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Extract combinations of *Curcuma zedoaria* and *Aloe vera* inhibit melanin synthesis and dendrite formation in murine melanoma cells

JR. Krishnamoorthy¹, MS. Ranjith², S. Gokulshankar²

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Received: April, 2010.

**Key words**: Melanin; Tyrosinase; Skin lightening; *Curcuma zedoaria*; *Aloe vera*;

---

**Summary**

The objective of the study was to determine the effect of role of combination of extracts of *Curcuma zedoaria* and *Aloe vera* in reducing melanin synthesis. Varying concentrations of the extracts of the plants were tested for melanogenesis and tyrosinase activity in murine melanoma cells.

Extract combinations at a concentration of 1-5µl showed 50-150% reduction in melanogenesis without altering the cell proliferation. Tyrosinase activity was very low in extract treated cells when compared to control.

---

**Riassunto**

L'obiettivo dello studio è stato quello di determinare il ruolo svolto dall'uso combinato degli estratti di *Curcuma zedoaria* e dell'*Aloe vera* nell'opposizione alla sintesi della melanina.

A questo scopo sono state controllate l'azione di diverse concentrazioni degli estratti vegetali sulla melanogenesi, in particolare sulla tirosinasi, utilizzando culture di cellule melanomiche murine.

L'azione degli estratti in concentrazione da 1 a 5 µl hanno dimostrato di ridurre il processo melanogenico dal 50 al 150% senza alterare la proliferazione cellulare.

L'attività sulla tirosinasi si è dimostrata molto bassa nelle cellule trattate se paragonata al controllo.
INTRODUCTION

Tyrosinase enzyme plays a major role in melanogenesis process. This rate limiting enzyme oxidizes the amino acid tyrosine to DOPA and then to melanin (1). Retarding the tyrosinase enzyme activity is considered to be the key approach for achieving skin lightening effect with most of the cosmetic skin lightening/whitening/fairness creams (2, 3). Further, this method of inhibiting the melanogenesis process is reversible in nature hence does not produce any permanent pigmentary problem to the skin. Melanocytes are dendritic cells and the transfer of melanosomes to the keratinocytes is aided by the dendrites in the melanocytes (4).

Qualitative and or quantitative changes to the dendrites in the melanocytes play a major role in the melanization of the skin. Dendrite modification is therefore inevitable for the regulation of skin colour besides modulating tyrosinase activity and melanin synthesis within the melanocytes. In the systems of Indian medicine, particularly in Siddha system, several plants have been recognized to have effect in modulating the skin pigmentation.

The glabradin, isolated from licorice, arbutin from mulberry and some other compounds of natural origin have been widely used in several skin lightening preparations all over the world. Curcuma zedoaria and Aloe vera, although used extensively in various cosmetic and drug preparations, the effect of the combinations of these extracts in inhibiting melanin synthesis is not studied in detail.

In the present paper, we discuss the role of the combination of extracts of Curcuma zedoaria and Aloe vera in reducing the melanogenesis through tyrosinase inhibition and along with their effect in modulating dendricity pattern in melanocytes.

MATERIALS AND METHODS

The rhizomes of Curcuma zedoaria and leaves of Aloe vera were extracted separately in propylene glycol: water at 1:1 ratio. The solid to liquid ratio was maintained at 1:100. The extracts were combined at 1:1 ratio.

The mixture of the extract at varying concentrations were used for testing the activity.

Cell Culture

B16F10 murine melanoma cells were cultured in Eagles minimal essential medium supplemented with 10% heat inactivated fetal bovine serum and 2mM L-glutamine at 37°C in a humidified atmosphere containing 5% CO₂.

Different concentrations of the extract ranging from 1-5 ul were added to the culture after the cells being seeded.

The cells were incubated for 24, 48 or 72 hrs and cell numbers (determined by counting in a haemocytometer chamber), melanin contents and tyrosinase activities were determined in triplicate for each treatment as detailed below.

Melanin Measurement

Melanin content was measured as per the method described as follows. Approximately 107 cells were pelleted by centrifugation at 1000 g for 5 minutes and then washed twice with phosphate buffered saline.

After further centrifugation, the supernatant was decanted, the precipitated cells were re suspended in 200 μl of distilled water, and 1 ml of ethanol-ether 1:1 was added to remove opaque substances other than melanin.

The mixture was stored and suspended at room temperature for 15 minutes.

After further centrifugation at 3000 g for 5 minutes, the precipitate was solubilized by treatment with 1 ml 1N NaOH/10% dimethyl sulfoxide at
80°C for 30 minutes in a capped tube. The absorbance was measured at 400 nm and the melanin content per cell was calculated and expressed as percentage of control (=100%).

**Tyrosinase Assay**

Tyrosinase activity was assayed as DOPA oxidase activity. Approximately $10^7$ cells were pelleted and then washed twice with phosphate buffered saline. After centrifugation, the supernatant was decanted. The cell pellet was dissolved in 1.0 ml of 0.5% sodium deoxycholate in distilled water and allowed to stand at 0°C for 15 minutes. Tyrosinase activity was assayed spectrophotometrically by following the oxidation of DOPA to dopachrome at 475 nm. The reaction mixture consisting of 3 ml of 0.1% L-DOPA in 0.1 M phosphate buffer, pH 6.8 was mixed with cell lysate. Assay was performed at 37°C in a spectrophotometer. The reaction rate was measured during the first 10 minutes of the reaction while it was linear. Corrections for auto oxidation of L DOPA in controls were made.

Specific activity was defined as the amount of DOPAchrome formed per 10 min per cell, and is expressed as percentage control (=100).

**Dendrite length and number measurement**

The extracts treated melanocytes were examined under microscope and the number and relative length of dendrites in each melanocyte were recorded at random and compared with untreated control.

**RESULTS**

Reduction in melanin content was observed in cell pellets incubated with the extract. However, the growth rate of B16F10 cells was not significantly altered by the extract treatment during 72 hr incubation period, both with controls and extract treatment. This clearly indicates that the melanogenesis modulation occurs in the cells without affecting the cell proliferation. The level of decrease of melanin synthesis in relation to the concentration of the extracts was 50% to 90% for 1-5 µl of the extract combinations respectively (Table I).

<table>
<thead>
<tr>
<th>Extract combination (µl)</th>
<th>Melanin inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
</tr>
</tbody>
</table>
Extract combinations of Curcuma zedoaria and Aloe vera inhibit melanin synthesis...

Optimum concentration of the extract that showed very high activity in decreasing the melanin synthesis was 4 µl. Higher than this level did not significantly retard the melanogenesis in the murine B16F10 cells.

The tyrosinase activity was recorded to be very low in cells treated by the extract combination when compared to control (Table II).

The extract up to a concentration of 50 µl did not show cytotoxicity when tested by MTT.

The number of dendrites in treated melanocytes were significantly lower when compared to the untreated control.

A total of ±15 dendrites were recorded in melanocytes under control group whereas the number of dendrites recorded in the treatment group was ±6.

Further, the length of the dendrites have reduced from 112 µm length in the case of control to 64 µm in the treatment group (Table III).

### Table II
Tyrosinase inhibition activity

<table>
<thead>
<tr>
<th>Extract combination</th>
<th>Tyrosinase inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 µl</td>
<td>28</td>
</tr>
<tr>
<td>2 µl</td>
<td>37</td>
</tr>
<tr>
<td>3 µl</td>
<td>44</td>
</tr>
<tr>
<td>4 µl</td>
<td>67</td>
</tr>
<tr>
<td>5 µl</td>
<td>72</td>
</tr>
</tbody>
</table>

### Table III
Dendricity modulation - DN= Dendrite Number - DL= Dendrite Length

<table>
<thead>
<tr>
<th>Study details</th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DL/micron</td>
<td>DN</td>
</tr>
<tr>
<td>Control</td>
<td>15 ± 3</td>
<td>112 ± 6</td>
</tr>
<tr>
<td>Test (Con.in µl)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data expressed as Mean ± SD
DISCUSSION

The present investigation clearly suggests that the combination of the extracts of *Curcuma zedoaria* and *Aloe vera* popularly referred as ‘Everfresh’ is very effective in decreasing the melanin synthesis when tested in murine melanoma cells.

The key mechanism of action in the extract combination was found to be through tyrosinase inhibition.

Tyrosinase, being the primary enzyme that plays a major role in the oxidization of tyrosine to melanin, most of the approaches for achieving the skin lightening effects through various cosmetic preparations were by inactivation of the above enzyme. Further, the above approach is reversible, which is very safe and does not cause any permanent damage.

However, melanin transfer inhibitors are also widely used for the above purpose. The melanin transfer inhibitors may or may not interfere in the melanogenesis but by their melanin transfer inhibition mechanism can significantly influence the skin pigmentation. In the present study, we have observed that combination of extracts of *Curcuma zedoaria* and *Aloe vera* possess dual mechanism of down regulating the melanin synthesis and its further transfer to the skin.

This is a unique phenomenon and was not reported or known.

*Curcuma zedoaria*, being a very old traditional plant used in India for skin care is also known to have antiseptic properties (5-7).

