied to cosmetics? Truly holistic cosmetics must be beneficial for the entire person - not only body, but mind and spirit as well. They cannot contain any harmful ingredients. To the contrary, holistic cosmetics should contain ingredients that are valuable for the whole person, ingredients that could be also used as supplements. Let’s concentrate on a few examples:

Green Tea is so healthy that it is called “China’s fountain of youth”. Because it is a great antioxidant, green tea is also used in cosmetics (7).

Glyceryl monolaurate is a main component of a self-preserving system that was discovered by

Prof. Dr. Jon J. Kabara many years ago. Glyceryl monolaurate was found in Mother’s Milk, Saw Palmetto, and other natural products (8). It helps support the immune system, and it is recommended as a supplement.

Pearls are admired in China and Japan. People from this region use cosmetics with pearl powder, and take pearl powder internally as a nutritional supplement. They believe that pearls can help them stay young longer. From my own experience I can add that good quality pearl powder is absorbed by skin surprisingly fast.

GEMSTONES IN COSMETICS

Gemstones have been used in cosmetics since ancient times. Egyptians were using powdered malachite as eye paint (9). Saint Hildegard from Bingen recommended amethyst for acne. Gemstones are still very popular in some regions.

In Europe - especially in Baltic countries – holistic medicine practitioners recommend amber elixir. Amber is also used in cosmetics. It is said to smooth and nourish the skin, add elasticity, and protect it from the sun’s rays.

In recent years, scientists have begun to show an interest in using gems in cosmetics. In 2002, the Journal of Cosmetic Science published the paper “Investigation of jewelry powders radiating far-infrared rays and the biological effects on human skin.” In this article, scientists from the Skin Research Institute, Pacific Corp. in Korea wrote about their experiments with tourmaline and jade powders. They obtained very promising results, and they said that these powders “show great potential for utilization as cosmetics’ raw materials” (10).

“Skin is alive, and lifeless chemicals cannot give life back to the skin”

Pratima Raichur (11)

According to the definition of holism, in addition to being beneficial for the whole body, holistic cosmetics should be beneficial to the mind and spirit. If a woman feels attractive, people will consider her to be attractive. Without a doubt, it is easier to feel good wearing cosmetics that contain uplifting essential oils.

Here we come to aromatherapy. Many women know that essential oils can influence our mood. Those who use these wonderful oils experience the refreshing properties of peppermint, the relaxing quality of lavender, and many other qualities from other varieties of these oils. And we know from history that Queen Cleopatra of Egypt was told to use roses to help her to seduce Mark Anthony.

Essential oils are very useful in skin care and hair care. Many of them have rejuvenating properties, and they can stimulate new cell growth and prevent wrinkles (e.g., lavender, neroli, and frankincense)(12). “Cellular regeneration is the key to a youthful skin, and essential oils provide a way of doing this which is far more pleasant than the most recent methods which involve using fetal cells” expressed Valerie Ann Worwood. It would be good to remember this statement today, when some companies market new anti-aging creams that are advertised as “stem cell technology”(13).

Essential oils are the most powerful free radicals scavengers. Scientists at Tufts University in Massachusetts, USA, have developed the ORAC (Oxygen Radical Absorption Capacity) test to compare the capability of different substances to fight free radicals. The higher the number, the greater antioxidant properties the substance has (2). Below are presented ORAC test results from

“Healing Oils of the Bible” by Dr. David Steward:

TABLE I

Antioxidant (ORAC) Scores for Selected Fruits and Vegetables

Carrots	210
Oranges	750
Beets	840
Raspberries	1,220
Strawberries	1,540
Blueberries	2,400
Wolfberries	25,300

TABLE II

Antioxidant (ORAC) Scores for Selected Essential Oils

Sandalwood	1,655
Juniper	2,517
Rosemary	3,309
Rose of Sharon (Cistus)	38,624
Cinnamon Bark	103,448
Thyme	159,590
Clove	10,786,875



Fig. 4 Scientists have proved that tourmaline can be a valuable ingredient in cosmetics.

As we see, essential oils have the highest free radicals scavengers' capability. This property alone should make them desirable cosmetics

components. Essential oils also have the highest electromagnetic frequencies of all known substances. The highest is Rose Oil at 320. The results for some other oils: Lavender - 118 MHz; German Chamomile - 105 MHz. and Peppermint - 78 MHz.

By comparison: fresh fruits and vegetables: 5-10 MHz, fresh herbs 20-27 MHz, processed food measured zero.

“Measurements on the human body found that a healthy person has a frequency around 62-68 MHz. When a person’s frequency dips to 58 MHz, cold symptoms can manifest. Flu symptoms start at 57 MHz. Cancer can begin when the body falls below 42 MHz. The process of dying begins at 25 MHz and goes to zero at death”. By applying essential oils we can increase our body’s frequency.

CONCLUSION

In order to make extraordinary cosmetics, most companies today seem to prefer the newest discoveries from the lab. Perhaps they should once again start looking to Mother Nature for natural, time-proven ingredients – like essential oils or gemstones.

We do have natural, valuable ingredients that can be used in cosmetics, and we don’t have to use harmful chemicals. As dr. Kurt Schnaubelt pointed in his book “Advanced Aromatherapy. The Science of Essential Oil Therapy”: “Most of the synthetic substances used in cosmetics today do not take part in metabolic processes of cell reproduction, and cause undesired or toxic effects if used over a significant period. In contrast, plant-derived products are active and have remarkable therapeutic effects if used with “the proper cosmetic base material” (*emphasis mine*) (14). Yes, this is exactly what we can do. We can’t change the cosmetics industry at once, but we should stop using harmful, toxic cosmetics ingredients.

And coming back to the definition of holistic cosmetics, one thing is certain. Even though there may be differences on the exact definition of "holistic", we will be hearing the term, “holistic cosmetics” more frequently. The same is true of holistic medicine, holistic lifestyle and holistic treatments. As a society we are now more aware that our future lies in a holistic approach to life, and to the environment.

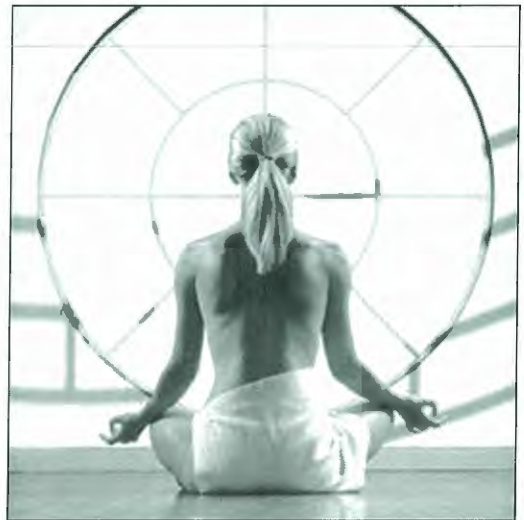


Fig. 5 Holistic approach to life is not limited only to meditation.

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CLINICAL APPLICATION OF INTENSE PULSED LIGHT IN ASIAN PATIENTS

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Key words: Intense pulsed light; Selective photothermolysis; Hyperpigmentation; Photoaging; Melasma; Acne and Rosacea; Asian skin;

Summary

Chinese skin is relatively darker than Caucasian skin, more apt to develop post-inflammatory pigmented changes, and to develop scar following the procedures that induces inflammation. In order to avoid the over damage of intense pulsed light to Asian skin, lower energy and mild treatment parameters are proposed.

A new IPL modality (Lumenis One, Santa Clara, USA), delivering more even distribution of the energy within each individual pulse and between sub-pulses, can result in safer and more effective treatments.

Patients with freckles got over 50% improvements after 2 sessions of IPL. With the succession of 4 treatments, 35%, 63%, 75% and 87% patients with café-au-lait spots obtained over 50% improvement. After 4 sessions of treatment, 69 of 89 melasma patients (77.5%) obtained 51-100% improvements, according to the over-all evaluation by dermatologists. IPL could also be tried in treating macules of port wine stain, which were resistant to pulsed dye laser. Half of them got over 50% of clearance in our clinical study. For rosacea patients, 81.18% had more than 60% improvement after 4 sessions. In 152 patients with photoaging, 91.44% got a score decrease of 3 to 2 after 4 sessions of treatment.

In conclusion, IPL has widely been used in treating a variety of skin disorders in Chinese population, with excellent improvements and limited side-effects.

Riassunto

La cute degli asiatici, a differenza della cute caucasica, è caratterizzata da melanosomi più grandi e maggiormente melanizzati. Per questi ed altri motivi sviluppa più facilmente iperpigmentazioni post-infiammatorie. In questo studio vengono riportati alcuni risultati ottenuti trattando con luce pulsata a bassa intensità (IPL) la cute di soggetti asiatici con lo scopo di attenuare i fenomeni iperpigmentari e infiammatori provocati da alcune comuni disfunzioni patologiche come, ad esempio, il melasma, l'acne, la rosacea ed il fotoinvecchiamento. I risultati che si incontrano con la IPL sulla popolazione cinese sono da considerarsi eccellenti.

INTRODUCTION

Asian skin is totally different from Caucasian skin in a variety of aspects. Asian skin is relatively darker than Caucasian skin. They are prone to get sun tanning, instead of sun burning, which are very common in Caucasian skin with Fitzpatrick phototype I to III. Most Asian skin belongs to Fitzpatrick phototype III or more. Asian skin is more apt to develop post-inflammatory hyperpigmentation and hypopigmentation following any procedure that induces inflammation.

Asians are far more likely than Caucasians to develop keloid. Thus more cautions should be given to Asian skin to avoid any damage to the integrity of the epidermis.

Furthermore, photoaging in Asians tends to occur at a later age and has more pigmentary problems but less wrinkling than in Caucasians [1]. This difference is partially due to the higher epidermal melanin content.

As has already been well documented, there are larger and more melanized melanosomes in Asian skin than in Caucasian skin. In order to avoid the over-damage to the epidermal melanin, lower energy and mild treatment parameters are proposed for Asian skin.

PIGMENTED DISORDERS

Ephelides

Ephelides, or freckles, are small, usually less than 0.5 cm in diameter, discrete brown macules that appear on sun-exposed skin. Histologically they demonstrate as melanocyte proliferation without nest formation along the basement membrane. Intense pulsed light (IPL) sources that emit a broad band of visible light and infrared light (400–1,200nm) from a noncoherent filtered flashlamp, affects pigmentation via photo-

thermal effects. IPL has been studied for the treatment of lentigines and ephelides with cutoff filters ranging from 550–590nm, a fluence of 25–35J/cm², and a pulse width of 4.0ms. These studies have been performed on Asian skin with surprisingly no PIH. This lower risk of PIH and the limited postoperative downtime have made IPL a popular choice. The patient should understand, however, that multiple treatments may be necessary. In our practice, for those who do not wish to have any downtime, or for those who wish to improve not only their pigmentation, but also pore size and skin texture, we offer IPL treatment combined with other laser modalities in the same treatment session to obtain a better outcome. The recommended parameter (Lumenis One, Lumenis) is single pulse (pulse width: 3–4 ms) at 12–14 J/cm² for fair skin, and double pulse (pulse width: 3ms, pulse delay: 20–30ms) at 15–17 J/cm² for dark skin. After 2 sessions, all the 69 freckle patients obtained over 50% clearance [2].

Café-au-lait macules (CALMs)

The use of Q-switched lasers (ruby 694nm, frequency-doubled Nd-YAG 532nm) [3] and pulsed dye laser (510nm) [4] in the treatment of CALMs has yielded variable results with a high risk of recurrence if pigment is left behind. Populations demonstrated a similar variable degree of repigmentation following a long-time follow-up period of up to 50%.

Yoshida et al performed the treatment of pigmented lesions with eurofibromatosis 1 by intense pulsed-radio frequency (IPL-RF) in combination with topical application of vitamin D3 ointment. Eight patients were treated in this study and the improvement was moderate to good in six cases (75%) [5].

We treated 58 Chinese patients of CALMs with Lumenis One IPL (560nm filter, single pulse, 3–4ms pulse width, 14 J/cm² for fair skin; 560nm

filter, double pulse, 3ms pulse width, 30ms pulse delay, 15-17 J/cm² for dark skin). With the succession of 4 treatments, 35%, 63%, 75% and 87% patient obtained over 50% improvement [2].

