Amiporine

Balancing Skin Moisture

Alban Muller
INTERNATIONAL

Summary

A.M.I. Your Ecoresponsible Partner

Expert in beauty since 1978, Alban Muller International develops its products in an eco-responsible approach: from the seed to the final product, each step is monitored, and everything is done to limit the impact on Man and the Environment

The Key Points of our **Ecoresponsible** Approach

Quality and Safety of our Supply

- Monitoring the origin of our plants, mostly cultivated close to our factory
- Monitoring our cultivating techniques in line with the *Guideline on Good Agricultural and Collection Practices* (GACP) *for Starting Materials of Herbal Origin* issued in 2006 by EMA *(European Medicines Agency)*
- Monitoring our plants after harvesting (identification, content in active principles and contaminants)

Monitoring our Impact on the Environment

- An ISO14001-certified manufacturing site
- Selecting renewable and biodegradable raw material
- Optimised, eco-clean manufacturing processes
- Reducing our water consumption
- Sustainable waste-management (recycling)
- Treating effluents through our Filtering Gardens
- Monitoring the carbon balance of our manufacturing site

"Ongoing Innovation in the Respect of Man and the Environment"

Partners who Share our Values

- The Herboretum Association
  www.herboretum.org
- The Cosmetic Valley
  www.cosmetic-valley.com

Water, the life origin

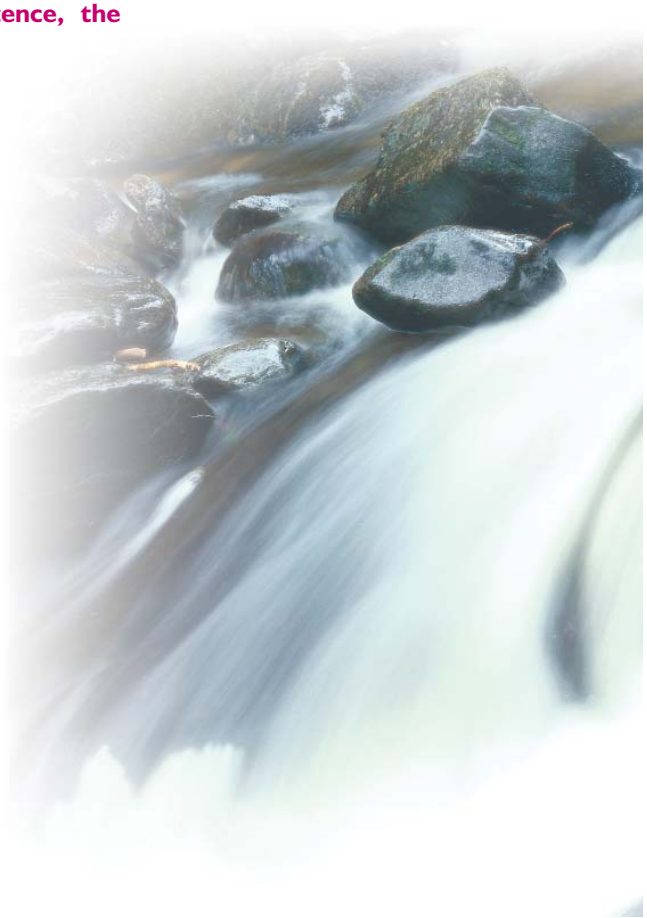
The life has first grown in the ocean. Then, the watery species left their medium little by little to conquer the terrestrial part of our Earth. By doing this, they set up completely interesting adaptive strategies in order to preserve their hydrous capital and to survive in their new environment.

In the animal world, the periophtalm of the mangroves is an illustration of this passage of the watery life to the terrestrial life: this small easily recognizable jumping fish with large protruding eyes is perfectly adapted to these 2 environments. It eats insects like the mosquitoes essentially and thus passes the majority of its existence out of water.

The periophtalm of the mangroves

In the vegetable world, one finds particular properties related to the adaptation of the species. One thus discovers aerial roots in the ficus, and of the fruit trees, like pomegranate tree, amazing properties to bloom in completely unexpected wet places!

Each reign, each specie, thus followed a progressive evolution enabling to preserve the source of its existence, the water.



Amiporine®, the youth strategy by A.M.I.

Our skin needs a sufficient water uptake, first to reconstitute the water pool in the dermis (to avoid dehydration and following decreasing skin elasticity and wrinkles appearance), and also to renew the hydrolipidic film that is essential for the epidermis barrier function.

The barrier function is vital since it allows the organism protection against potential dangerous external agents (pollution, temperature variation, UV, etc.). The epidermis also takes part in general homeostasis regulation by limiting water loss across the skin.