Similarly, *Aloe vera*, the other widely used medicinal plant for various cosmetic and drug preparations all over the world.

This plant is also known to have sunscreening effect and hence the use of this plant extract in skin creams offers sun protection benefit (8).

The potent skin lightening effect what we established for the combination of the extracts of *Curcuma zedoaria* and *Aloe vera* assumes great importance as both plants are widely used for various skin benefits.

Retarding the melanogenesis known to make the skin relatively more vulnerable to UV damage, and that is why most of the skin lightening creams contains sun screeners. Use of the combination of the above extracts offers dual benefit of skin lightening effect as well as sun protection to the skin.

This approach also would provide the advantage of eliminating the chemical sun screeners in the product, thereby can ensure total benefit come purely from herbal source.

This is the first report to our knowledge that the combinations of extracts of *Curcuma zedoaria* and *Aloe vera* having the dual inevitable property of inhibiting the melanin synthesis and also act as melanin transfer inhibitor to the keratinocytes.

The above findings validates the astute science of the ancient Siddha system of medicine as the formulation and its benefits are well documented in the literature of Siddha system of India but the exact mechanism of action was not known.
Extract combinations of Curcuma zedoaria and Aloe vera inhibit melanin synthesis...

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Tiliroside and Dihydroxy Methylchromone: from Nature to Cosmetic Applications

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Key words: Tiliroside; Dihydroxy Methylchromone; Anti-aging; Anti-Inflammatory;

Summary

The biological activities of the natural ingredients Tiliroside and Dihydroxy Methylchromone were investigated for cosmetic applications. Tiliroside offers an example to demonstrate the possible transfer of protective benefits from plants to humans in cosmetics as shown in a placebo-controlled \textit{in vivo} study on human volunteers. Inflammation on human skin was provoked by UV-radiation using a solar simulator. The development of erythema was examined after the application of the respective test substances at the measuring times prior to treatment and after different time points in comparison with both a negative and positive control. This study provides an example where traditional use of a medicinal plant is supported by the identification of the active principle. Tiliroside is a potent ingredient for normal and sensitive skin and offers a multitude of claim opportunities for cosmetics. Examples are calming, soothing skin or anti-inflammatory.

Dihydroxy Methylchromone is found in numerous medicinal plants but in such a low concentration that its isolation on an industrial scale remains prohibitive. It was synthesized and found to possess anti-aging properties by preserving the integrity of the extracellular matrix. It induces the synthesis of hyaluronic acid in keratinocytes \textit{in vitro} and collagen in the dermis \textit{ex vivo}. Dihydroxy Methylchromone was shown to inhibit key enzymes in the aging process, including elastase and hyaluronidase and has the ability to reduce the release of MMP-1 in non irradiated fibroblasts \textit{in vitro}. These results were supported by a 28 days \textit{in vivo} experiment which demonstrated the anti-wrinkle and smoothing activities of this active ingredient.
Con questo lavoro si sono volute controllare le attività biologiche di ingredienti naturali quali la tiliroside ed il diidrossimetilcromone, utilizzati per la formulazione di prodotti cosmetici.

La tiliroside rappresenta un esempio sul come i benefici protettivi riscontrati possano essere trasferiti dalle piante all'uomo mediante studi condotti in vivo su volontari.

L'inflammazione topica è stata provocata sull'uomo mediante l'utilizzo di un simulatore solare in grado di emettere radiazioni UV. È stato così possibile controllare lo sviluppo dell'eritema indotto prima e dopo l'applicazione topica delle sostanze in studio, in confronto con controlli non trattati. Questo studio vuole essere un esempio di come l'uso tradizionale di alcune piante possa essere collegato alla presenza nelle stesse di principi attivi ben identificati.

La tiliroside è un importante principio attivo che può essere utilizzato con versatilità per il trattamento della cute normale e della cute cosiddetta sensibile, in quanto svolge dalla semplice attività calmante alla più complessa attività anti-inflammatoria.

Anche il diidrossi-metilcromone è stato rintracciato in molti estratti fitoterapici ma la sua concentrazione è così bassa che il relativo isolamento su scala industriale ne rende il costo prohibitivo. Si è così preferito sintetizzarlo e dagli studi ne è emersa una interessante attività antiage. Il diidrossi-metilcromone è, infatti, in grado di preservare l'integrità della matrice extracellulare e di indurre, in vitro, la sintesi di acido ialuronico a livello dei cératinociti e, in ex vivo il collagene a livello del derma.

È stato dimostrato come questo principio attivo sia in grado di inibir enzimi chiave che intervengono nel processo di invecchiamento, quali l'elastasi e la ialuronidasi, e come sia anche in grado di ridurre (in vitro) la produzione del MMP-1 (metallo proteinasi-1) nei fibroblasti non irradiati. Questi risultati sono stati confermati da uno studio in vivo della durata di 28 giorni, che ne ha dimostrato l'attività anti-rughe ed anti-invecchiamento.
INTRODUCTION

Mankind had turned to nature for solutions to health problems since ancient times. One of the earliest historical documents describing treatment of skin diseases is the Ebers Papyrus from 1550 B.C. (1), which recorded the use of henna (Lawsonia inermis) as a medicinal plant. The discipline of ethnomedicine focuses on valuable traditional knowledge of medicinal plants and the relationship between people and plants. Documents describing the use of medicinal plants are found very early in recorded history. In A.D. 78, the Greek surgeon Dioscorides authored De Materia Medica, a catalog of approximately 600 plants from the Mediterranean area used for medicinal purposes. If this knowledge can be transferred to cosmetics, ethnobotany could prove to be an important key for discovering new cosmetic ingredients.

Bio-guided fractionation is a good strategy for elucidation of a single active principle. Ideally, the candidate of interest is isolated from a medicinal plant or another plant source is identified from which the pure ingredient can be extracted. The advantages of such products as a cosmetic ingredient are obvious: the identity of the ingredient can be precisely described by a chemical structure, the mode of action can be elucidated and the constant quality of the ingredient can be monitored.

The bioflavonoid kaempferol 3-O-β-D-(6-O-coumaroyl)glucopyranoside (Tiliroside) (Figure 1) is given as an example where the activity of a variety of different medicinal plants such as Tilia, Malva or others can be explained mainly by a single compound. The synthesis of such a molecule in a low-cost industrial process is difficult, knowing that a five-step process is required for this purpose (2). The best approach in this case is to extract the flavonoid directly from a plant source. Tiliroside can be found in the hairy shields and young leaves of plants growing in tropical regions of the world. It protects these plants from UV-induced and environmental stress. The first part of this paper seeks to demonstrate the possible transfer of protective benefits from plants to humans in cosmetics as shown in a placebo-controlled in vivo study on human volunteers.

Another strategy is to identify natural compounds and screen their potential biological activities with available in vitro assays. One example is Dihydroxy Methylchromone (5,7-Dihydroxy-2-methylbenzopyran-4-one) (Figure 2), also known as Noreugenin. This natural product can be found in numerous medicinal plants. First isolated from the Japanese plant Nauclea orientalis (3), it has also been identified in Schumannophytton magnificum, Stevia purpurea, Angelica polymorpha and Rheum emodi, to name just a few. The biological activity of Noreugenin could not be deduced from the traditional use of the medicinal plant containing this entity. In fact, the healing properties of the majority of these plants cannot be explained by a single compound but are based instead on the synergetic effects of several ingredients.

Even though Dihydroxy Methylchromone (or
DHMC) is present in nature, its isolation is difficult due to its low abundance in the aforementioned plants and side compounds with similar polarity and often similar molecular weights are present as well. The time-consuming effort necessary to isolate this molecule from plants would be prohibitive on an industrial scale.

Consequently, this natural ingredient was synthesized in the laboratory in very high quality and large amounts. A detailed investigation of the synthesized molecule showed that DHMC possesses interesting biological properties which led us to develop this compound in the field of anti-aging as described in the second part of this paper.

**MATERIAL AND METHODS FOR TILIROSIDE**

**In vivo study design for Tiliroside**

The test was carried out on a total number of 20 healthy female volunteers with dry skin. The blinding of the tested verum and reference emulsion was maintained throughout the study. The participants were asked not to change their skin care habits during the prescribed study time. Compatibility of the test products was checked prior to the efficacy study. Test products did not induce existing sensitization that might have been triggered by the substances contained in the products.

All volunteers received detailed information listing each single parameter relevant to the study. Each volunteer was required to submit a written declaration of consent for their participation in the study.

Pregnancy and breast-feeding, intake of medication, which might influence the outcome of the study, sunbathing or the use of sun-beds were exclusion criteria.

**Evaluation of the anti-inflammatory efficacy of Tiliroside**

Inflammation is provoked by UV-radiation. In this test, erythema is generated on the inner forearms of each volunteer test subject using a solar simulator (1 MED, SOL3 Hönle, Munich, Germany). In this study erythema was documented by means of two measuring methods during a period of three days. The applied measuring methods are color measurements (reddening index a-value) with the Minolta Chromameter CR 300, and the determination of capillary flow by means of the Laser-Doppler Flowmeter. The erythema threshold is determined for each test subject individually, using different light intensities. The development of erythema is examined after the application of the respective test substances at the measuring times prior to treatment and after 6, 24, and 48 hours in comparison with an untreated reference (negative control) and a 1% hydrocortisone cream (positive control). One test area remains untreated (empty field), i.e. it is neither irradiated nor treated.