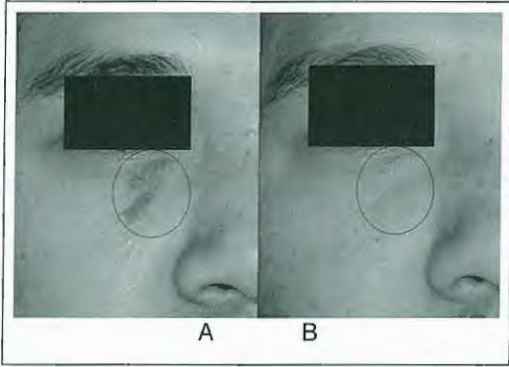


Fig. 2 A patient with CALMs. **A:** prior the treatment; **B:** after 3 sessions of treatment (560nm filter, double pulse, 3ms pulse width, 35ms pulse delay, 14J/cm²).

Melasma

Melasma is commonly seen in Asian population. Traditional therapies are less effective and may cause adverse effects. We tried IPL (Lumenis One, Lumenis) in 89 women with melasma. Subjects received a total of four IPL treatments at a 3-week interval (560/590/615/640nm filter, pulse width of 3-4ms, pulse delay of 25-40ms, fluence of 13 to 17 J/cm²). Changes in facial hyperpigmentation and telangiectasis were evaluated using a Mexameter, the melasma area and severity index (MASI), and a global evaluation by the patients and blind investigators. Sixty-nine of 89 patients (77.5%) obtained 51-100% improvements, according to the over-all evaluation by dermatologists. Self-assessment by the patients indicates that 63 out of 89 patients (70.8) considered over 50% or more improvements. Mean MASI scores decreased substantially from 15.2 to 4.5. Mexameter results

demonstrated a significant decrease in the degree of pigmentation and erythema beneath the melasma lesions. Patients with the epidermal-type melasma responded better to treatment than the mixed-type. Adverse effects were minimal [6].

Postburn hyperpigmentation

Ho et al has tried IPL in the treatment of postburn hyperpigmentation to assess its efficacy and side effects. Multilight™ (ESC Medical Systems Ltd., Yokneam, Israel) of the IPL family was used to treat these patients at intervals of 3-4 weeks for three to seven treatments. Patients were treated with an energy fluence of 28-46 J/cm², pulse width of 1.7-4 ms, double pulse mode, and a delay of 15-40 ms. Among the nineteen Chinese patients, over 78% showed more than 50% clinical clearance and nearly 32% of the patients were able to achieve more than 75% clearing. Although two patients had no clinical response, one patient had 100% clearing. Three patients developed blisters and one patient had erythema that all resolved within 1 week without leaving permanent marks. They have been followed-up from 11-32 months and there was no recurrence of the hyperpigmentation [7].

Port wine stain

Asian patients with more melanin in the epidermis are at a higher risk of adverse effects after laser treatment of vascular diseases. Although the pulsed dye laser is the gold standard in treating port wine stain, at times patients find the results disappointing and the various side effects, such as pronounced purpura and pigmented changes, to be disturbing.

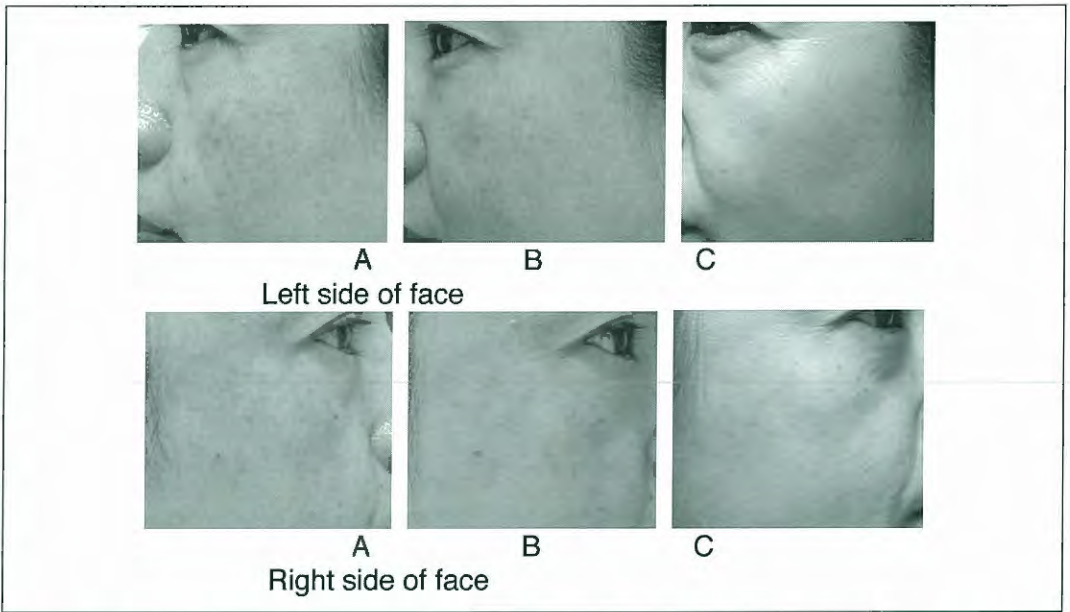


Fig. 3 A patient with melasma. **A:** pretreatment; **B:** after 4 sessions; **C:** at 3 month follow-up visit (590nm filter, triple pulse, 3ms pulse width, 30ms pulse delay, 15-17 J/cm²). **Upper trace:** left side of face. **Lower trace:** right side of face.

Bjerring et al used IPL system for the treatment of port-wine stains (PWS) resistant to multiple pulsed dye laser (PDL) treatments. Fifteen PWS patients, who were previously found to be resistant to multiple PDL treatments, were treated four times with a second generation IPL system. Patients with dye laser resistant PWS could be divided into two groups: responders to IPL treatments (46.7%) and non-responders (53.3%). All responders obtained more than 50% reduction, and 85.7% of the responders obtained between 75% and 100% reduction of their lesions. The IPL treatment modality was found to be safe and efficient for the treatment of PWS, except for those located in the V2 area [8].

We tried also IPL (Lumenis One) in treating port wine stain and got very good results. See Fig. 4 for details.

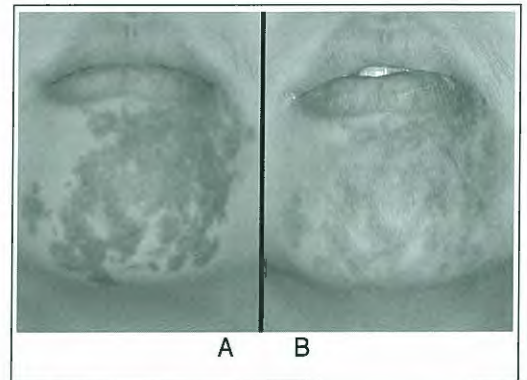


Fig. 4 A patient with port wine stain. **A:** prior the treatment; **B:** after 4 sessions of treatment (560nm filter, triple pulse, 3ms pulse width, 50ms pulse delay, 20-24 J/cm², 2 passes).

ACNE AND ROSACEA

A rising number of laser- or light-based therapies are addressing the need for effective acne treatments with minimal downtime. In order to evaluate the efficacy of IPL in the treatment of acne, Chang et al performed IPL equipped with a 530- to 750-nm filter on 30 female Korean patients (mean age, 25.7 years) with mild-to-moderate acne. All patients experienced the reduction of inflammatory lesion counts in both sides of face. There was no significant difference between IPL-treated and untreated sides of the face for mean papule plus pustule counts, 3 weeks after three sessions. As to red macules, 63% obtained good or excellent results on the laser-treated side compared to 33% on the untreated side. Improvement of irregular pigmentation and skin tone was detected on the laser-treated side than the untreated side [9].

We have used IPL in treating acne patients and rosacea patients at a 3-week interval. It could significantly reduce the inflammatory papules, pustules, acne scars, and red macules.

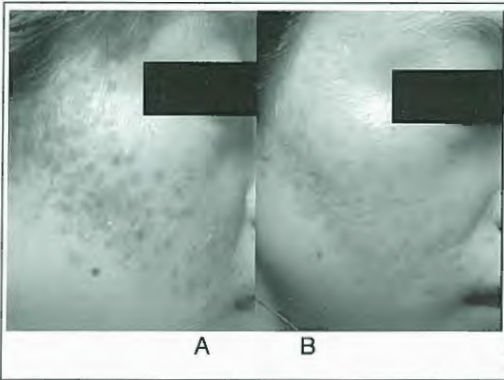


Fig. 5 A patient with inflammatory acne. **A:** prior to treatment; **B:** after 3 sessions of treatment (590nm filter, triple pulse, 3ms pulse width, 30ms pulse delay, 17-19 J/cm²).



Fig. 5 A rosacea patient received 3 sessions of treatment on the nose (the perioral region was spared). **A:** prior the treatment; **B:** after 3 sessions of treatment (590nm filter, triple pulse, pulse width 3ms, pulse delay 35ms, 17-20J/cm², 2 passes).

PHOTOAGING

Cumulative exposure to sun is the main reason for skin aging. Photoaging skin is characterized by fine and coarse wrinkles, dyspigmentations, telangiectasia, sallow color, dry and rough texture, laxity, increased pore size, and a leathery appearance in habitually sun-exposed skin. Bitter et al has reported that the noncoherent IPL device could efficiently solve all the above problems at the same time [10].

One hundred and fifty-two Chinese women with photoaging skin were treated with IPL (Lumenis one) in our open-labeled study. Subjects received a total of four IPL treatments at a 3- to 4-week interval. One hundred and thirty-nine of 152 patients (91.44%) experienced a score decrease of 3 or 2 grade, according to the dermatologist. One hundred and thirty-six of 152 patients (89.47%) rated their overall improvement as excellent or good. The mean MI and EI values decreased with each session. MI on forehead and EI on cheilion decreased most significantly. Adverse effects were limited as mild pain and transient erythema. IPL treatment is a safe

and effective method for photoaging skin in Asian patients. Adverse effects were minimal and acceptable [11].

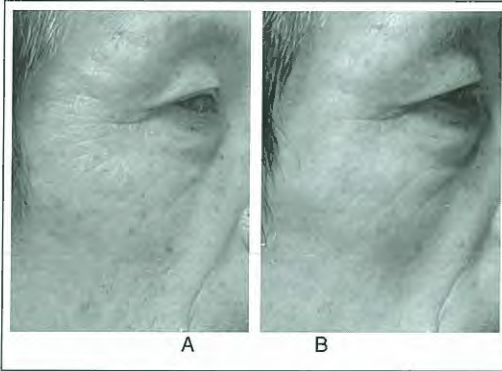


Fig. 7 A patient of photoaging. A: prior to treatment; B: after treatment (640nm filter, triple pulse, 3ms pulse width, 30ms pulse delay, 17-19J/cm²).

CONCLUSION

In conclusion, IPL has widely been used in treating a variety of skin disorders in Chinese population, with excellent improvements and limited side-effects.

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CHITIN-NANOFIBRILS: A NEW ACTIVE COSMETIC CARRIER

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Key words: Chitin Nanofibrils; Lutein; Melatonin; Ectoin; Skin penetrability; Skin Enhancer;

Summary

The cosmetic industry has evolved over the last 50 to 60 years from an era of secret formulas, elusive promises and false hope to an entirely new industry based on science. No longer are the cosmetic giants isolated scions, but there is an ever growing interactions and interdependence among cosmeceutical, pharmaceutical, biochemical, and medical community. Thus new development has been successfully translated into more effective treatments as well as preventive treatments of dry or aging skin, as example.

At this purpose, active compounds of new cosmetic products has to be carefully selected to obtain the best efficacy and safeness. Thus innovative cosmetics have become more sophisticated in both formulations and presentation meanwhile new tests have been developed to ensure not only quantity and safety but also the validation of products claims.

Different *in vitro* and *in vivo* tests are reported to show the capacity of *chitin nanofibrils* to be used both as penetration enhancer and active compound as anti-ageing agent.

Riassunto

Negli ultimi 50 anni l'industria cosmetica si è trasformata da produttrice di formule basate su false promesse a produttrice di prodotti innovativi basati su studi scientifici. La cosmetologia è così diventata scienza multidisciplinare collegata con la biologia, la farmaceutica e con la comunità medica.

Sono state prodotte con successo nuove formulazioni utili per impedire, ad esempio, l'instaurarsi di xerosi o per prevenire il fotoinvecchiamento cutaneo.

A questo scopo è indispensabile che siano attentamente selezionati sia i principi attivi che i veicoli

utilizzati per ottenere i migliori risultati di efficacia e sicurezza d'uso dei prodotti cosmetici formulati. Sono nati così cosmetici innovativi più sofisticati sia nelle formulazioni che nelle loro prestazioni, mentre contemporaneamente sono state sviluppate metodologie di controllo necessarie per supportarne i relativi messaggi pubblicitari.