Nevertheless water applied on the skin moisturises or re-moisturises under two main conditions:

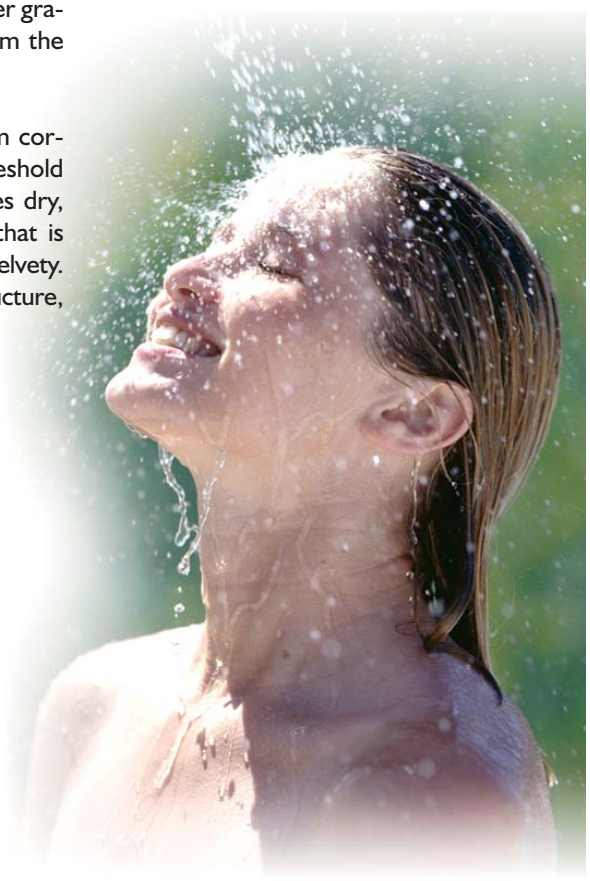
- the water has to be fixed by lipids that prevent evaporation;
- the water has to circulate in the whole skin tissue

In a normal and young skin, the water content of the epidermis is about 10 to 15% in the stratum corneum and about 60 to 70% in the deeper levels of the skin. There is a water gradient maintained by the water flow from the more hydrated cells to the less ones.

When the water content of the stratum corneum decreases under the critical threshold of 10%, the epidermis surface becomes dry, tends to crack and lose the elasticity that is essential for a normal skin softness and velvety. Indeed, the more dry is the keratine structure, the less supple it is.

As a matter of fact, a drying up of the skin is noticed when it is getting old: the skin loses its sooth aspect, it is wizened and furrowed with wrinkles.

To bring an answer to this phenomenon, Alban Muller International (A.M.I.) has created Amiporine®, the first restructuring active that restores water circulation in the skin, and thus prevents ageing.



Nature to serve Beauty

The capacity developed by some plants to preserve their water capital is due to specialised proteins: aquaporins which are the cells "water channels".

A.M.I. is true to its principles and by looking at Nature was inspired by the mechanisms developed by terrestrial organisms to take and keep their environmental water.

Pomegranates

Indeed, maintaining the water capital is a main constrain for the growth and development of terrestrial plants. Particularly, they might be submitted to hard environmental conditions such as cold, dryness or soil salinity, which are dangerous for their essential balances.

The fundamental role played by cell membranes in the plants water capital preservation was highlighted when aquaporins have been discovered. These proteins are water channels vital for some plants functioning.

As a response to environmental changes, aquaporins expression, reveals an extremely performing adaptive strategy developed by species.

Pomegranate tree is bearing pomegranate and perfectly illustrates this adaptive phenomenon.

This tree preferentially grows in southern countries and on the opposite of most of fruit species, humidity excess favours its totally fulfilling.

Nevertheless, Pomgranate tree can also survive in the cold countries if the temperature does not fall -13°C below!

Aquaporins: a source of youth

Aquaporins are proteins with 6 domains, anchored in the membrane forming a specific communication channel

Aquaporins were discovered for the first time by electronic microcopy in 1974. They were really characterised in 1991 by Peter AGRE, who received the Nobel Prize in Chemistry in 2003 for this work.

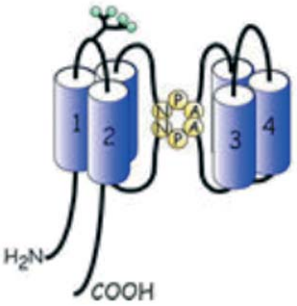
Up to date, 200 aquaporins were listed in the animal kingdom of which 10 in the human organism. These molecules are generally named AQP followed by a number corresponding to their discovery time. The aquaporin 3 (AQP3) is the only one known to be expressed in the epidermis.

Aquaporins are inter-membrane molecules forming, specialised tunnels for water flow in the cell wall.