The respective development of the study for the test substances for reddening of the skin and capillary flow are presented graphically in comparison to the untreated control and the 1% hydrocortisone cream. This allows for documentation of a possible anti-inflammatory effect.
**Study Treatment**

The following emulsions were tested as shown in Table I.

**RonaCare® Tiliroside**

A common characteristic of all bioflavonoids is their yellow color (lat.: flavus = yellow). The pure active Tiliroside (Figure 1) has a natural yellow color which is retained as a light yellow colour in the combination of Sorbitol and Tiliroside known as RonaCare® Tiliroside used in this study. The blend was made by a specific procedure which delivers the active material well distributed on a special Sorbitol. This blend is easy to handle because it can be incorporated into the water phase which eventually delivers optimum distribution of the active ingredient after emulsification.

**Statistical methods**

In order to validate the measuring results statistically, the pre-post differences to the baseline at the individual measuring times (after 6, 24, and 48 hours) was calculated for both measuring parameters (skin reddening and change of the capillary flow) and for all test fields. Furthermore, a statistical evaluation according to the AUC procedure (Area Under the Curve) was done for the whole test period. All measured data of the different treatment groups were compared in pairs by means of the Wilcoxon Sign Rank test. For all tests a p-value \( \leq 0.05 \) was fixed as statistically relevant.

**MATERIAL AND METHODS FOR DHMC**

**Release of Matrix Metalloproteinase-1 (MMP1, collagenase)**

Human fibroblasts were cultivated at 37°C and 5% CO₂ with a culture medium containing DMEM (Life Technologies). At confluency the culture medium was changed for fresh medium containing (i) test compound, (ii) dexamethasone as positive control and (iii) a standard culture medium.

<table>
<thead>
<tr>
<th>Phase</th>
<th>INCI</th>
<th>Placebo [% (w/v)]</th>
<th>Verum [% (w/v)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Glyceryl Stearate, Steareth-25, Ceteth-20, Stearyl Alcohol 8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Stearyl Alcohol       1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dicaprylyl Ether      6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isostearyl Isostearate 4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Propylene Glycol      1.9</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aqua</td>
<td>ad 100</td>
<td>ad 100</td>
</tr>
<tr>
<td></td>
<td>Tiliroside</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Sorbitol</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Propylene Glycol, Diazolidinyl Urea, Methylparaben, Propylparaben 0.5</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>
Each experimental condition was conducted in triplicate. After incubation for 24 h, the MMP-1 content of the supernatant was measured using the specific quantitative BIOTRACK Human MMP-1 ELISA Kit (Amersham). The viability of the culture was performed using a standard MTT assay.

**Elastase assay**

Different concentrations of the test compound and elastase (from human leukocyte, Sigma) diluted at 100 mU/ml in 500 µM Tris buffer, pH 6.8, were pre-incubated for 10 min on ice. Elastin (fluorescent DQ-Elastin, Interchim) was then added (5 µg/well final concentration) and plates were incubated at 37°C for 120 minutes. 100 ml of each reaction mixture was filtered through individual exclusion spin columns (MicroSpin G-25, Pharmacia) according to the manufacturer’s protocol. The filtrate was collected in microtubes containing the inhibitor AAPV (methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone) and was immediately placed on ice. This filtration system allows the separation of the large fluorescent elastin peptides (excluded from the column) from the compound (trapped at the top of the column). Each sample was measured at 485 nm (SpectroMax Gemini, Molecular Devices). The results were expressed in percent of enzyme activity.

**Hyaluronidase assay**

Different concentrations of test compound (Figure 7) were dissolved in 0.1 M phosphate buffer, pH 5.3, and hyaluronidase (HYAL, Sigma type IV-S) was added at a final concentration of 1mg/ml. Hyaluronic acid (HA, Sigma) was added and the mix was incubated at 37°C for 1 hour. Residual HA was precipitated with bovine serum albumin (BSA, Sigma, diluted at 1% in acetate buffer 0.5 M, pH 4) and photometrically quantified at 540 nm (SpectroMax Gemini, Molecular Devices).

**Hyaluronic acid synthesis assay**

Normal human epidermal keratinocytes (NHEK) were cultivated in a 96-well-plate in Keratinocyte-SFM culture medium (Invitrogen) for 24 hours at 37°C and 5% CO₂. The medium was aspirated and the cells were incubated with the indicated concentration of the positive control (retinol) or DHMC for 72 h. All treatments were performed in triplicate. At the end of the incubation, the hyaluronic acid concentration was evaluated in the supernatants by ELISA according to the procedure of the manufacturer (R&D Systems) and cell viability was evaluated using a standard MTT reduction assay.

**Ex-vivo test: staining of total collagen on explants**

57 biopsies of an average diameter of 10 mm were prepared from skin of cosmetic surgeries from a 46-year-old woman. These explants were cultivated in BEM culture medium (BIO-EC’s Explants Medium) at 37°C in a humidified, 5% CO₂ atmosphere. The placebo formulation and the formulation containing 2% Ronacare® Luremin™ (a mixture of DHMC and sorbitol in a 5 to 95 ratio) were applied topically on the basis of 2 mg/cm² per explant, using a small spatula, on day 0, 1, 2, 5, 7 and 8. The control explants did not receive any treatment. On day 0, 3 explants from untreated batch (T0) were collected, cut in two halves and frozen at -80 °C.

On day 8, 3 explants from each batch were collected and processed in the same way. Staining of total collagen was performed on 12 explants from the following batches:

- Untreated on day 0 (3 explants)
- Untreated control on day 8 (3 explants)
- Placebo (E) on day 8 (3 explants)
- Formulation containing 2% RonaCare® Luremin™ (corresponding to 0.1% DHMC) on day 8 (3 explants).

The collagen from 2 different areas of an explant of each batch was stained in histologies using micro-sirius red. Microscopic observations were made using a Leica Orthoplan microscope with a 20x objective. Pictures were taken with a Sony DXC 390P camera. 9 microscopic fields were analyzed for the 2 areas of each explant and the surface percentage occupied by collagen in the papillary and upper reticular dermis was determined by image analysis with the Leica QWin software. The results of the 18 microscopic fields were then averaged for each batch. The difference between 2 batches is significant if p≤0.05.

In vivo test: evaluation of the anti-wrinkle effect of a formulation containing DHMC in comparison to the corresponding placebo formulation.

The in vivo test was performed in a double-blind and inter-individual study with 40 female volunteers. 20 volunteers (between 47 and 65 years) tested the placebo and 20 volunteers (between 45 and 62 years) tested the product DHMC at a concentration of 0.1%. The assessment zone was the crow’s feet. The formulations were applied twice daily (morning and evening) in normal conditions of use during 28 days.

To evaluate the anti-wrinkle effect of this cosmetic ingredient, 3D Primos® was used to study the variations of the cutaneous relief parameters. This technique consists in calculating a phase image from interference fringe projection images. The acquisition software allows 2D and 3D measurements, and enables the roughness parameter on the profile and the depth of the wrinkle to be determined. An automatic system of repositioning allows the precise re-identification of the same measurement zone.

The following parameters were analyzed:
- Average roughness (Ra): ratio between the integrated surface around the mean value and the length of skin evaluated.
- Average relief (Rz): on five regions of the profile (mean value of these different maxima, obtained on five successive regions of the profile. It reflects local differences).
- Maximum relief amplitude (Rt): maximum difference between the highest peak and the lowest point of the wrinkle(s) registered over the entire profile. In order to refine the Rt value, the length L of sweeping is divided into five equivalent segments. It is calculated, in the same way that for Rt and for each interval, the height corresponds to the difference between the maximum peak and the deepest hollow. This parameter is called Rzi. The average value of Rzi is Rz.

The following variations were defined:
- \( \delta \text{Ra} = \text{Ra(day0)} - \text{Ra(day28)} \)
- \( \delta \text{Rz} = \text{Rz(day0)} - \text{Rz(day28)} \)
- \( \delta \text{Rt} = \text{Rt(day0)} - \text{Rt(day28)} \)

A positive delta Ra expresses a smoothing effect. The higher delta Ra, the better is the smoothing effect.
A positive delta Rz (or delta Rt) expresses an anti-wrinkling effect. The higher delta Rz (or delta Rt), the better is the anti-wrinkling effect.

RESULTS AND DISCUSSION

The aging process of the skin is a complex biological phenomenon consisting of two components: intrinsic aging and extrinsic aging.