In questo lavoro vengono riportati studi *in vitro* ed *in vivo* necessarie ad evidenziare le capacità possedute dalle *nanofibrille di chitina* quali promotrici della penetrazione transcutanea e quale principio attivo utile, ad esempio, per la formulazione di cosmetici anti-età.

INTRODUCTION

The central tenets of current and upcoming molecular biology, nanoscience and innovative ingredients will continue to play an ever increasing role in the cosmetic and skin care industry (1-3). On one hand the increasing demand for anti-aging personal care products and the need to understand these product's mechanism of action continuously provides a scientific challenge to the industry formulator and expert evaluator (4,5).

On the other hand informed consumers want to know which topical treatments are a viable alternative to invasive surgical procedures, and which active ingredients and carrier systems are able to preserve and even regenerate a youthful, healthy look. Numerous studies show, in fact, that present day personal care products provide the greatest efficacy only after months of regular and repeated application (6). Thus the technologically advanced delivery and controlled-release of highly functional ingredients, offered by the new chitin-nanofibril, makes them the most reliable way to achieve those desired results. (7-9).

Aims

We aimed to examine both *in vitro* and *in vivo* the activity of some well known antioxidant compounds transported through the skin layers by a nano-emulsion carrier based on chitin-nanofibrils, previously used in our group's research (10-12).

In order to understand the mechanism of action of these compounds against free radicals and pollutants at the skin level, we compared a mixture of melatonin, lutein and ectoin pre-linked with chitin-nanofibrils (CN) and embedded in a CN-nano-emulsion, in accord with other studies already published by our group (13,14)

MATERIALS AND METHODS

Experimental Section

METHODS

In Vitro Activity. *Regenerative activity*

As it is known, the dermis represents the fundamental and supporting portion of the skin. Its papillary portion contains a high amount of collagen and elastic fibers required to give firmness and elasticity to the skin. Fibroblasts, contained in high amounts in the papillary dermis, continuously produce these fibers (15). Cutaneous aging causes a thinning of the dermis and a qualitative and quantitative reduction of the fibroblasts, which no longer produce collagen efficiently. Thus the effect of chitin nanofibrils on the growth of a fibroblast culture was tested both alone and in association with some antioxidant and immunostimulant compounds.

The skin regeneration process is, in fact, very efficient in young people and in healthy skin, but drastically reduced with aging. It is also influenced by stress, loss of sleep, and air conditioning, capable of reducing the normal cellular turnover, and increasing both age spots and wrinkles (16). All these phenomena, contributing to the general aging of skin, may be examined with different methods, e.g. by assessing the energy required for various metabolic processes, stored as ATP; or, by measuring fibroblast activity and collagen and melanin synthesis, thereby verifying the hyperpigmentation activity that contribute to the formation of age-spots.

Fibroblast activity

Fibroblasts of NB1RGB strain were used (2×10^5 cell/ml) and suspended in the α -MEM culture medium placed in 8 Petri dishes (containing

10% foetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin) (17).

To 6 cultures were added, respectively a 10 mg/ml concentration of:

1. melatonin;
2. chitin-nanofibrils (CN)
3. melatonin-lutein;
4. melatonin-ectoin;
5. melatonin-lutein-ectoin;
6. melatonin-lutein-ectoin-chitin-nanofibrils while two were left as control.

The results obtained are reported in Fig. 1, illustrating the medium percent of cell proliferation with respect to the control value.

All biochemical processes require energy that is accumulated in the form of ATP (adenosyn-tri-phosphate). ATP was measured on a culture of keratinocytes irradiated with 4J/cm² UVA + 04

J/cm² UVB (SOL500 lamp – Munich, Germany) and compared with ATP levels on keratinocytes irradiated and additioned with the products under study. As known, irradiation causes a drastic reduction of the ATP present and it is dose-dependent (18).

Measuring ATP activity

Of the 8 dish cultures, 6 received 10 µg/ml of the different substances to be tested 24 hours before UV irradiation, whereas two served as control.

The ATP level was detected by using ATP Lite-M (Chemiluminescent kit, Packard).

Results are reported in Fig. 2, illustrating the residual medium percent amount of ATP per dish.

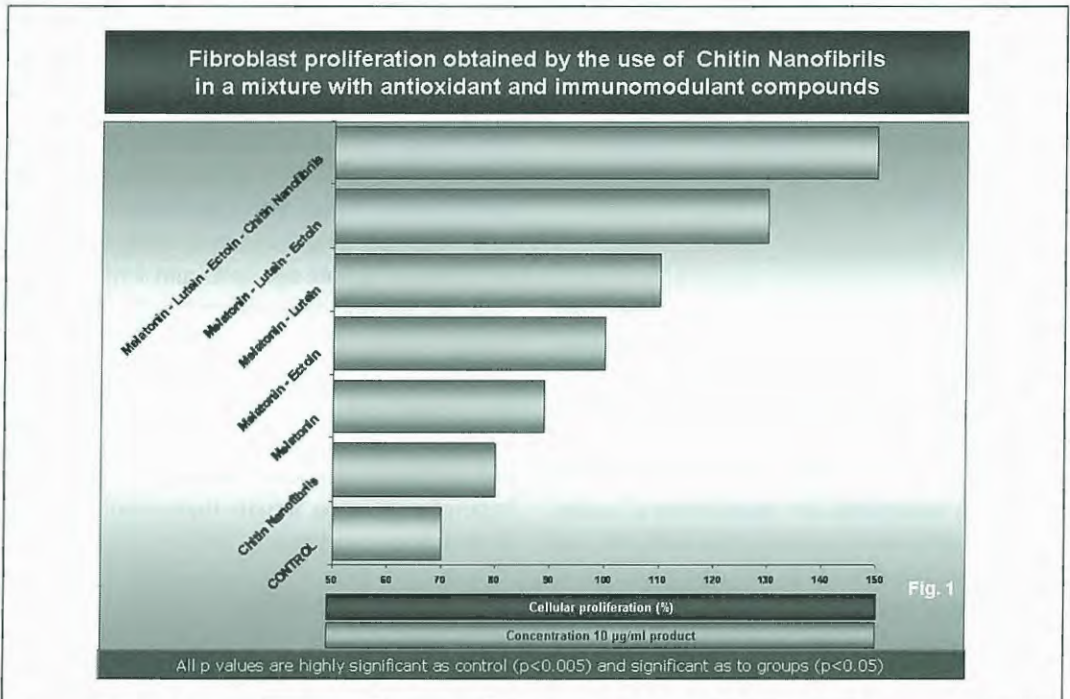


Fig. 1

Stimulation of collagen synthesis

A continuous and regular collagen synthesis is of fundamental importance for the ECM (extracellular matrix) structure, and therefore for skin elasticity, firmness and wrinkle reduction. A decrease in the rate of collagen Type-I production, and expression of the genes coding for collagen Type-II and III, can be observed during aging. The rate of collagen Type-I secretion was measured by the use of specific antibodies (Elisa method) on 8 cell cultures, 6 of which enriched with 10 µg/ml of the various substances directly introduced in the culture medium. Two served as control (19). Measurements were done after 6 days of incubation.

Results are reported in Fig.3, illustrating the medium percent increase of collagen with respect to the control value.

Depigmentation activity

The depigmentation activity present in the various mixtures of products was verified on B16 melanoma cells (5x10 cell/ml) suspended in MEM culture medium (10% FBS, 1000 I.U. (International Units) /ml penicillin and 100 µg/mg streptomycin) containing 2 mM teophylline. The suspension was subdivided into 8/500 - µl portions. To each portion, placed in suitable bars, 50 µg/ml of the various mixtures of active agents were added. Two were the untreated controls.

Post-incubation: 300 µl PBS were added, then all samples were ultrasonicated. An increase or decrease in the presence of melanin was measured by 415-nm spectrophotometer (20). The various composts examined showed significant decreases in melanin formation.

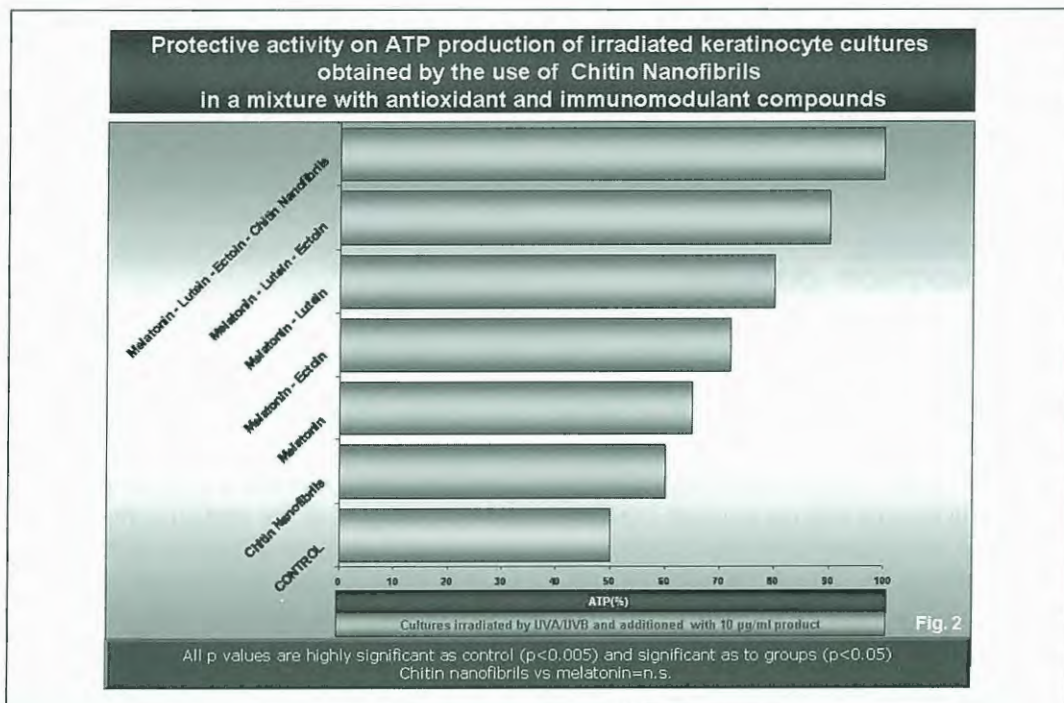


Fig. 2

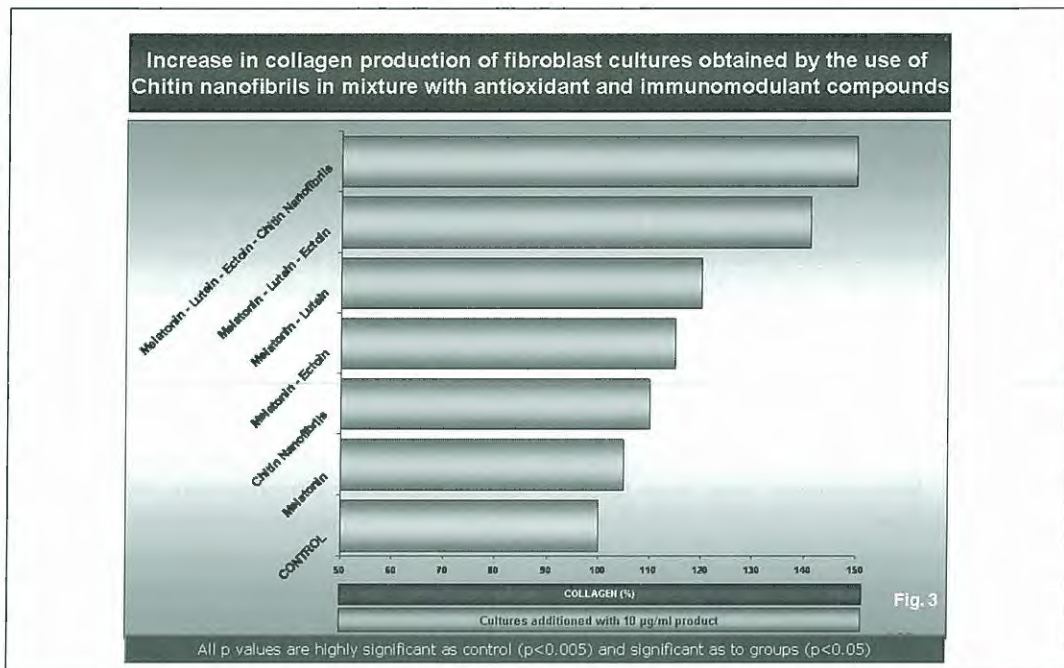


Fig. 3

The average results obtained are reported in Fig.4, illustrating percent values with respect to the control value.

In Vivo Activity.
Skin absorption -potential

Before starting the other *in vivo* studies, the absorption potential of CN was controlled, as probable skin penetration enhancer of our active compounds, in comparison with the vehicle alone. The dansyl chloride labelling technique was used, in keeping with our previous experiences (21-24). A 5% dansyl chloride concentration finely triturated was added into four formulations:

1. vehicle alone
2. vehicle + CN
3. melatonin-lutein-ectoin + vehicle(product B)
4. melatonin-lutein-ectoin-CN + vehicle (pro-

duct A).

The formulations were applied indifferently on the right (product 1 and 2) and/or the left (product 3 and 4) volar forearm of 10 women volunteers, and kept under semi-occlusive dressings for 24 hours.

The day after, the area was cleansed using a lotion (Idroskin latte) and soft tissue paper. The dried stratum corneum (SC) surface was removed using 15 strips of an adhesive tape (Sellotape®) in succession.

Using the correct methods it is possible to obtain successive layers of the stratum corneum, each one of single thickness. On all the SC-layers, the level of fluorescence was measured by UV illumination, using an arbitrary scale of 0-8. The level of redox-balance (not reported) was also detected on the different layers treated by mixture 3 and 4, in accord with our previous studies (23,24).

The obtained mean final results are reported in Fig.5.

External aggressive agents provoke an immune response, always accompanied by inflammatory reactions. When these reactions are excessive, the cascade that produces the pro-inflammatory mediators can become abnormal.

Interleukin 8 (IL-8) is the one most responsible for the permanence of the inflammatory state (25).

Anti-Inflammatory Effect IL-8 examined

10 volunteers suffering from cutaneous dryness of atopic origin, ranging in age from 15 to 20 , with elevated interleukin 8 (IL-8) expression were selected. Then lymphocytes were isolated. The 8 collected blood samples of lymphocytes, 6 pre-supplemented and 2 non-supplemented (control) with the components under study (1

µg/ml), were subdivided into 8 Petri dishes, and further supplemented with 10 mg/ml Tumor Necrose Factor α (TNF-α). The TNF-α addition caused a marked increase in IL-8 production, whereas the substances under study were supposed to reduce said increase. IL-8 amounts were determined photometrically, by specific antibodies (26,27).

The results obtained are reported in Fig.6, illustrating the relative medium amounts of free IL-8 with respect to the control values.

Experimental Project

To a group of 40 women volunteers ranging in age fom 25 to 35, suffering from photoaged dry skin, were distributed under double-blind conditions two different typologies of cream to be applied by light massage indifferently on the left or right arm, in the morning and in the evening, as well as on the two hemi-sides of the face.

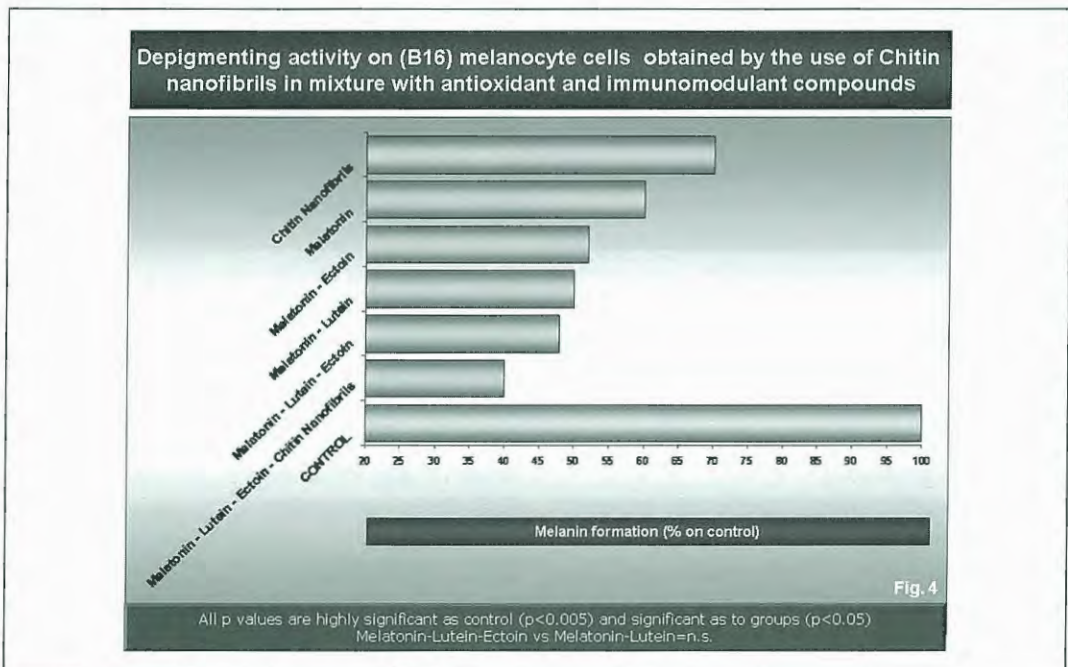


Fig. 4

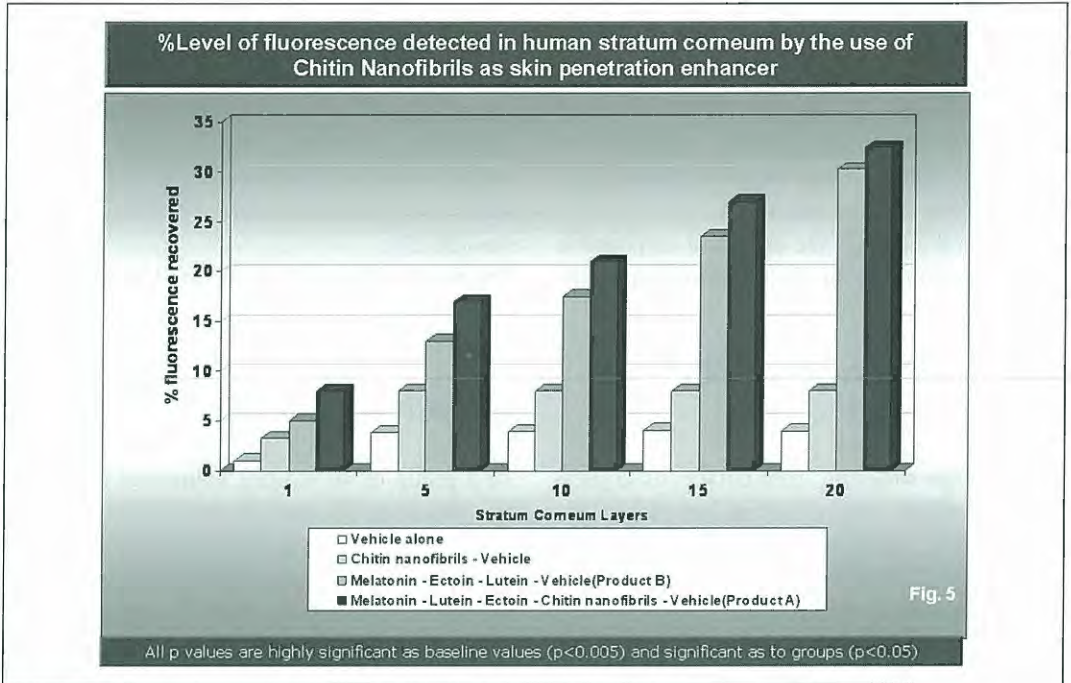


Fig. 5

The two creams, contained in differently-coloured tubes, were sufficient for 60 days of treatment.

Initially and throughout the entire experiment the groups were subdivided as follows:

10 women treated with product (emulsion carrier with melatonin –lutein-ectoin-CN) and carrier only, (product 1)

10 women treated with product (emulsion carrier with melatonin –lutein-ectoin) and carrier only, (product 2)

10 women treated with emulsion with CN and carrier only (product 3)

10 women treated with the carrier only (group 4) (control group)

15 days before, at the starting and 60 days after the control period, skin surface lipids, hydration, and TEWL, were verified by the 3C System (28); the lipid peroxides by the MDA method (29) All the values were the average of 3

assessments.

MEASUREMENT EQUIPMENT

Skin surface lipids

The skin surface lipid levels were measured with the 3C System (Dermotech S.r.l., Rome, Italy). Determination is based on photometric measurement of light transmission through a skin surface imprint obtained by applying a frosted pastic foil to the designated skin area. It allows adherence of skin lipids in a 1 cm² area calculated digitally in µg/cm² (28).

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 sec.), the capacitance was displayed digitally in arbitrary 3C units. The results reported are expressed as mean values of the measurements performed on four different right or left sites (cheek, forehead, chin and nose).

Transepidermal water loss (TEWL)

All evaluations were performed after a 30-minute acclimatization period in a room at $22\pm 2^{\circ}\text{C}$ with 50% humidity.

Water evaporating from the skin surface was measured quantitatively with the 3C System methodology.

The 3C System probe consists of a cylindrical open chamber measuring system, with diameter

14 mm, height 10 mm and a distance from skin area $0,95\text{ cm}^2$. Two sensor units, containing thin capacitive film transducers, were placed in the probe at 3 and 7 mm distance from the skin surface. TEWL is calculated digitally in $\text{g/m}^2\text{ h}$.

Lipid peroxides

Lipid peroxides were checked determining the presence of these derivatives by the malonyl dialdehyde (MDA) method (29).

The global results obtained are reported in Fig. 7-10, illustrating the percent decrease of skin lipid peroxides and TEWL (Fig. 7 and 8) and the percent increase of skin hydration, and superficial skin lipids (Fig 9 and 10). All the values were calculated with respect to the starting values.