Intrinsic aging, also termed chronoaging, is an inevitable change attributable to the passage of time alone and is manifested primarily by physiologic alterations with subtle but undoubtedly important consequences for the healthy skin. Intrinsic aging is largely genetically determined.
Extrinsic aging is caused by environmental exposure, primarily to UV light, and is more commonly termed photoaging. In fact sun exposure is considered to be by far the more significant deleterious to the skin. It is believed that 80% of facial aging is due to chronic sun exposure (4). Oxidative stress by increasing hydrogen peroxide and reactive oxygen species (ROS) is thought to play a central role in initiating the cellular response following UV irradiation (5). Plant derived flavonoids are known to inhibit processes initiated by oxidative stress. Their antioxidant mechanisms include the inhibition of enzymes involved in the formation of ROS such as protein kinase C, lipooxygenase or cyclooxygenase. Additionally flavonoids act as chelators of free iron or copper which are potential enhancers of free radical formation (6).

**TILIROSIDE**

By means of the UV model an experimental stress reaction of the skin was induced. By measuring the reddening of the skin with the Minolta Chromameter, and the capillary flow by means of Laser-Doppler Flowmetry, the development of the stress reaction can be monitored. The impact of Tiliroside on this reaction was studied to evaluate this product’s potential ability to protect the skin. An increasing capillary flow and reddening are signs of radical stress. These endpoints are also suitable readout parameters for inflammation of skin.

**Results of the color measurements with the chromameter (reddening index, a-values)**

The test field of the untreated control showed an increase of skin reddening at the measuring time of 6 hours after irradiation. This value increased slightly at the measuring time after 24 hours. At 48 hours reddening of skin slightly subsided (Figure 3, A). The placebo formulation showed a very weak efficacy in this model. The increase of skin reddening is only minimally below that of the untreated control. The verum formulation containing 0.1% Tiliroside leads to a decrease of skin reddening. After 6 and 48 h the effect is statistically significant versus the placebo. After the statistical evaluation of the whole test period by means of the AUC procedure the effect of the verum differs significantly compared to the placebo (Figure 3, B and Table II A).

![Graph](image_url)
TABLE II

Statistical analysis of in vivo results. The pre-post difference to baseline at 6, 24 and 48 h was calculated for redness (A.) and capillary flow (B.). The UV-control was only irradiated and not treated with any test product. The analysis of the whole test period was done according to the AUC procedure (n.s., not significant, p-value > 0.05; AUC = area under curve).

<table>
<thead>
<tr>
<th></th>
<th>6 h</th>
<th>24 h</th>
<th>48 h</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) redness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>verum vs UV-control</td>
<td>p = 0.011</td>
<td>p = 0.003</td>
<td>n.s.</td>
<td>p = 0.015</td>
</tr>
<tr>
<td>verum vs. placebo</td>
<td>p = 0.024</td>
<td>n.s.</td>
<td>p = 0.021</td>
<td>p = 0.048</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>6 h</th>
<th>24 h</th>
<th>48 h</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B) capillary flow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>verum vs UV-control</td>
<td>p = 0.007</td>
<td>p = 0.001</td>
<td>p = 0.002</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>verum vs. placebo</td>
<td>n.s.</td>
<td>p = 0.009</td>
<td>n.s.</td>
<td>p = 0.002</td>
</tr>
</tbody>
</table>

Results of capillary flow

In the reference field of the untreated control, the capillary flow had increased to a maximum 24 hours after UV-irradiation. This reaction was followed by the placebo product, which showed the lowest efficacy in this test as well (Figure 4, A). The Tiliroside-containing verum suppresses the capillary flow after 6, 24 as well as 48 h after UV-irradiation. The calculated p-value of about 0.002 underlines the statistical significance of this effect after AUC procedure (Figure 4, B and Table II B). No significant changes were found in the empty field (non-irradiated and untreated). The findings of the presented in vivo study clearly identify Tiliroside as a skin protecting ingredient derived from nature. Different research groups have shown that in cell cultures of mononuclear cells Tiliroside down-regulates the overproduction of NO and TNF-α all known to be involved in inflammation (7,8). These findings described in literature support evidence not only for an anti-oxidative but also for the anti-inflammatory effect of Tiliroside on skin in vivo.

DHMC

The biological activity of DHMC was investigated on the 3 primary structural components of the extracellular matrix: collagen, elastin and glycosaminoglycans. Alterations in number and structures of these key components as a consequence of intrinsic and extrinsic aging are believed to be responsible for wrinkle formation. Collagen, the main structural component of the dermis, is responsible for conferring strength and support to human skin. 70% of dry skin mass is composed of collagen (9). The most abundant types of collagen in the skin are collagen I and III. With age, the amount of collagen in the skin tends to decline due to lower levels of collagen synthesized by aged fibroblasts as well as increase in the production of matrix metalloproteinases (MMPs) (10). Considering that collagen predominates in the skin, any ingredient able to stimulate its synthesis in the dermis is of particular interest. An ex vivo study was carried out to evaluate the effect of a formulation containing 2%
RonaCare® Luremin™ (corresponding to 0.1% concentration of DHMC in the formulation) in comparison to a placebo formulation on the stimulation of collagen synthesis in an 8-day study. The RonaCare® Luremin™ and placebo formulations were applied on the basis of 2 mg/cm² at day 0, 1, 2, 3, 5, 7 and day 8. Then the collagen in the explants was stained with picror-sirius red (a standard red dye). The surface percentage occupied by collagen in the papillary and upper reticular dermis was determined by image analysis. It was observed on 2 separate areas that the treatment with RonaCare® Luremin™ induced a significant increase of 7% and 8% respectively in the surface percentage occupied by collagen compared to the batches treated by the placebo formulation (Figure 5 shows the averaged results of the two areas for each batch).

![Graph A](image.png)

**Fig. 4** Anti-inflammatory efficacy of Tiliroside as a function of time. (A.) The capillary flow is determined by Laser Doppler flowmetry and the results for each time are presented (* statistically significant vs. placebo: p-value < 0.05). (B.) The results of all time points are shown as area under the curve (p-value = 0.002).

![Graph B](image.png)

**Fig. 5** Effect of a formulation of RonaCare® Luremin™ (2%) on the density of collagen ex vivo on the sections of 2 separate zones. * Statistically significant vs placebo (p<0.05) and vs untreated control (p<0.05).
In parallel to the stimulation of collagen synthesis, a complementary approach would be to inhibit the matrix metalloproteinases (MMPs), in particular MMP-1 (collagenase) which degrades collagen into small pieces leading to further degradation of this structural protein. The limitation of collagen degradation by MMP-1 in conjunction with the stimulation of the collagen synthesis is a good approach to slow down the skin aging process. At this level, although DHMC is not an inhibitor of MMP-1, it significantly reduced at 40 µg/ml (corresponding to a concentration of 0.2 µM) the release of MMP-1 from not irradiated fibroblasts by 40%, p<0.01 in comparison to negative control.

Elastin is one of the main constituents of elastic fibres in the papillary and reticular dermis. It is a minor component of the dermis but it has an important function in providing the elasticity of the skin. It accounts for 2-4% of the extracellular matrix. Upon intrinsic aging dermal elastin is altered. The major alterations concern the architecture of the elastin network. There is a progressive disappearance of elastic tissue in the papillary dermis not only due to a down-regulation of elastin gene expression (12) but also to the presence of degrading enzymes such as elastase which leads to the observed slow reduction of elastin. Consequently skin elasticity is gradually lost with age (13). It is believed that the phenomenon of sagging skin observed in the elderly man may be due to this loss of elasticity. In an in vitro assay it was shown that DHMC inhibited leukocyte elastase with an IC$_{50}$ of 200 µM. Thus DHMC could slow down the degradation process of elastin in the skin by limiting the proteolytic activity of elastase.

As previously mentioned, the degradation of the extracellular matrix plays an important role in the development of wrinkles and other signs of skin aging. Structural proteins (collagen or elastin) are important but other components of the extracellular matrix such as glycans, a class of glucose-based polymers including glycosaminoglycans and proteoglycans, are necessary for a healthy skin.

A very important glycan in the skin is hyaluronic acid (HA). Hyaluronic acid is a polysaccharide composed of repeating alternating units of D-glucuronic acid and N-acetylglucosamine. The molecular domain of HA encompasses a large volume of water that hydrates tissues and is responsible for skin moisturizing. Additionally HA has a wide range of functions such as regulation of the osmotic pressure or the regulation of cell-matrix adhesion, for example (14). It also increases viscosity and contributes to the suppleness of the skin.

The skin content of hyaluronic acid decreases with aging. Though dermal HA is responsible for most skin HA, epidermal cells are also able to synthesize HA. The most dramatic histochemical change observed in senescent skin is the marked decrease of epidermal HA. In aged skin, HA is still present in the dermis, while the HA of the epidermis has disappeared entirely (15). This contributes to the loss of moisture; the skin becomes thinner and less supple.

One way to increase the skin content of HA would be to stimulate its synthesis in the skin, in particular in the epidermis. In an in vitro experiment, DHMC tested at 20 µM significantly increased the secretion of hyaluronic acid in keratinocytes by 73%, p<0.01 compared to the negative control. As expected, in the same assay, retinol used as a positive control and tested at 1 µM significantly increased the production of hyaluronic acid in keratinocytes by 76% (Figure 6).