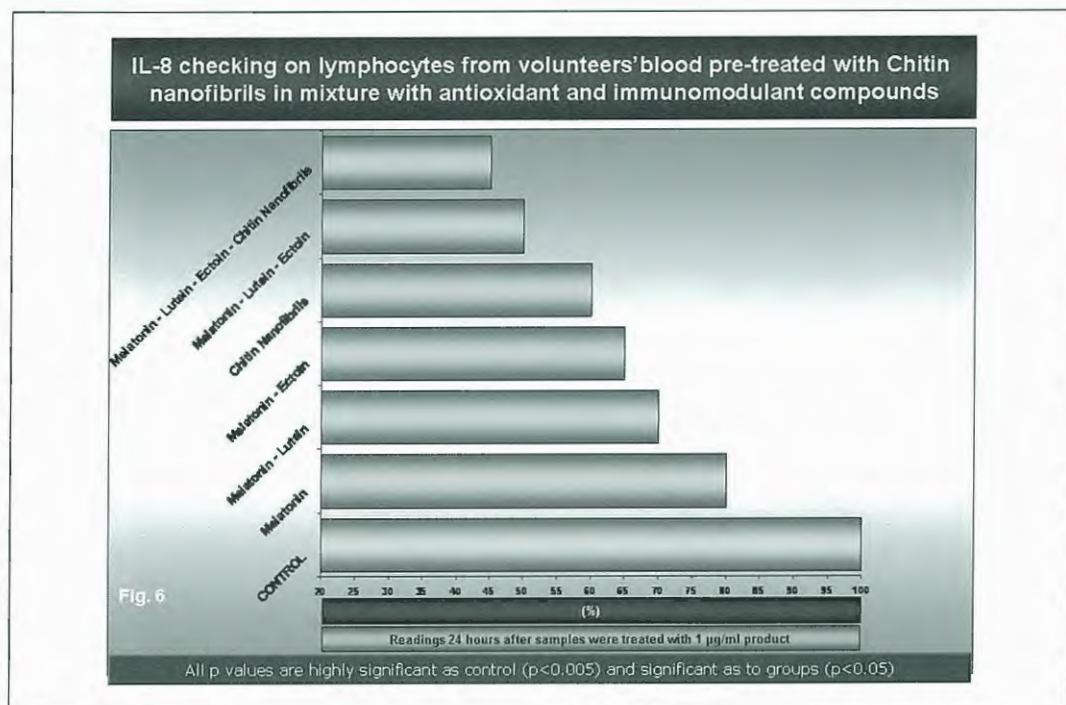


Fig. 6

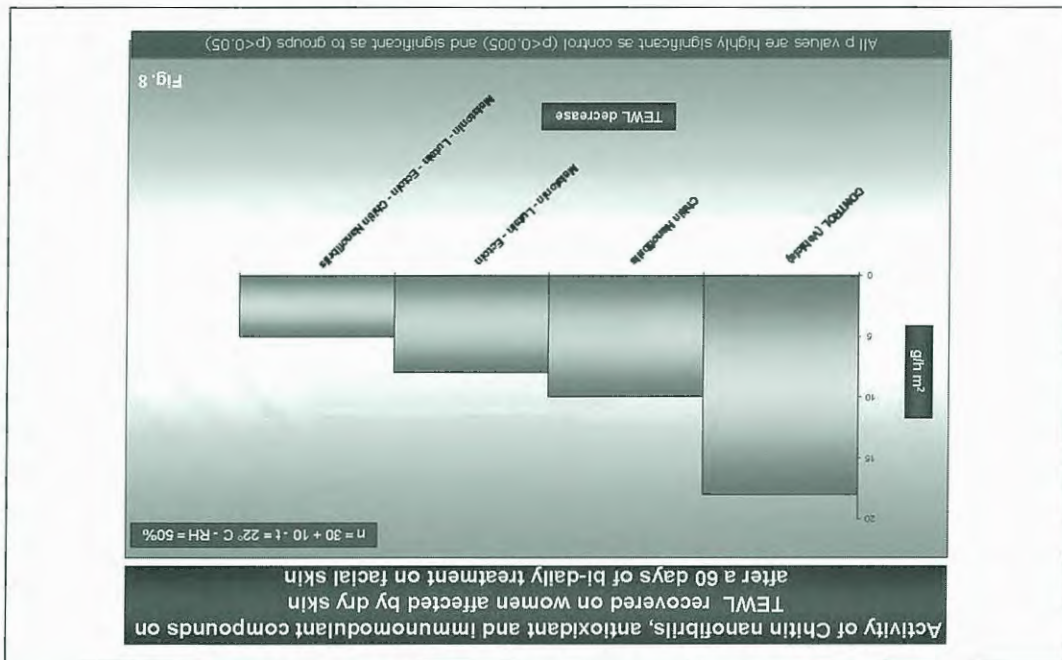
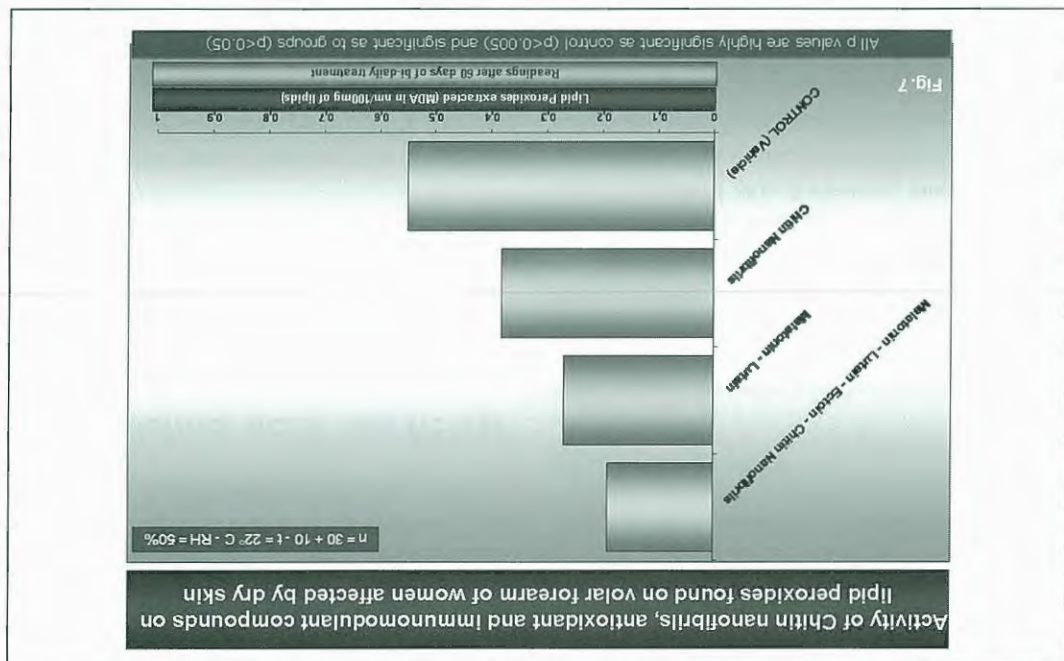
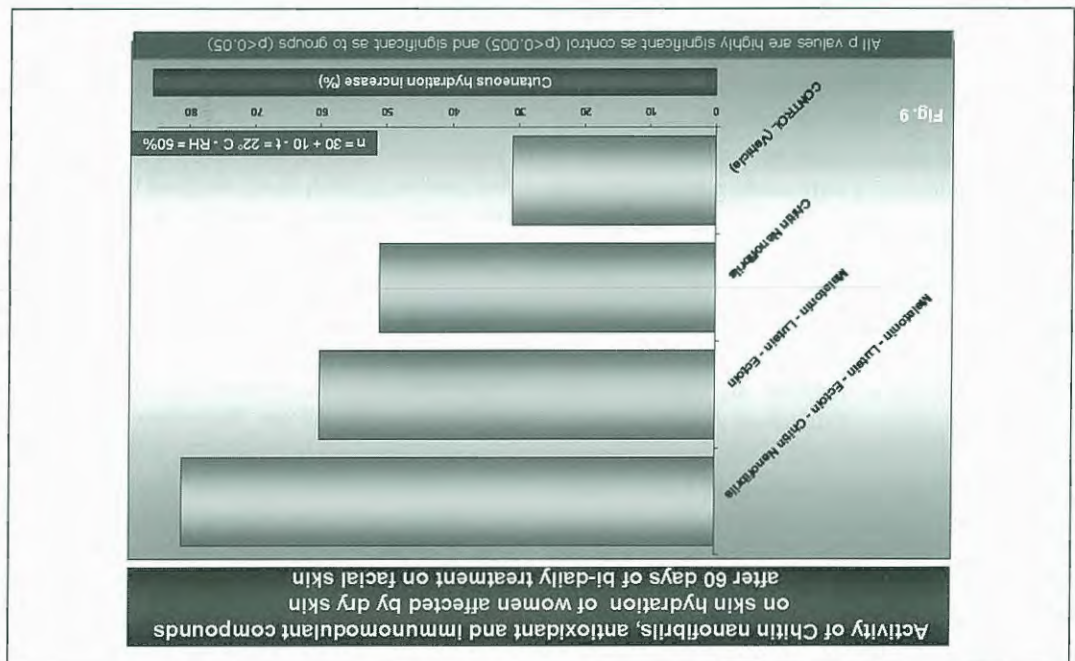
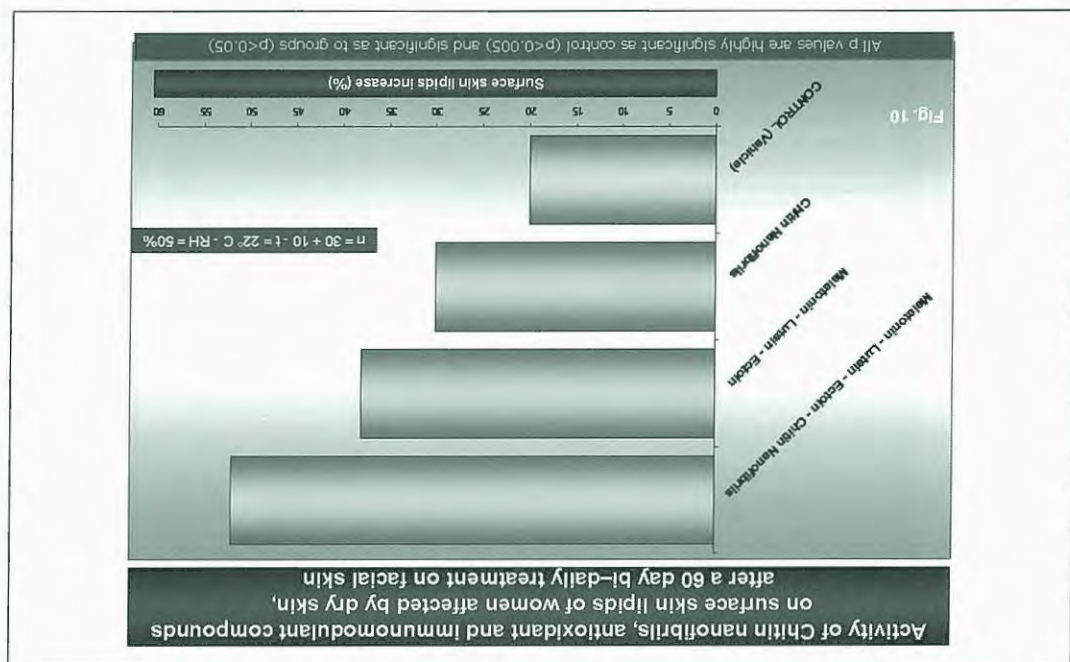


Fig. 7





Anti-irritation effect

A sub-clinical skin erythema, obtained from a 1MED of UVB irradiation (by a Multiport 601 150W Solar Light Simulator, - Solar Light Co, INS Philadelphia, PA, USA), was measured by a laser Doppler. It is considered the most sensitive method for measuring sub-clinical changes in the skin's microcirculation (30, 31). Depending on the site being analyzed, subjects were required to be seated, or to recline, during the equilibrium period.

During testing, the probe applied to the skin surface using double sided adhesive tape, was maintained in position for at least 30 second and measurements were repeated at least two times, averaging the data.

In keeping with the test procedure the skin of the volar forearm was divided in 8 areas of 1 cm² each.

- a) untreated
- b) placebo treated
- c) pre-treated by product A (emulsion carrier of melatonin-lutein-ectoin-CN) prior to UV-exposure (preventive treatment)
- d) pre-treated by product B (emulsion carrier of melatonin-lutein-ectoin) prior to UV-exposure (preventive treatment)
- e) post-treated by product A soon after UV exposure (curative treatment)
- f) post-treated by product B soon after UV exposure (curative treatment)
- g) post-treated 2 hours after UV-exposure by the product A (delayed curative treatment)
- h) post-treated 2 hours after UV-exposure by the product B (delayed curative treatment)

An increase in microcirculation is correlated to an increase in sub-clinical erythema, whereas a decrease in microcirculation is indicative of a decrease in clinical erythema. The obtained results are reported on the Fig.11.

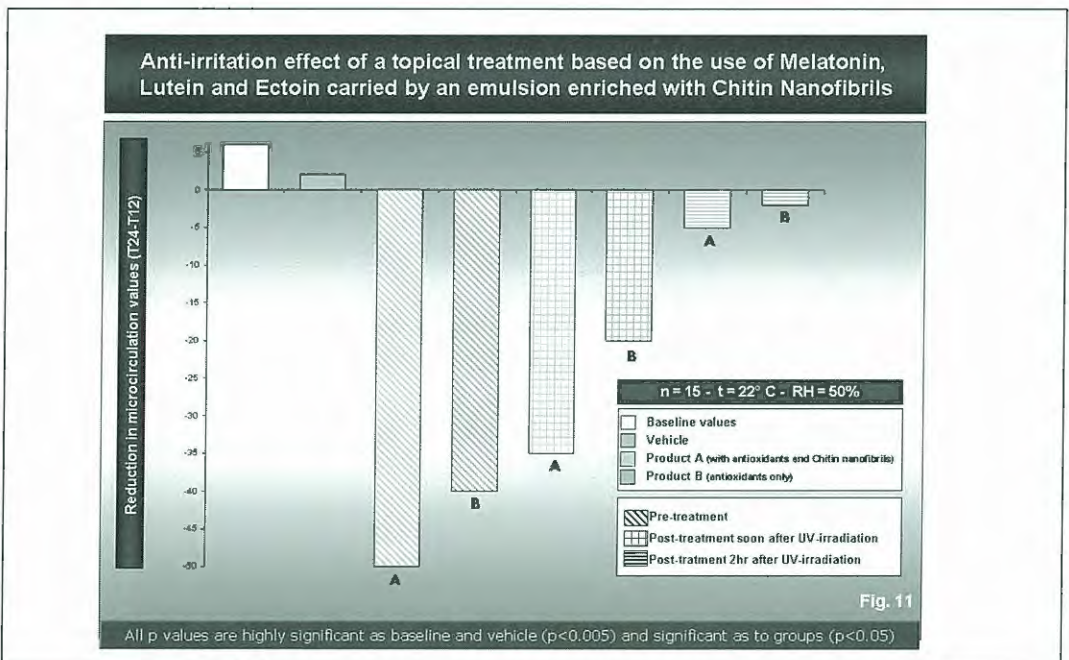


Fig. 11

STATISTICAL ANALYSIS

The Student's Test was used in evaluation of all the data before and after the treatment period. All the analyses were done using the SAS statistical package, version 5.18 (SAS Institute Inc., Cary, N.C.).

Probabilities less than 0.05 were considered significant.

RESULTS AND COMMENT

As clearly evident from Fig.5 the chitin nanofibrils (CN), used in all the formulations as skin penetration enhancer, seem to increase the antioxidant efficacy of the active compounds selected to reduce oxidative stress. Recently it was demonstrated that CN is capable of penetrating throughout the skin layers in conjunction with other active compounds, facilitating their penetration power (13).

Probably this polyglucoside compound drives and activates some specific biochemical processes such as desquamation, modulation of extracellular lipid lamellae and sebum secretion, facilitating the possible interactions between the emulsion, the selected active compounds and the skin.

It may also lead to a reversible deformation in the bilayer structure that allows the creation of various types of *openings* in the skin bilayers. These *openings* can trigger a thermodynamic alteration within the lipid domains leading to increased lipid fluidity or creation of actual microscopically visible pores.

This of course may explain the activity observed *in vivo*. On the other hand the increased activity *in vitro*, may be explained by a reversible interference by CN with the cell's metabolic activity. As it is known the papillary portion of the dermis contains a high amount of collagen fibers required to give firmness and elasticity to the skin. Fibroblasts are assigned to the continuous pro-

duction of collagen while consuming ATP as energy.

Then the effects some antioxidant/immunomodulant compounds mixed with CN would have on the growth of fibroblast cultures and collagen production were studied.

As evident, in fact, in Fig. 1, the *in vitro* activity of fibroblasts is normally increased by the use of the antioxidant lutein/melatonin and the immunomodulant ectoin. Also fibroblast growth is increased when CN is added to this mixture of active compounds, with the consequent increase in collagen production. (Fig.3).

The same results are obtained when the enzymatic ATP activity after UV-irradiation was examined (Fig.2). In fact, when fibroblasts are irradiated by UV, oxidative reactions occur affecting both oxygen-sensitive substances and ATP activity with the result of obtaining a decrease of the ATP content and an increase of lipid peroxides. The *in vivo* studies have confirmed these data.

The antioxidant/ immunomodulant compounds used have demonstrated, therefore, an interesting hydrating (Fig.9) and whitening activity (Fig.4), normalizing also the surface skin lipids (Fig. 10) of subjects suffering from a particular photoaged dry skin.

What is interesting to underline is the capacity the emulsion has, to simultaneously reduce the TEWL (Fig.8) and the lipid peroxides (Fig.7), thus demonstrating an interesting global antiaging activity.

Last but not least, the right combination of these antioxidant/ immunomodulant compounds carried by CN have demonstrated interesting anti-inflammatory effects both on people affected by atopy and on normal subjects. The formulation, in fact, easily decreased the elevated interleukin-8 (IL-8) of some volunteers affected by atopy (Fig.6), but also seemed to be able to decrease sub-clinical erythema due, for example, to UV exposure (Fig.11). It was demonstrated therefore, that this formulation has the possibility to

decrease the microcirculation, while reinforcing the skin's vascular system, and highly decreasing sub-clinical erythema, when applied before UV exposure. Moreover its activity is increased about 20% by the addition of CN.

If the formulation is applied just after the UV irradiation, the reduction in sub-clinical erythema is still significant, but not as great as when used in the pre-irradiation period.

This difference of activity supports the conclusion that CN and the active ingredients used in the formulation, are all powerful free radical scavengers and., therefore, more active at the time of free radical production. For this reason also the right combination of melatonin, lutein and ectoin carried and empowered by the use of the chitin nanofibrils can help to prevent the long-term adverse effects of solar radiation and environmental pollution on the skin, including photo-aging, wrinkling and sagging.

FINAL CONCLUSION

The significance of these findings, together with the numerous recent reports of the bio-activity of chitin nanofibrils (CN) indicate this natural polyglucoside as a very promising active carrier for innovative cosmetics, diet supplements and bio-materials.

The level of fluorescence detected in the different skin layers (Fig.5) has demonstrated that CN may be used as a penetration enhancer of different active compounds also. The level of redox activity detected, in fact, on the skin treated with antioxidant compounds (product A and B) is further proof of this activity (20,24).

The CN nanosize, composed of innumerable nanoparticles, has interesting film-forming properties, efficient in delivering enhanced moisturization by reducing TEWL. Moreover, their capacity to easily entrap active ingredients, gives them the possibility to diffuse gradually into the skin, from the site of application. The CN's good

stability at different pH (from 0 to 12) and temperatures (from 0 to 240°), its ability to stimulate collagen synthesis, to protect the ATP production and its inherently good antireactive and wound healing properties, when associated or not with other natural active compounds, or used as penetration enhancer, opens up a great number of possibilities to create future innovative cosmeceuticals, nutraceuticals, medical devices and health fibers. Other studies showed CN able to improve the SPF values of sun-protective emulsions by its booster activity, water-resistance and hydrophobic character, increasing also skin hydration as moisture absorber (32-36). Moreover, it seems able to reinforce the extracellular matrix (ECM) helping in promoting and improving skin firmness and elasticity reducing the appearance of wrinkles. Finally, CN has bacteria inhibiting properties, and promotes also longer perfume endurance through a tighter adherence between skin and perfume, as well as improves dermatological compatibility of preservation agents, bacterial and anti perspirant agents, used, for example, in deodorant formulas (36, 37).

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BIOTECHNOLOGY IN COSMETICS. Concepts, Tool and Techniques.

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More innovation is key to securing the future of the chemical industry, first of all the skin care one such as Cosmetic.

Innovation concerns new products, but can also be based on new applications, new services or new processes.

While innovation is more than R&D, the link between research in chemistry and related sciences, and innovations in the cosmetic industry is particularly strong. Meanwhile the chemical industry has a key role through its enabling function for the entire industry, the cosmetic industry has a key and functional role in all the sectors of well-being. This means that chemistry, and the cosmetic industry in particular, will always have a strategic and social importance.

Cosmetic industry, in cooperation with academia, should intensify efforts to forecast the consumer's requirements of well-being, but needs also to develop a more effective dialogue with Society and Medical Community based on natural understanding and trust.

Listening and understanding the consumers' needs is essential to an effective two-way communication, and it's the key to developing the trust needed to support skin-friendly environment.

Innovation needs the confidence of investors, customers, workers, and consumers in the sustainability and safety of products and processes.

Nevertheless, more economical and intellectual investments are needed in R&D to reducing the time to bring innovations to market, thus shortening the payback periods. Therefore, innovation is indispensable to overcome all the challenges of our society and, biotechnology may avail the related opportunities and ensure the further success to the cosmetic industry.

This book, organized in **five parts** and **37 chapters**, gives an interesting overview not only on biotechnology involving cosmetics, but also opens a window on the last knowledge discovered the skin and the new active ingredients used on skin aging. Thus it may represent an irreplaceable provider of biotechnological innovations to *cosmetic* industries and a key component of value claims that end with the great majority of consumer products on wellness.

Today, in fact, consumers are becoming more sophisticated and demanding to use cosmetics that *actually do something* on their skin. Therefore we are living the era of cosmeceuticals, dermaceuticals, dermocosmetics or Clinically Correct Cosmetics®.

This is because of the existing direct relationship between the pharmaceutical and the cosmetic industry in knowledge, technology and market trends, that has led to the coming age of *biotech*

cosmetics. But the ability to assess potential safety concerns and develop reliable means for evaluating claims for personal care products, remains a critical component of the product development pathway. Therefore, the increasing demand for performance-driven personal care products and the need to understand a product's mechanism of action provide a scientific challenges to the industry formulators, toxicologists and expert evaluators.

Interesting discussion on these important topics are clearly reported on the first four chapters of Part I of this book.

By the advent of the complete transcribing of the human genome we are becoming to understand the very small variations in DNA that make the difference between each individual.

These variations are known as single nucleotide polymorphisms or SNPs. Thus the scientists become to isolate SNP that control various skin conditions including oxidative stress on human skin.

While humans will continue to punish their skin and health through their lifestyle choices, the foundation of what makes each of us resistant to the detrimental effects of our lifestyle choices will gradually become clearer. Thus the cosmetic industry can begin to respond in kind with products scientifically-designed to help provide what we lack.

The skin is the body's first line of defense against the world outside and this inevitably leaves its marks. The sun's ultraviolet rays in particular, as well as dry air, smog, and cigarette smoking are all stress factors that can accelerate the extrinsic aging process of the skin.

The outermost layer of the skin, also known as the *stratum corneum*, consists of dead cells embedded in lipid lamellae. It prevents external foreign bodies from penetrating the skin.

Therefore it is clear that this protective *mantle would* challenge skin penetration of compounds and repel outside insults. For these reasons, over the years scientists have attempted to find compounds or systems that will allow overcoming this barrier and interaction with deeper sub-tissues or tolerating permeation to the circulation system.

We still do not have, in fact, a complete understanding of the function of topical products-especially the role of emerging theories like the *metamorphosis* of the vehicle after application to the skin. In the immediate future, especially in the pharmaceutical field, it seems that totally occlusive vehicles will continue to be used to protect the skin, until chemical penetration enhancer or particulate-scavenging topical vehicles have been perfected and their toxicity has been fully evaluated.

Thus, understanding the possible routes for penetration can provide tools for the design of an appropriate system that will deliver the molecule to the desired target of action.

On **Chapter 5** all the possible and different ways of penetration throughout the skin are reviewed and discussed.

The skin, in fact, is a very viable tissue which includes many metabolic systems aimed to drive biochemical processes such as desquamation, secretion of extracellular lamellar sheets, programmed cell death and sebum sweat secretion.

These systems, as the enzymes, may attack active or inactive compounds as they penetrate, and convert them into an inactive, active or toxic form. When studying the penetration properties of a compound it is important to understand its possible penetration pathways. Understanding the details of the interaction of penetration with the skin of a compound, or changing its molecular properties, choosing a delivery system or a carrier formula, one can find ways to prevent its drawbacks or alter its preferred route of penetration.

Skin is the mirror for our mental state. Well being and physical relaxation go, in fact, hand in hand,

complementing each other. Thus, in its role as a system for the surveillance of environmental changes and perception of that environmental changes, the skin generates stimuli that are transmitted to the nervous system via mediators. Even surprise, consternation or anger translate into reactions that affect the skin: raising the eyebrows and frowning lead eventually to expression lines, while repeated muscle contractions cause deep wrinkles on the face. This phenomenon has attracted recent attention with the aim of treating deep wrinkles with muscle relaxing agents such as specialized peptides. Therefore, owing to the fact that the body uses peptides for communication between cells, it was theorized that perhaps engineered peptides might be able to up-regulate or down-regulate cutaneous functions that had decayed with time due to the cumulative effects of aging. Moreover various classes of constitutive and inducible antimicrobial peptides are instrumental in protecting the organism against infection and maintaining the skin homeostasis. Thus, it is very clear now that antimicrobial peptides are in the center of epithelial defense mechanism and form an important part of the immune system.

However, the better knowledge of the skin immune system is a vital key to understand how cosmetics can promote healthier skin at the cellular level.

Keratinocytes are able, in fact, to synthesize cytokines, interfering with immunological processes. Moreover they locally synthesize catecholamines (CH) also. These hormones as well as the cytokines, contribute to the interconnection of the three systems that control homeostasis: the nervous system, the immune system and the endocrine system.

Such a findings suggest that skin reacts to different types of environmental stress by secreting hormones that regulate vascular reactivity locally and the blood flow through the microvascular circulation.

The similarity between keratinocytes and lymphocytes should indicate that epithelial cells play an active role in the immune defense of mucous membranes and skin surfaces, either through their physical role or by secreting soluble factors aimed at adapting the responsiveness of cells located in the local microenvironment to the needs triggered by internal or external factors.

Aging and environment insults, such as UV and blue light, perturb this equilibrium, altering the immune system and/or increasing the activity of some enzymes, such as the metalloproteinases (MMP). Such unusual activities may result in a collapse of the meshwork in the extracellular matrix (ECM), contributing to the visible effects of UV damages: wrinkling, loss of elasticity and dilation of surface micro-capillary vessels.

MMP inhibitors can be part of several cosmetic formulations destined for specific skin types. The reinforcement of the ECM could help in promoting skin tone, improving skin firmness and elasticity reducing appearance of wrinkles and ameliorating the integrity of skin structural and functional components, such as micro-capillaries.

However, the slow, inevitable skin-aging process is characterized by a progressive degeneration of the skin tissue as well as by a variety of attendant visible changes in the skin surface. The skin acquires a new appearance as wrinkles form and become increasingly conspicuous, the epidermal layer thins, and the skin decreases in firmness and elasticity. Such visible effects can be seen in humans well before age of 30 and result from major changes in skin cells and the structures supporting the tissue.