Furthermore, as hyaluronic acid is degraded by enzymes called hyaluronidases, the inhibition of this enzyme would limit the decrease of HA in the skin. In an in vitro assay, it was observed that DHMC inhibited the HA-degrading enzyme hyaluronidase with an IC$_{50}$ of 20 µM (Figure 7).
Fig. 6 Stimulation of the synthesis of hyaluronic acid in keratinocytes.

Fig. 7 Effect of DHMC on the activity of hyaluronidase.
**In vivo assay with DHMC**

Finally, to confirm the observed *in vitro* and *ex vivo* anti-aging activity of DHMC, an *in vivo* study was carried out with 40 female volunteers. This study was conducted as a double-blind and inter-individual study. 20 volunteers (between 47 and 65 years) tested the placebo and 20 volunteers (between 45 and 62 years) tested a verum formulation containing the ingredient DHMC at a concentration of 0.1%. The assessment zone was the crows' feet area. The formulations were applied twice daily (morning and evening) in normal conditions of use during 28 days. To evaluate the anti-wrinkle effect of this cosmetic ingredient, 3D Primos® was used to study the variations of the following cutaneous relief parameters.

Delta Ra for the placebo corresponding to Ra at day 0 subtracted to Ra at day 28 was lower than delta Ra for the verum formulation, indicating a clear smoothing effect of DHMC. Similarly, delta Rₜ and delta R₂ were lower for the placebo than for the verum, expressing the good anti-wrinkle effect of DHMC (Figure 8).

In other words, the results of this *in vivo* study showed that no significant anti-wrinkle effect was noticed with the placebo formulation. By contrast, a significant anti-wrinkle effect with the DHMC formulation (Figure 9) was observed with a significant decrease of 12% in the average roughness (Ra) in 80% of the volunteers, a significant decrease of 11% in the average relief (R₂) in 85% of the volunteers and a decrease of 8% in the maximum relief amplitude (Rₜ) in 80% of the volunteers.

**Fig. 8 Improvement of the parameters Ra, R₂ and Rₜ after 28 days of application of DHMC (0.1%).**
CONCLUSION

Nature remains an endless source of inspiration to identify potential compounds for the cosmetic industry. Once identified, natural ingredients such as Tiliroside can be isolated directly from a plant. If isolation is impossible and synthesis economically feasible, they can be synthesized instead, such as the case with Dihydroxy Methylchromone (DHMC).

The described anti-inflammatory study with Tiliroside provides an example where traditional use of a medicinal plant is supported by identification of the active principle, providing the basis for a new cosmetic ingredient. In this case, it was possible to obtain a secondary plant metabolite directly from nature and to provide a pure compound rather than an extract. Tiliroside is a powerful ingredient for normal and sensitive skin. It offers a multitude of cosmetic benefits such as calming, soothing skin or anti-inflammatory properties.

DHMC induced the synthesis of hyaluronic acid in keratinocytes in vitro and the synthesis of collagen ex vivo. Furthermore, DHMC was able to inhibit several key enzymes in the aging process, such as elastase or hyaluronidase, and to reduce the release of MMP-1 in non-irradiated fibroblasts in vitro. All these results were supported by a 28-day in vivo experiment which demonstrated the anti-wrinkle and smoothing properties of DHMC.
References


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Paul Klee and His Illness

by Hans Suter

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For the first time a specialist in dermatology and known collector and patron of the visual arts has written a book on the famous artist Paul Klee, describing the links between his art and illness.

Paul Klee, born on December 18, 1879 near Bern, died in 1940 at the age of 60 of a disease which, at the time, remained undiagnosed (it was only ten years after his death that the illness was given the name Scleroderma).

He was not only good at drawing but was also a talented violinist, deeply interested in literature and theatre. As painter "he was not only avant-garde in his art, but also, in the literal sense of the world, someone who was in advance, someone who could foresee world events". For this reason it is possible that the paintings he produced on the theme of fear, really portend the fear, misery and degeneration of those who suffered oppression and persecution in Nazi Germany. Thus he was able to conceptualize his personal experiences and transform his emotions into arts.

Klee was, in fact, an excellent teacher who loved his profession, but just three months after the Hitler election as Chancellor, was suspended from his position as Distinguished Professor at the Düsseldorf Academy of Fine Arts. His work was discredited, he was labelled as Galician Jew, and was publicly denounced in the touring exhibition entitled Degenerate art.

For many months Paul Klee tried to stay in Germany, where he had an excellent reputation as an artist and art teacher, but in December 1933 he had to definitively return as an emigrant to his home town in Bern. The disappointing about his dismissal from the Düsseldorf Academy and the illness that never leave him, greatly influenced his art suffering above all, psychologically and emotionally. However, he bears his pain and suffering with great fortitude, being able to conceptualize his personal experiences and transform his emotions into art. Thus, he was capable to handle his terrible disease, as well as he handled his deformation and dismissal by the Nazis.

In any way Paul Klee produced cryptic diary-like drawings which have given a clue of his mental state during the course of his illness. Its paintings show anxiety and depression, but also a sense of hope, revival and optimism.

He was, however a wise man capable to see into the future invoking it in his own way through drawing and printing by a very vivid imagination. Upon finished a printing, Klee gave it a pointer to help interpret his work. At the same time, he was very creative in his use of language, so that in around 9,800 works he very rarely repeated a title.

This interesting and well written book, while describes in a clear way the complex disease of
Scleroderma affecting Klee for all his life, arranges and interprets more than 90 of his late works, linking them with the disease in progress. All the comments regarding the different Klee paintings are very interesting, not only for the artistic point of view but also for the consequent medical diagnosis.

In my opinion, this unique book has to represent a key stone of the personal library for all the medical and/or artistic communities that like art and wish to better understand the link that always join beauty, health, and happiness with any artistic creation.

P. Morganti
Editor-in-Chief
Colloids in Drug Delivery

By M. Fanun

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Enhancement of skin permeation, absorption, and relative efficacy of drug, cosmetics and food locally applied or taken by oral route is the main objective of all the products. Moreover many of the bioactive ingredients used need also to be shielded from the environment or need to be protected from rough standardised production processes.

The ultimate goal of skin permeation and/or mucosal absorption is to ensure that the compounds are delivered at a specific rate and at the right concentration, to remain at the topical site or to reach the systemic circulation. The overall efficacy of a product containing functional ingredients is due, in fact, to the intrinsic activity of the ingredients and their delivery to the right site of action. Both must be optimized in order to obtain an effective product. Thus, according with J. Wiechers: “the role of a delivery system is to ensure that the right concentration of the right chemical is reaching the right site in the body for a sufficient long and correct period of time”.

Differently from drug, the cosmetic delivery has to be more selective. It must avoid as much possible a transdermal delivery, keeping the functional molecule in specific skin layers of the epidermis. At this purpose the microencapsulation technology can release the ingredient at the proper site along the skin (cosmeceuticals) or digestive tract (nutraceuticals), preventing ingredients oxidation, losing functionality and unwanted interaction, but also masking taste, smell or colour. What microencapsulation means?

It consists of embedding an active substance inside a microparticle so that, the resulting particle may have a different structure such as a solid sphere, a liquid core surrounded by a membrane, a coated solid core or a hydrogel bead.

At the base of the modern technology of encapsulation of drug, cosmetic, and food delivery, there are colloidal systems. These systems have evolved from use in the enhancement of solubility and protection of labile substances, to reduction in the toxicity of drugs, and/or to improve the therapeutie performance, by targeting drugs to the site of action, and by increasing the patent life of the product also.

Colloids in Drug Delivery is a book in 25 chapters, examining all the topics necessary to understand the present and the future applications, and the different biotechnologies based on the use of colloidal compounds.

Colloidal systems and microencapsulation has proven to be a valuable tool for food, cosmetic and pharmaceutical industries. The efforts have been in alteration of the surface properties and particle
size distribution of active ingredients incorporated into colloidal curriers to achieve a diverse array of therapeutic delivery objectives, such as controlled and targeted drug/food/cosmetic delivery. Thus, the analysis of the encapsulated product is a crucial component of encapsulation research and development, as well as it is a critical point for determining capsule morphology, composition and performance. At this purpose, microsphere and microcapsule have formulation advantages and disadvantages. It is, therefore, important to fully characterize their physical properties. Smaller particles, for example, yield a larger surface area in the bulk product that can lead to faster release rates of an encapsulated material, while wider size distribution of capsules can provide problems for injectable particles. Shell thickness for the microcapsule structure has, in fact, a large impact on the overall payload percentage, in addition to controlling diffusion rates through the shell.

Payload distribution in matrix particles can also impact release rates and generate small burst releases if large chams of active ingredient exist in the microsphere.

In any way to know the weight percent of active ingredients in a microcapsule and determine the production process encapsulation efficiency, it is of fundamental importance to meet the performance of the final product. The constituent surfactants and polymers allow for the controlled and targeted drug, cosmetic and food delivery, enhanced effective solubility of the chemical and facilitation of its cellular uptake and minimization of its degradation and toxicity through understanding so that the understanding of the physicochemical properties and interfacial consequent implementation behaviour of surfactants can provide a major impetus for any chemical delivery. Surfactants in fact, being amphiphilic molecules with a polar hydrophilic head and a non-polar hydrophilic tail tend to accumulate at varies interfaces, and reducing the contact and the free energy of the oil/water boundary, form varied structures such as micelle, microemulsions and liquid crystalline phases. The foundation of all advances in colloidal drug delivery science has been, therefore, based on the use of surfactants and polymers.