Therefore, the request of cosmetic products, the so called *cosmeceuticals*, combining the aesthetic appeal and benefits of traditional cosmetics with a therapeutic component. Consumers are getting, in

fact, more and more sophisticated, just as technology and the products.

“Therefore it is hard to tell which came first, as with the chicken and the egg”.

But one thing is clear: consumer research technique, natural products and innovation. Consumers believe that *natural* is good for their health but are searching for personal care that really works. Thus the era of natural and botanically derived cosmeceuticals is generating novel materials that really do reduce wrinkles, help the skin resist the trauma of environmental attack and improve its appearance and health.

Power is therefore shifting to the customer, and so the marketer's focus must follow.

The key to maintaining a youthful look might be in the power of new active *natural* (?).

But while consumers formulate their own ideas about healthy skin, new active and advanced delivery systems are perpetuating the notion that you can buy the solution in a bottle. Marketers have, in fact, the ability to research, develop, communicate, and sell on a personal, one-to-one basis, taking the axiom *it's not just what you know, but who you know*, to a whole new level of meaning. Thus, “products developers have an unprecedented opportunity to meet personal needs and preferences even more with multifunctional products by enabling consumers to help design the functional and feature bundles that suit them best”.

And topical alternatives for skin have become the *mantra* of the majority of women and men seeking to beautify without invasive procedure, but by the use of cosmetic products having a global body approach.

For all these reasons topical skin care has become highly technical and the processes by which actives are delivered to the skin run the gamut. From time-release of *chitin nanofibrils*, to heat activation, to nanosphere bursts to film technology, each advance is a potential panacea to a customer seeking to combine the best of hard science and aesthetics for healthier, more beautiful skin. However consumers are increasingly choosing products based on their content of certain high-tech and natural ingredients, and avoidance of certain synthetic ingredients.

Thus natural products are perceived as superior to many conventional offerings and consumers give emphasis on what is consumed internally translating to concern about what is being applied externally. Although currently, the natural and organic category holds 2% of the global market for health and beauty products, it is enjoying growth rates of 14-20% compared with the total market's 1-5%. For these reasons enough space of this book is dedicated to the description of the natural ingredients of plant or sea origin; it is, however, to underline that consumers are becoming very aware of what is blatant green, natural or bio cosmetic. The international situation is, in fact, complex; because now each label has its own definition of *organic* or *natural* and its own philosophy and vision of the market.

US and EU directives don't include any specific regulation concerning organic, natural or bio-cosmetic! Therefore the ultimate goal will be a worldwide regulation defining first of all the percentage of organic or natural ingredients in a given product, together with the relative processing and analytical control methods, to guarantee both manufacturers and consumers.

Many others are the topics reported on this interesting book provided from well-known experts from industry and academia.

The formulations, the high tech and natural ingredients described and discussed, the last testing methods focused, give to the reader a correct and a modern vision of the applied biotechnology in the cosmetic field, necessary to understand in the better way the progress we assisted in the last 20

years and will continue to assist in the next future.

Cosmetology is now an indispensable part of the global science and this book has to be considered as a key stone and a general desk reference for all the specialized book shops for both medical and chemical communities that intend to know better where biotechnology applied in cosmetic science is going.

It also provides physicians, intellectual properties lawyers, and investors with an important survey of emerging ideas and technologies useful to improve health, ameliorating our way of living.

P. Morganti
Editor-in-Chief

cifying the characteristics that the offspring shall have. Thus the phenomenon of *heredity* is central to the definition of life.

However, most living organisms are single cells, others, as mammals, are multicellular cities in which groups of cells perform specialized functions and are linked by intricate systems of communication. Thus, they reproduce themselves by transmitting genetic information to their progeny. The cell is the minimal self-reproducing unit, and the vehicle for transmission of the genetic information is stored in the same chemical form, as double-stranded DNA (deoxyribonucleic acid). It replicates its information by separating the paired DNA strands and using each as a template for polymerization to make a new DNA strand with a contemporary sequence of nucleotides. The same strategy is used to transcribe portions of the information from DNA into molecules of the closely related polymer, RNA (ribonucleic acid).

These in turn guide the synthesis of protein molecules by the more complex machinery of translation, the ribosome. However, proteins are the principal catalysts for almost all the chemical reactions in the cell. The specific function of each protein depends on its amino acid sequence of a corresponding segment of the DNA, the genes that codes for that protein. In this way the genome of the cell determines its chemistry, providing for the synthesis of DNA, RNA, and protein.

This the chemistry of life. It is special for at least three reasons. First, it is based overwhelmingly on carbon compounds, and therefore, on organic chemistry. Second, cells are 70% water and their life depend on chemical reactions that take place in aqueous solutions. Third, all chemistry is enormously complex.

Most of the carbon atoms in cells are incorporated, in fact, into enormous *polymeric molecules*.

In conclusion, life hinges on the property of water. However, living organisms and cells are autonomous, self-propagating chemical systems. They are made from a distinctive and restricted set of small carbon-based molecules. Each of these molecules is composed of a small set of atoms linked to each other in a precise configuration through covalent bonds. They are usually found free in solution and have many different fates. Some are used as *monomer* subunits to construct the giant polymeric *macromolecules* (proteins, nucleic acids and polysaccharides) of the cell. Others act as energy sources and are broken down and transformed into other small molecules in a maze of intracellular metabolic pathways.

The main categories of these molecules are sugars, fatty acids, amino acids and nucleotides. Sugars are a primary source of chemical energy for cells.

Fatty acids have most critical function in the formation of cell membranes. Amino acids constitute the bulk polymers known as proteins.

Finally nucleotides play a central part in energy transfer and are the subunits for RNA and DNA.

To live a cell requires matter, as well as free energy. Thus, animals depend on plants for supplies of organic carbon and nitrogen compounds. Plants, in turn, although they can fix carbon dioxide from the atmosphere, lack the ability to fix atmospheric nitrogen, and they depend in part on nitrogen-fixing bacteria to supply their need for nitrogen compounds.

Therefore mammals obtain their energy by eating organic molecules and oxidating them in a series of enzyme-catalyzed reactions that are coupled to the formation of ATP (adenosine triphosphate), the common currency of energy in the cell.

ATP is, in fact, a currency store of energy used to drive a variety of chemical reactions in cells. When required, ATP gives up its energy packet through its energetically favorable hydrolysis to ADP (ade-

nosine diphosphate) and inorganic phosphate. The energetically favorable reaction of ATP hydrolysis is coupled to many otherwise unfavorable reactions through which other molecules are synthesized. However other activated carriers or coenzymes, as, for example, NADPH (reduced nicotinamide adenine dinucleotide phosphate), pick up and carry a chemical group in an easily transferred, high energy linkage. Thus, enzymes provide the intricate molecular surfaces in a cell that promote its many chemical reactions. But enzymes are catalytic proteins that speed up reaction rates by binding the high-energy transition states for a specific reaction path, also performing acid and base catalysis for other specific reactions.

What is the protein function?

Proteins embedded in the plasma membrane form channels and pumps that control the passage of small molecules into and out of the cell. Other specialized proteins act as messengers, signal, integrators, antibodies, hormones, elastic, fibers, etc.

From a chemical point of view, proteins are by far the most structurally complex and functionally sophisticated molecules known. And a protein molecule's physical interaction with other molecules determines its biological properties. Thus, antibodies attack to viruses or bacteria to mark them for destruction, the enzyme hexokinase binds glucose to ATP for energy, and so on.

All these topics are reported in a detailed form on the first **3 chapters of the Part I** of the book, where *Cells and genomes*, *Cell Chemistry and Biosynthesis*, and *Proteins* are described and discussed to give the right global idea on the fascinating general characteristics and functions of the cell and living organisms.

However, as we have seen, the properties and functions of a cell are determined largely by the proteins that it is able to make. "They serve, in fact, as building blocks for cell structures and form the enzymes that catalyze the cell's chemical reactions. But they regulate gene expression also, and they enable cells to communicate with each other and to move."

Thus, life depends on the ability of cells to store, retrieve and translate the genetic instructions required to make and maintain a living organism. This *hereditary* information is passed on from a cell to its daughter cells at cell division, and from one generation of an organism to the next through the organism's reproductive cells. These instructions are stored within every living cell as its *genes*.

As a result, we now know the order of the 3 billions DNA subunits that provide the information for producing a human adult from a fertilized egg, as well as DNA sequences of thousands of other organisms.

Part II of the book, by **chapters 4-7**, deals with the *Basic Genetic Mechanisms*, the ways in which the cell maintains, replicates, expresses, and improves the genetic information carried in its DNA.

A gene is a nucleotide sequence in a DNA molecule that acts as a functional unit for the production of a protein, a structural RNA, or a catalytic or regulatory RNA molecule. The genetic information stored in a organism's DNA contains the instructions for all the proteins the organism will ever synthesize and is said to comprise its genome. The human genome contains 3.2×10^9 DNA nucleotide pairs, divided between 22 different autosomes and 2 sex chromosomes. A chromosome is formed from a single, long DNA molecule that contains a linear array of many genes.

Genetic information is carried in the linear sequence of nucleotides in DNA. Each molecule of DNA is a double helix formed from two complementary strands of nucleotides held together by hydrogen bonds between -G-C (guanine-cytosine) and A-T (adenine-thymine) base pairs. Thus duplication of the genetic information occurs by the use of one DNA strand as template for the formation of a com-

plementary strand.

Maintaining the genetic stability that an organism needs for its survival requires, therefore, not only an extremely accurate mechanism for replicating DNA, but also mechanism for repairing the many accidental lesions that occur continually in DNA.

And the importance of DNA repair is evident from the large investment that cells make in DNA repair enzymes. For example, p53 protein, plays an important role in the response to UV, accumulating in human skin, to repair the UV damages. However the ability of p53 to promote growth arrest/repair and thus survival on the one hand, but also apoptosis and thus cell death on the other hand, raises the question of what determines the choice between these two opposite events. However, the recombination (repairing) event is guided by a specialized set of proteins.

But how cell read the genome?

Much of the DNA-encoded information present in genomes specifies the sequence of aminoacids for every protein the organism makes. The aminoacids sequence in turn dictates how each protein fold to give a molecule with a distinctive shape and chemistry. When a cell make a particular protein, it must decode accurately the corresponding region of the genome.

Additional information encoded in DNA of the genome specifies exactly when in the life of an organism and in which cell types each gene is to be expressed into protein. Since proteins are the main constituents of cells, the decoding of the genome determines not only the size, shape, biochemical properties, and behavior of cells, but also the distinctive features of each species on earth.

However, decoding genome is not a simple matter. Although the DNA sequence of the human genome is known, it will probably take at least a decade to identify every gene and determine the precise aminoacid sequence of the protein it produces. It is to remember that the cells as our body do this thousands of time a second!

Thus, a long-range goal is to obtain a complete understanding of what take place inside a cell as it responds to its environment and interacts with its neighbours.

Part III, chapters 8 -9, is dedicated to present the principal and latest methods used to study the molecular components of cells, particularly proteins, DNA and RNA.

Therefore DNA cloning, cutting chromosomal sequence together with genetics and genetic engineering provide powerful tools of the study of gene function in both cells and organisms. Many of these and other complementary microscopy methods are, thus, reported and discussed. Techniques are now available, in fact, for detecting, measuring and following almost any desired molecule in a living cell. Virtually, for example, protein of interest can be genetically engineered as fluorescent-fusion protein, and then imaged in living cells by fluorescence microscopy. Radioactive isotopes of various elements can be also used to follow the fate of specific molecules both chemically and microscopically.

Moreover using computational method, either multiple images or views from different directions can be combined to produce detailed reconstructions of macromolecules and molecular complexes through the techniques of electron tomography and single-particle reconstruction, often applied to cryo-preserved specimens.

Cell membranes are crucial to the life of the cell. **Part IV** is entirely dedicated to this topic (**chapters 10 to 18**).

Despite their differing functions, all biological membranes have a common general structure, made of a very thin film of lipid and protein molecules, held together mainly by non-covalent interactions.

The lipid molecules are arranged as a continuous double layer about 5nm thick. This lipid bilayer provides the basic fluid structure of the membrane and serves as a relatively impermeable barrier of the passage of most water-soluble molecules. Protein molecules, that span the lipid bilayer mediate nearly all of the other functions of the membrane transporting specific molecules across it. Thus the main constituents of biological membranes are: lipids and proteins.

The lipid bilayer is fluid, and their molecules are amphiphilic. Cells contain 500-1000 different lipid species with three different major classes: phospholipids, cholesterol and glycolipids.

Whereas the lipid bilayer determines the basic structure of biological membranes, proteins are responsible for most membrane functions, serving as specific receptors, enzymes, transport proteins, and so on. However, cells have way of immobilizing specific membrane proteins, as well as ways of confining both membrane protein and lipid molecules to particular domains in a continuous lipid layer.

Because of its hydrophobic interior, the lipid bilayer of cell membranes prevents the passage of most polar molecules. This barrier function allows the cell to maintain concentrations of solutes in its cytosol that differ from those in the extracellular fluid and in each other of the intracellular membrane-enclosed compartments.

But every cell must eat, communicate with the world around it, and quickly respond to changes in its environment. To help accomplish these tasks, cells continually adjust the composition of their plasma membrane in rapid response to need. They use and elaborate internal membrane system to add and remove cell-surface proteins embedded in the membrane, such as receptors, ion channels, and transporters.

Through the process of *exocytosis*, the biosynthetic-secretory pathway delivers newly synthesized proteins, carbohydrates, and lipids to either the plasma membrane or the extracellular space. Cells also use *endocytosis* to capture important nutrients, such as vitamins, lipids, cholesterol, and iron; these are taken up together with the macromolecules to which they bind and are then released in endosomes or lysosomes and transported into the cytosol, where they are used in various biosynthetic processes.

At this purpose, there are two classes of membrane transport proteins: transporters and channels. Each type of transporter has one or more specific binding sites for its solute. It transfers the solute across the lipid bilayer by undergoing reversible conformational changes that alternatively expose the solute-binding site first on one side of the membrane and than on the other. Unlike transporters, channel proteins form hydrophilic pores across the lipid bilayer and allow inorganic ions of appropriate size and charge to cross the membrane down their electrochemical gradients at rates of about 1000 times greater than those achieved by any known transporter.

The channels are gated and usually open transiently in response to a specific perturbation in the membrane, such as a change in membrane potential or the binding of a neurotransmitter.

Thus, ion channels work together in complex ways to control the behavior of electrically excitable cells.

Unlike a bacterium, which generally consists of a single intracellular compartment surrounded by a plasma membrane, an *eucariotic* cell is elaborately subdivided into functionally distinct, membrane-enclosed compartments. Each compartment, or *organelle*, contains its own characteristic set of enzymes and other specialized molecules. The main types of organelles are the endoplasmatic reticulum, Golgi apparatus, nucleus, mitochondria, lysosomes, endosomes, and peroxisomes. Thus proteins

confer upon each compartment its characteristic structural and functional properties, catalyzing the reactions that occur in each organelle and selectively transporting small molecules into and out of its interior. Because the lipid bilayer of organelle membranes is impermeable to most hydrophilic molecules, the membrane of each organelle must contain membrane transport proteins to import and export specific metabolites. Each organelle membrane must also have a mechanism for importing, and incorporating into the organelle the specific proteins that make the organelle unique.

However, each cell has elaborated an its own cell cycle of life. The core of this system is an ordered series of biochemical switches that initiate the main events of the cycle, including chromosome duplication and segregation. The only way to make a new cell is, in fact, to duplicate a cell that already exists. In addition to duplicating their genome, most cells also duplicate their organelles and macromolecules.

This is why cell division usually begins, with duplication of all the cell's contents, followed by distribution of those content into two daughter cells.

Cell division balances the programmed cell death also. This cell death program, not confined to animals but occurring in plant also, is known as *apoptosis*.

In this way, animal cells that are irreversibly damaged, or are a threat to the organism can be eliminated quickly and neatly. Apoptosis depends on proteolytic enzymes called *carpases*, which cleave specific intracellular proteins to help kill the cell. Thus, programmed cell death functions is a quality-control process in development, eliminating cells that are potentially dangerous.

Of all the social interactions between cells in a multicellular organism, the most fundamental are those that hold the cell together to form an organized multicellular structure. How is this possible?

Part V by chapters 19 to 25 replies to this enquiry.

The mechanisms of cohesion govern, in fact, the architecture of the body. Thus the answer lies in two basic building strategies. One strategy depends on the strength of the *extracellular matrix*, a complex network of proteins and polysaccharide chains that the cells secrete. The other strategy depends on the strength of the cytoskeleton inside the cell and on *cell-cell adhesions* that tie the cytoskeletons of neighboring cell together.

In animal tissues the two categories are represented by the *connective tissue*, where the extracellular matrix is plentiful and cells are sparsely distributed within it.

On the other hand matrix is rich in fibrous polymers (i.e. collagen), that bears most of the mechanical stress to which the tissue is subjected.

By contrast in *epithelia tissues*, such as epidermis, cells are closely bound together into sheets called *epithelia*. The extracellular matrix is scanty. Within the epithelium, the cells are attached to each other directly by cell-cell adhesions, mediated by transmembrane proteins that are anchored intracellularly to the cytoskeleton.

Thus, human epithelia constitute the body's contact zone with a hostile environment, full of pathogenic microbes ready to attack the body. Moreover, the stratum corneum (SC) being the outer permeability barrier of the skin, is permanently exposed to contacts with physical and chemical agents from the environment, which in turn may cause a continuous loss of water and proteins. Therefore, as a highly specialized structures, it is essentially impermeable for water, except for a small but vital flux, serving to maintain its hydration and its flexibility. This flux is regulated from the intercellular lipid-like substance of the SC, the so called *lipid lamellae*, composed of long-chain lipids organized as bilamellar structures. These lipid lamellae are designed to keep many substances outside the body,

thus acting as permeability barrier.

For the stabilization of the intercellular lipid bilayer structure, the hydrophobic envelope formed on the surface of the corneocytes plays an important role. The water impermeability is, in fact, the result of the ordering of the corneocytes embedded in these lamellae.

However, the skin, like almost all tissues, is a complex of several different cell types. To perform its basic function as a barrier the outer covering, as previously described, depends on a variety of supporting cells and structures many of which are required in most other tissues also. It needs mechanical support, provided by a framework of extracellular matrix, mainly secreted by *fibroblasts*. It needs a blood supply to bring nutrients and oxygen and to remove waste products, and this requires a network of blood vessels, lined with *endothelial cells*. These vessels also provide access routes for cells of the immune-system to defend against infection; as *macrophages*, *dendritic cells* and *lymphocytes*.

Nerve fibers are needed too, to convey sensory information from the tissue to the central nervous system, and to deliver signals in the opposite direction for glandular secretion and smooth muscle contraction. Therefore, all these skin's processes can be maintained only if the basal cell population of the epidermis is self renewing. Thus it must contain some cells that generate a mixture of progeny, including daughters that remain undifferentiated like their parent, as well as daughters that differentiate.

These are the *stem cells*, always required wherever there is a recurring need to replace differentiated cells that cannot themselves divide. And thus phenomenon happens in many different tissues.

Many other are the topics treated in this interesting book on the cell as the cancer process and its molecular basis or the problem of sexual reproduction and fertilization, just for citing some. It is really difficult to discuss and report all the subjects, always detailed and clearly treated.

We try to report and summarize some one of the many chapters describing the cell and its life, just for giving a general idea about the organization of this treatise-book.

For the full details I invite all the readers to an accurate reading of all the chapters, of which the interesting Media DVD-Rom, provides movies and animations for a better understanding of the different topics.

It was very interesting for me also to remind myself the complex molecular machinery of the living cells. We know a lot about the complexity of our body, but not in a sufficient way for understanding the intimate mechanisms that make life possible.

In my opinion, not only students but all the scientists involved in wellbeing as well as the readers of this journal, should have this book in their personal library.

The deeper knowledge of the living cell has to be considered, in fact, at the base of the science of Cosmetic Dermatology and Wellbeing.

P. Morganti
Editor-in-Chief




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According to a survey of the bfai (German National Association for Foreign Economics) the demand for cosmetic products in Poland is steadily increasing. Polish manufacturers such as the "Kolastina Gruppe" are active not only in their home market, but increasingly abroad as well. Companies like Eris, Dermika, Dax Cosmetics, Inglot, Bell and others are continually expanding their market positions. Again, other cosmetic manufacturers such as Avon, Nivea Polska (Beiersdorf), L'Oréal, Procter & Gamble and Alberto Culver (Soraya) strongly invest in new production facilities in Poland.

Similar developments can be observed in the cleaner and detergent market.

The demand for suitable raw materials and ingredients is increasing and it is vital for distributors and service providers of this business to be present in Central and Eastern Europe.

The hpci-congress, organised by SOFW and supported by the Polish Society of Cosmetic Chemists and SEPAWA e.V., offers the ideal forum to establish and cultivate interesting and important contacts in the Central and Eastern European market. The event includes a scientific conference on the subjects cosmetic, household cleaning, and specialities.

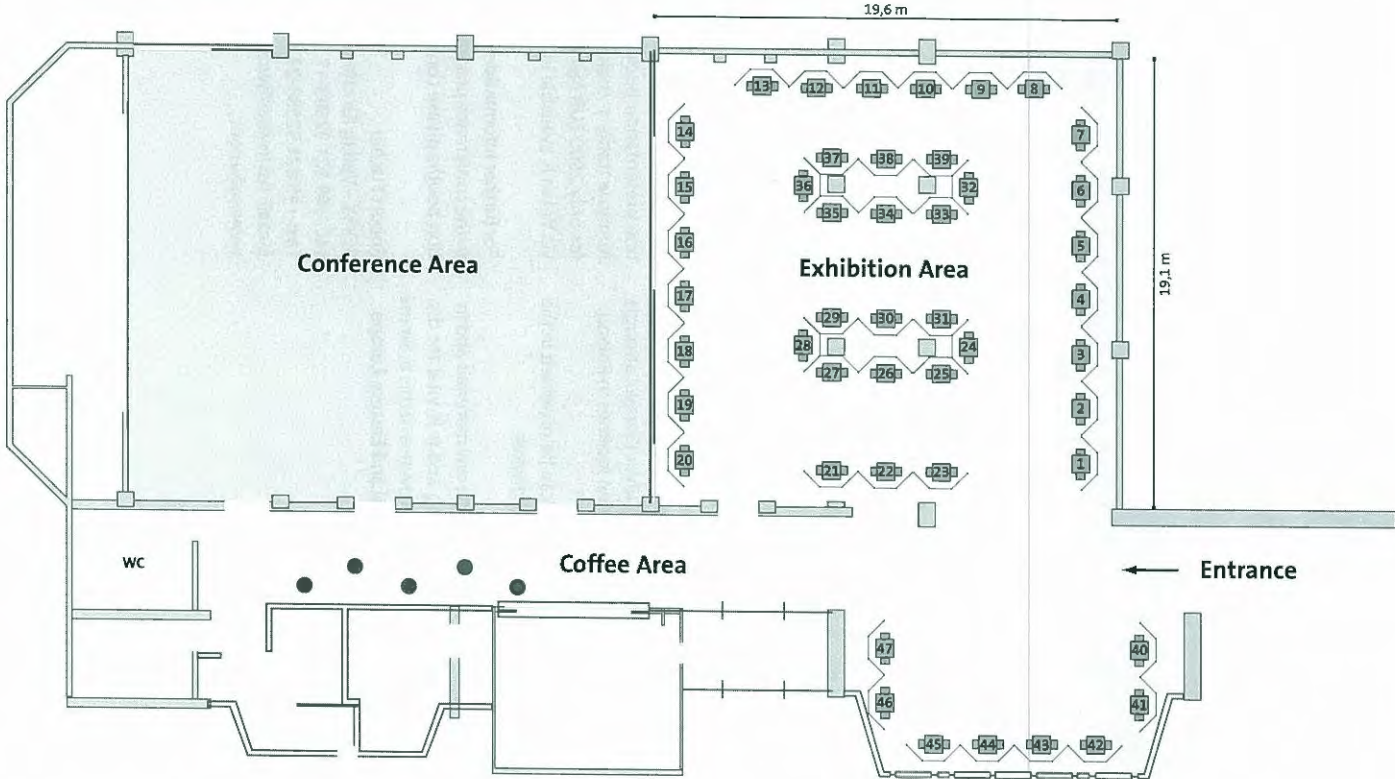
At the same time there will be an exhibition for suppliers and service provider. We offer one presentation booth with 3 display walls, furniture (table + chairs), light and electricity for only 2500 EUR (excl. tax). Internet access via W-lan is available in the exhibition.

For further information to the event as well as to the conference programme and the exhibition booths please contact:

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Cristalli di Luteina al microscopio elettronico a scansione (SEM). *Su gentile concessione* dell'Istituto di Morfologia Umana Normale, Università Politecnica delle Marche, Ancona, Italia.

Chrystals of Lutein, scanning electron microscopy (SEM). *On kind permission of* the Institute of Human Normal Morphology, Università Politecnica delle Marche, Ancona- Italy

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