As pharmaceutical, cosmetic and food carriers, colloids can however, he classified as self-assembled lipid systems (emulsions, liposomes, solid lipid nanoparticles, etc.) polymer systems (micelles, dendrimers, conjugates, etc) drug nanoparticles and precollloid systems (self-emulsifying oral delivery systems, and liquid crystalline systems).

Thus, as previously written the characterization of any prepared dispersion is an important prerequisite to confirm its reproducibility and to control its dispersion property and effectiveness. The morphological properties of colloidal particles greatly influence, in fact, the morphological characteristics of the delivery system, so that any alteration in their size, shape and surface area may alter the stability biocompatibility and bio-distribution of these chemical carrier systems.

The theoretical aspects, characterization and control of the colloidal delivery systems are reported on Chapters 1 and 2.

The use of hard colloidal drug delivery systems formed by surfactants, polymers, proteins and lipids has provided interesting results. Microencapsulation is one of the most promising technologies for protecting and controlling the release of actives in drugs, cosmetics and food.

Encapsulation may also be used to create new structures and functions.

Traditionally different microencapsulation techniques are used, and in the last years it has been shown that materials aspects and structural features down to the nanoscale, affect the efficacy of nanoencapsulation systems. The small size in combination with the chemical composition of the encapsulation system in general and more particularly with surface, provides the unique properties
of the nanostructured encapsulation systems (NESs).

Concerning the classes NESs, are distinguished into two classes: lipid-based systems and protein-based systems.

**Chapters 3 to 5** provide the main scientific issues and challenges to the development of new classes of delivery systems, review their day to day status outlookiing their future, support the development on new applications, as smart bio-textiles. The topical delivery of compounds to the surface and deeper layers of the skin, and the transdermal delivery of pharmacologically active substances represent topics of great interest for professionals, consumers and readers of this journal. Skin delivery systems are being evaluated to maintain the concentration of drugs, but also of cosmetic products as sunscreens, deodorants and all the beauty products.

The skin barrier function is recognized as the most important facet to defend the body from the many interior and exterior environmental aggressions. It, as protective organ, consists of various viable cell layers, the keratinocytes, covered by the Stratum Corneum (SC). This outermost non-viable layer, comprising corneocytes embedded and surrounded by lamellar lipids, form the barrier of the skin with a big impact to the deeper skin layers concerning drug and cosmetic delivery. It consists, in fact, of cornified desquamating cells with a high level of keratin linked by lipids and desmosome-intercellular connections. The corneocytes have a continuum turnover of about four weeks and below the viable epidermis maintains its activity.

Fundamentally the skin consists of 3 viable layers: Epidermis, Dermis and Hypodermis covered by the outermost non viable layer, the Stratum Corneum.

The Dermis supports the epidermis and its annexes (nails, hair, glands). It is composed by cells, fibres, and ground substance (extra cellular matrix) and, unlike the epidermis, it is richly innervated and vascularised. The fibres (collagen and elastic fibres composed of aminoacids support the internal structure of the skin, while the ground substance is the cementing component of dermis (macromolecules, as glycosaminoglycans, hyaluronic acid, etc) with the ability to bind numerous water molecules.

The extensive vascular network plays a role in the nutrition of skin tissue and in thermoregulation of the human body, as well as the lymphatic system is important for removal of large molecules, and nerve connections are necessary to connect the skin with the entire body.

Other dermic structures, as sweat and sebaceous glands, and hair follicles are also to be considered in terms of drug/cosmetic transport.

Cosmetic and drug delivery have, in fact, three principal routes of skin penetration: transepidermal, transdermal and transfollicular. Chemical species, may diffuse through the keratinocytes or between them through the intercellular lipid lamellae: hydrophilic substances should diffuse intracellularly, whereas hydrophobic ingredients through the intercellular region. New reports characterize the possibility for hydrophilic chemical transport by the intercellular with hydration of the intercellular lipid lamellae and reversible degeneration of the desmosomes.

Many applications in many domains, for many different purposes, and concerning many different active ingredients lead to the development of a large number of different encapsulation methods clearly described in these chapters. Classifying these methods is a complex task and it is difficult to find and select the more suitable technologies.

Soft colloidal systems including micelles, multiple emulsions, dermoemulsions and microemulsions are reported and discussed from **Chapters 6 to 12.**
Polymeric micelles are becoming a powerful nanomedicine platform for the therapeutic application because of their small size, good biocompatibility, stability, and successful use in pharmaceuticals to solubilise insoluble chemicals, in general, and thus water insoluble drugs.

The nanoscopic dimension, (stealth properties induced by hydrophilic pelimeric brush on the micellar surface), capacity for stabilized encapsulation of hydrophobic drugs offered by the hydrophobic and rigid micellar core, together with the possibility for the chemical manipulation of the core-shell structure, have made polymeric micelles one of the most promising carriers for drug delivering, targeting, and imaging. These potential carriers for poorly water-soluble drugs are particularly attractive because of their ability to deliver large payloads of a variety of drugs and chemicals, their improved in vivo stability compared to other colloidal carriers, and their nanoscopic size that allows for passive accumulation in diseased tissues, such as solid tumors.

Multiply emulsions are complex polydispersed systems in which water-in-oil (W/O) and oil-in-water (O/W) emulsions exist simultaneously. Their stabilization requires both hydrophilic and hydrophobic surfactants in the formulation, in the right ratio. In addition to pharmaceutical applications, multiple emulsions have shown potential use in agriculture, cosmetic, food, separation sciences, and nutriceutical industries.

One of the unique advantages of these carriers is the easy and inexpensive method of their preparation, meanwhile the challenge is to increase their bioavailability and long-term stability by the use, for example, of complessing gelling and polymeric agents. However, because of their physical characteristics, multiple emulsions can be used as carriers for both hydrophilic and lipophilic drugs/chemicals depending on the type of application, route of administration, and feasibility of formulation. Moreover, they have been recognised as an intermediate step in the formulation of microspheres, nanospheres, nanoparticles, microencapsules and so forth.

Nanoemulsions (NEs), defined as extremely small droplet emulsions, are of two types (a) thermodynamically stable systems and (b) metastable systems, depending on the method of preparation. Despite their metastability NEs can persist over many months or years because of the presence of stabilizing surfactant micelles. However, to make a stable and reproducible emulsion, a large number of factors must be controlled, such as selection of an appropriate composition, controlling the order of addition of the components, applying the shear to obtain the droplets rupture, and measuring the energy input.

One of the preparation methods for NEs is a high-energy technique that include, for example, high-pressure homogenization. This emulsify technique depends on the formulation of nanometre-sized droplets in the presence of a surfactant or a surfactant mixture, carried out using a high shear mixer to obtain a coarse emulsion. This raw emulsion is successively passed through a high-pressure homogenizer several times to obtain a homogeneous dispersion of small inner phase droplets. The homogenization pressure, number of homogenization cycles, and homogenization temperature affect the droplet size of the NE.

Microemulsions, known also as a small emulsion-like structures or structured solutions, are colloidal dispersions usually consisting of a water phase, an oil phase, and one or more amphiphiles; the physicochemical properties of the constituents and their concentrations are balanced to form thermodynamically stable liquids at a given temperature. These carriers are homogeneous on a macroscopic level, but they are heterogeneous on a microscopic level because the structure consists of different microdomains. The specific properties of microemulsions, such as thermodynamic stability,
ultralow interfacial tension, small droplet size, low viscosity, and high interfacial area between the oil and water, provide tremendous benefits for their pharmaceutical applications including easy formation with little energy input (beat or mixing), long-term shelf life, filterability, sprayability, high solubilisation capacity for drug molecules, and dose uniformity.

The most important features of microemulsions colloidal systems are increased drug solubilisation capacity and the dynamic character of their microstructures. To increase these characteristic properties have been developed by the self-microemulsifying drug delivery systems (SMEDGs).

SMEDDGs (microemulsion preconcentrates) are described as isotropic mixtures of oils, surfactants, and co-solvents with a solubilised drug substance, which form a microemulsion by diluting with aqueous medium on mild agitation or by mixing with biological fluid in vivo.

These pre-concentrates form W/O and O/W microemulsions only within a specific and narrow range of water concentrations.

Because of their unique combination of properties, microemulsions have been used as reactors for the synthesis of nanoparticulate carriers of drugs such as SLNs or polymeric nanoparticles. Nanoparticles obtained by this technique usually have diameters of less than 100 nm.

The main disadvantage of the microemulsion approach for nanoparticle synthesis is the high content of surfactants and co-surfactants. Differently from SMEDDs, oil of long-and medium-chain triglycerides with different degrees of saturation have been used for the formulation of self-emulsifying drug delivery systems (SEDDs). One feature of these mixtures is the ability to form oil-in-water (O/W) emulsions with only gentle agitation when they exposed to aqueous media. The surfactants that are commonly used to formulate SEDDs are non-ionic with a relatively hydrophilic-lipophilic balance (medium-chain mono-and diglycerides, polyoxyl 35 castor oil, tween 80, etc.), and the concentration usually used to form stable emulsions ranges between 30% and 60% (W/W). It seems that the mean droplet size may be reduced by increasing the concentration of the surfactant. Usually the surfactant concentration in ointments and creams is significantly lower than in surfactant gels.

The microstructure of both these structures may consist of liquid crystals (LCs), as long as liquid crystalline network or matrix as formed by amphiphilic molecules. To obtain a liquid crystalline matrix, amphiphilic surfactants that form lyotropic LCs at room temperatures have to be selected. They are, in fact, intermediate state of matter or mesophases, which are halfway between an isotropic liquid and a solid crystal. It is to remember that their mesomorphic properties change, with the temperature, pressure, and relative concentrations of the different components of the mixture.

In nature, many structures are composed of bilayers characterized by hydrophilic and hydrophobic lamellar phases presenting mesomorphic states. These lamellar-non lamellar transitions are fundamentally important in modulating the physicochemical properties of cell membranes and consequently in regulating different biological processes. It is therefore important to understand how lipid composition and protein contenten modulate the membrane structure and its vital function.

Chapters 13 and 14 are focused to describe and understand the biocompatibility and stability of the LCs and the lipidic nanoparticulate systems such as cubosomes, hexosomes, etc., useful to enhance the drugs bio-assimilability.

The biomedical application of self-assembling non ionic amphiphiles, the niosomes, and the aggregation of cationic liposomes by appositely changed linear polyons, are reported respectively on Chapters 15 and 16.

Niosomes, produced from entirely synthetic and well-defined non ionic surfactants, have the advan-
tage of a low cost, stability, easy storage, industrial scale production, and interesting biological performances. On the other hand, *Cationic liposomes*, based on the supramolecular structures formed between changed polymers and appositely charged particles, show promising potential for their modularity and flexibility. This system, in fact, allows to obtain both positively and negatively charged aggregants (clusters) of the desired size by simply changing the basic liposomal vesicles (positively or negatively charged) and by using appropriate appositely charged polyns. Within the clusters, the liposomes, which are simply glued together by the polyns, are able to maintain their structural integrity.

Apart of the increased stability of the liposomal structure, the chemical compositions can be varied to a large extent and adjusted to meet the requirements of different applications.

In any way, liposomes are typically made on natural, biodegradable, nontoxic, and non-immunogenic lipid molecules that can encapsulate or bind a variety of chemical molecules within or onto their membranes. Usually they are composed of natural and synthetic lipids (phospho and sphingolipids) and may also contain other bilayer constituents such as cholesterol and hydrophilic polymer-conjugated lipids. The net physicochemical properties of the lipids composing the liposomes, such as membrane fluidity, charge density, steric hindrance, and permeability, determine the interactions between liposomes and the human tissues component after topical/systemic administration.

Liposomes of different sizes and characteristics usually require different sizes and methods of preparation, as well as drugs or chemicals to be encapsulated may be included in the aqueous hydration buffer for hydrophilic drugs or in the lipid film for lipophilic drugs.

This is the interesting topic discussed on Chapter 17, where the correlation between the different liposomal preparation strategies are reported.

Chapter 18 reviews and demonstrates the feasibility of usnic acid loaded liposomes with high encapsulation efficiency.

**Dendrimers** with their highly branched, nanoscale architecture and high functionality, and **microspheres** with their characterisation and preparation techniques are reported as new and important drug carriers on Chapters 19 and 20. Dendrimers provide a unique platform for developing nanostructured deep delivery systems, because of their great structural adaptability and functionality to meet specific pharmaceutical and biomedical needs. Assembling therapeutic and bioactive molecules on the dendrimer surface through proper covalent linkage has become, in fact, a common way to develop dendrimer-based drug delivery systems. These dendritic polymers are capable of evolving with the science of medicine to incorporate state-of-the-art functionalities as they are discovered, in order to develop potent nanomedicines.

**Microspheres**, as colloidal drug delivery systems, have given good results as inhaled medications. At this purpose, particle size and size distribution are important factors in efficient aerosol drug delivery for local and systemic disorders, as well as the understanding of the complex factors influencing aerosol delivery in the treatment of respiratory diseases, as focused on Chapters 21 and 22.

Colloidal carriers in dental tissue engineering is the topic of Chapter 23.

The functional life of tooth is determined by the instability of the dentin-pulp complex. The pulp maintains, in fact, tissue homeostasis after tooth development and underpins the defence reactions taking place in response to injury leading to time regeneration. At this purpose, growth factors are a group of molecules responsible for signalling a variety of cellular processes and various aspects of tissue regeneration.
The application of growth factors in tissue engineering necessitates the use of a delivery system. The colloidal microspheres seem to be the most promising carriers in pulp healing and dentist regeneration.

Nanocarriers for cancer therapy and imaging applications are focused in the final Chapters 24 and 25. One of the major problems encountered in cancer treatment is that the cytotoxic agents are non-selective between normal and cancer cells. Thus, effective drug delivery systems are appearing as a result of more accurate targeting of pathological tissue, obtained by the use of new colloidal drug delivery systems (CDDGs). For the same reasons the ability to quickly visualized anatomical information or diseases tissues or organs by various imaging modalities, such as Scintigraphic Imaging, Magnetic Resonance Imaging, Computed Tomography, Positron Emission Tomography, etc. is today another important medical necessity. But non invasive imaging are generally conducted by the use of contrast agents necessary to help establish the appropriate signal intensity. Thus, the real success of medical imaging depends upon the selective and targeted delivery of these imaging agents, generally based on new colloidal nanocarriers.

In conclusion, these nanocarriers result useful not only for enhance the diagnosis by the use of different imaging techniques, but also for targeting the drug to the specific tumoral cells, diminishing the adverse effects, while maintaining and increasing the therapeutic efficacy in cancer patients.

This book reports and discusses the more innovative colloidal systems used up today to formulate, and produce drugs, cosmetics, food, and other varied range of natural and high tech products, opening new frontiers in the field of chemical delivery.

The well organised strategy and description of the different colloidal carriers reported also in all the enriched updated references, give a fully documented key of lecture of the fascinating topic *Colloids in Drug Delivery*.

For all the scientists and/or students involved in the Medical and the Chemical Community, as well as in the fields of Biochemistry and Physical Chemistry, this book must represent an indispensable key stone of their personal library, to better understand where colloid systems are going, and how they may be used to ameliorate the performance of drugs, cosmetic and food formulations.

P. Morganti
Editor-in-Chief
By advancements in technology and dissemination of information, the today consumers want to know what's included in the products they are buying and how they may affect the environment. Eternal youth and robust health have become an ongoing quest for the increasing aging population so that beauty and well-being from within is the new demand of consumers.

Hence, antiageing products have become a powerful driving force in the global cosme-nutri-ceuticals market, even in today’s economically depressed world. Senior citizens, as a proportion of the population is growing, and the desire to look younger is not only the preserve of old; youth and beauty are, in fact, inversely coveted. However the nutricosmetics’ promises of youth must be increasingly be backed by solid science.

This interesting book, divided in 7 parts and 25 chapters, reports the more innovative researches on nutrients for which there is evidence of benefits for the skin from within.

According to the 2006 Euromonitor International, the global nutricosmetics market was of about US$ 2.1 billions representing only 3% of the overall skin care market of about US$ 200 billions. But it expected growth ranges from 7% to 12% 3 times, more than the today global cosmetic market prevision of increase. According to Datamonitor 2009, the antiaging skin-care market is expected to grow by 20% at inflation-adjusted prices during the next 5 years.

The media obsession with health and youth ensure, in fact, that young and old (alike) are looking to prevent the ageing process, and “nutricosmetics embraces the idea that beauty can be enhanced through the consumption of functional dietary products that may support an healthier skin also”

The skin is, in fact, the largest and most extensive organ of our body, drawing the line between the end of the human organism and the beginning of the world outside. To perform its protective function the outer covering of the skin (the epidermis) depends of a variety of supporting cells and structures. It needs a) mechanical support provided by a framework of extracellular matrix; b) blood supply to bring nutrients and oxygen and to remove waste products and carbon dioxide, provided by a network of vessels; c) a defend network against infection provided by the immune system; d) a convey sensory network to send and deliver signals from and to the central nervous system by nerve fibers.

The epidermis is the fundamental component of the skin and its appendages, such as hair, fingernail, sebaceous and sweat glands, represent specializations of the epidermis.

In anyway, in direct contact with the environment, the skin undergoes ageing faster than other organs as consequence of environmental damages. Most of these damages are assumed to be the result of
oxidative stress mediated by the UV rays and leading to generation of reactive oxygen species (ROS) with consequent damages to collagen, and DNA mutagenesis.

The mechanism by with UV radiations interfere with collagen synthesis is not yet known. However, DNA mutations leads to a situation that can best be described as defective powerhouse, where inadequate energy production leads to chronic oxidative stress. In the dermis, as functional consequences of direct DNA damages and aberrant ROS production, could be an altered gene expression pattern at level of human fibroblasts affecting neovascularisation and collagen metabolism, and possibly the generation of an inflammatory infiltrate, as well as the oxidation of intracellular proteins, and inhibition of the proteosome.

The ability to resist to this aggressions is however the ultimate goal for an healthy skin.

All these topics are reported in Part 1 by the first 4 chapters.

To counteract oxidative injury of structural lipids, carbohydrates and proteins, human skin is equipped with a network of enzymatic and non-enzymatic antioxidant systems responsible for maintaining the right equilibrium between pro- and antioxidant compounds. Therefore, the use of cosmetics (beauty from the outside) associated with diet supplements (beauty from the inside) aimed to maintain the cells’ antioxidant power by the synergistic activity of this network of protective compounds.

With the evolution of the health concept, we are entering in the era of beauty outside in, so that the term wellness become synonymous with beauty, well-being and longevity. For these reasons, to use Cosmetic and Functional Food is a common place for consumers to support their Beauty and Wellness, participating the body from an environment every day more hostile to humans.

Staying healthy is thus the new message driven by cosmetics and diet supplements and innovative cosmetic and food products have not only to increase the general health of the body but also to stimulate the imagination and produce emotions by combining exciting images, sensual fragrances and tastes. Therefore, many cosmetics that strongly appeal to the senses often evoke delicious food, as well as the food may represent the most efficient emotional way to obtain a sensual dimension. This is the reason why our self-esteem and physical confidence are largely influenced by how we perceive our body and consequently the skin.

Research studies, concentrated on the skin activity of innovative cosme-nutri-ceuticals have shown, in fact, that immune and cutaneous systems are strictly connected to the nervous and endocrine systems, in such a way that these four-way communication appear vital for our global wellness. The necessity to look for and achieve this objective has led to the NICE concept, in which the Nervous, Immune, Cutaneous and Endocrine systems work all together activating the skin physiological cell signals both from inside and outside by the use of specific Cosmeceuticals and Nutraceuticals. Because of this skin-brain connection, the first mind-body skincare products are appearing on the market.

This fascinating topic is reported in Part II, chapter 5.

In order to be active in skin, cosmetic and dietary’ bioactive ingredients must be able to cross respectively the stratum corneum and the intestinal barrier, being available for metabolic processes or to be stored in the body.

Thus the extent of bio-availability depend on class and chemical structure of the bioactives used, consumer habits, dose solubility, and competition, or synergy between the different molecules present in the formulation. The administration of many active ingredients by the oral route offers several advantages over their topical application: the intestines absorb bioactives which are sometimes
compromised in topical application due to their low stability or slow skin penetration. Moreover, these molecules rich the entire skin of the body and are distributed and allowed their bio-efficacy to all the skin compartments such as, epidermis, dermis, hypodermis, blood vessels and sebum. However, the oral administration of diet supplements could be complementary to all the topical applications.

The human organism depends on an adequate energy supply provided by major dietary components, protein, carbohydrates and lipids. Thus, minor constituents such as vitamins, minerals and specific fatty acids are required in a healthy diet. Among them, the polyphenols, as carotenoids, tocopherols, and flavonoids are known to be best and more efficient antioxidant micronutrients. Moreover, Co Q10 plays a key role in ATP synthesis (viz biological energy production) and has an important antioxidant effect throughout the body in combination with hydrophobic tocopherols, vitamin E, and hydrophilic ascorbic acid, and vitamin C.

All these topics are focused on Part 3 and 4 by 8 chapters.

Aminoacids are chemical entities that have been recognized as the building blocks of life. They form strings of linked chains called peptides, polypeptides, and proteins, constituting muscles, tendons, organs, glands, skin, hair and nail. Growth, repair, and maintenance of all the cells are dependent on their continuous supply in the body, so that replenishment of aminoacids can help retain normal skin structure, enhancing its beauty and healthy condition.

The dermis consists, in fact, of the structural protein type-1 collagen, accounting for about 90% of the dry weight of the skin. While the dermis supports the epidermis and serves as a cushion that gives elasticity to skin, the epidermis is responsible for the barrier function. But to maintain an healthy skin, it is important to continuously maintain its cell turnover, eliminating old tissues and producing new one. But the cell division process requires a large amount of energy, and glutamine is frequently used. It appears that glutamine serves to help cell proliferation by participating in the process of donating amino groups, thereby having the effect of promoting also wound healing in the skin.

Both the cutaneous permeability and the antioxidant barrier, which are critical for life in a hostile environment, reside in the epidermis’ outer layers, the Stratum Corneum (SC). The SC cells, composed of corneocytes embedded in lipid lamellar membranes, represent the first antioxidant body defence. This barrier, has also the function to bind both lipids and proteins to hydrophilic substances, such as NMF (normal moisturizing factors) present in the SC, thus maintaining the skin hydration. Moreover any oxidative modification of the skin’ biomolecules may result in loss of structural and/or functional integrity of key components of the epidermis barrier.

In anyway, aminoacids play important roles in skin beautification. Proteins such as keratin and collagen act, in fact, as moisturizers together with the NMF, and also serve as protectors and building blocks of the skin.

The skin barrier regulates, in fact, skin hydration maintaining its water content and protecting itself from the environment’ aggressions. Anything that disorganizes this structure leads to skin damage, dysfunction and pathological diseases. This is why rehydration of the skin is the goal of all moisturizing formulations both from outside and inside, as well as the mean to maintain the integrity of skin barrier. Achieving the proper hydration, is therefore the key to maintain a general beauty and wellness. At this purpose, oral and topically applied chemicals and plant-derived compounds are used, being effective in protecting skin from the environment assaults.

Antioxidant compounds, as tocotrienols are reported, for example to be effective in protecting the
skin from UV damages, as well as glucosylceramides from rice, converted to ceramide, are used to hold moisture in the stratum corneum. As intercellular messengers in the sphingomyelin cycle, ceramides provide, in fact, not only the skin barrier functionality, but also regulate the lipid biosynthesis at level of SC lamellae.

All these topics are reported and discussed in Parts 5 and 6 by chapters 14 to 19.

A smooth, blemish-free healthy, and youthful skin tone is exactly what consumers want and wish to hold onto. The link between eating habits, health, and physical appearance is becoming increasingly evident and has made consumers all over the world open to concept of beauty from within. Thus oral supplementation with lactoferrin-whey protein seems to result in decrease in skin blemishes and redness in acne patients, as well as ingested probiotic bacteria seems to accelerate the recovery of cutaneous immune homeostasis after UV exposure, playing an important role in UV-induced skin damages.

Dietary soy consumption has been show to have beneficial effects on several aspects of human health, improving plasma lipid profiles and bone health and, reducing menopausal symptoms, enhancing cognitive function, and potentially reducing the risk of breast and prostate cancers. Soy, in fact, is rich and contains a variety of micronutrients such as calcium, iron, zinc, riboflavin and folate. Thus, in addition to the beneficial of soy and/or soy isoflavones for skin care, recent research has suggested its benefits effect for hair health as well. Finally, the dietetic use of green tea and silibinin as chemo-preventive agents against skin cancer and photoaging are worldwide known.

Therefore, nutriceuticals, are not only focusing on topical creams and other preparations that help the skin look younger and slow down the aging process, but also offer solutions for attacking free radicals and protect the immuno competent cells inside our body by providing simultaneous supplements of natural antioxidant and immunomodulant compounds.

The book ends with Parts 7 and 8 describing by other 6 chapters what the natural support for a healthier complexion and protection from photocarcinogenesis. To achieve an higher efficacy of Cosmetic Products and Beauty Food it should be necessary not only to use ingredients with a demonstrated biological activity at level of keratinocytes, fibroblasts, and/or other skin cells, but also to be capable to activate and modulate all the mediators produced from the nervous, immune, cutaneous and endocrine systems, constituting a unique net protecting the skin.

Moreover, adding cosme-nutri-ceuticals to the normal dermatological treatment programs can ameliorate the final aesthetic look, also improving the therapeutic result of the drugs, giving the psychological benefits of looking better while receiving medical care.

For all these reasons this interesting book may be useful to the daily work of Dermatologists, Plastic Surgeons, Cosmetic Chemists, Nutritionists and all Scientists from the Chemical, Biological and Medical Communities that wish to deeper know the significance of nutricosmetics for the today health.

P. Morganti
Editor-in-Chief
In copertina / Front cover

Architettura delle fibre di chitina, parte fondamentale dell’esoscheletro dei crostacei.
Foto al microscopio elettronico a scansione (SEM). Su gentile concessione del Prof. Dierk Raabe
Max-Planck-Institut fuer Eisenforschung, Max-Planck-Str. 1, 40237 Duesseldorf, Germany.

Architecture of chitin-protein fibers forming the honeycomb structure.
Scanning Electron Microscopy (SEM) micrographs. On kind permission of Prof. Dierk Raabe,
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