In the human body, water flow in the skin is ensured by a specific aquaporin known as aquaporin 3 (AQP3). This protein is expressed by epidermis cells and is essential in the general skin metabolism. It is actually a glycero-aquaporin, that is to say this protein is permeable both to water and glycerol (or glycerine) which play an important role in the hydro-lipid barrier formation.

Aquaporins allow to preserve a water balance in the skin even in unfavourable conditions (opposite osmotic pressure): they facilitate water and small solutions (glycerol for example) diffusion across the membranes.

When aquaporins metabolism is altered, there is a water gradient modification leading to a bad water circulation. The skin is then deprived of its water capital and loses its suppleness and young aspect.



Scheme of an aquaporin

Aquaporins quaternary organisation: four channels optimise water circulation from one compartment to another (illlustration from the work of R.J. LAW)

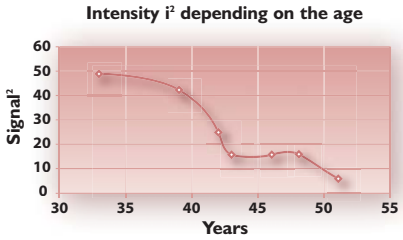
Ageing and water flow: the role of aquaporins

Skin ageing is associated to a decreasing aquaporins expression

1 - The experiments are obvious

The A.M.I. research team has performed a study on aquaporins 3 expression depending on the age. The results demonstrate the dramatic decreasing of their presence in the skin when subjects are 40 to 45 years old.

The aquaporins expression decreases with age. When these "water channels" do not work anymore, the whole skin metabolism is disturbed.



Aquaporins 3 (AQP3) expression depending on the age

2 - *Ex vivo* study of the aquaporin 3 expression in old and young skins

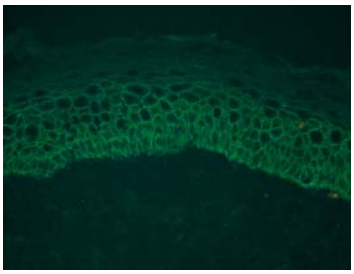
Analytical method: indirect immunofluorescence

Skin explants were obtained by surgery on 3 and 53 years old subjets. They were sectioned using a cryostat and fixed on glass slide.

The aquaporins expression is highlighted using a specific antibody for human aquaporin 3. This antibody is revealed using a second one coupled with fluoresceine.

The experiment reveals the difference of aquaporins expression between a young (1) and an old (2) skin.

Obviously, by maintaining its water capital aquaporins play an essential role to preserve the young and tonic aspect of the skin.



(1) Young skin organisation
The stratum corneum is steady and thick, keratinocytes of the epidermis widely show aquaporin 3. This presence is gradual and more intense in the deep levels than in the external level of the epidermis.

(2) Old skin organisation
The skin organisation appears widely modified with age: the stratum corneum is thin and the aquaporin 3 is weakly expressed by keratinocytes. The staining noticed in the dermis matrix is a non-specific staining.

Anti-ageing moisturising: the Amiporine® revelation

Wiser for these results on aquaporins, A.M.I. has developed a purified pomegranate extract able to rehabilitate the natural water flow by stimulating the skin specific aquaporins (AQP3).

By restoring water balance, Amiporine® brings a renewed youth to skin: the epidermis is perfectly moisturised and the stratum corneum regains its suppleness and brightness.

From pomgranate to Amiporine®: the A.M.I. experience at the service of beauty and youth

1 - Effect of Amiporine® increasing doses on aquaporins expression by keratinocytes

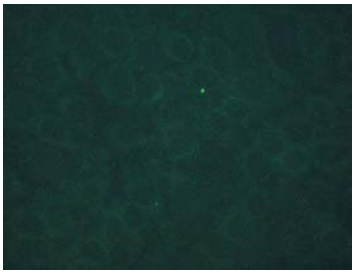
Analytical method

Keratinocytes used for these experiments are from HaCat cell line. One night after being cultured, the media is exchanged with a new one containing Amiporine®.

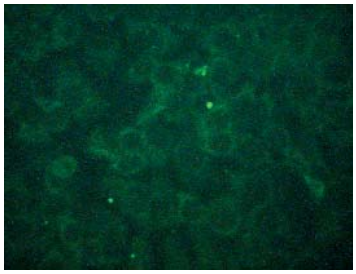
After a 72-hour incubation, cultures are rinsed and fixed for immunostaining.

This experiment highlights the Amiporine® effect: aquaporins expression increases when the active concentration increases.

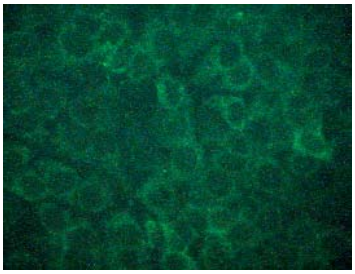
Control



Amiporine® 5 mg/ml



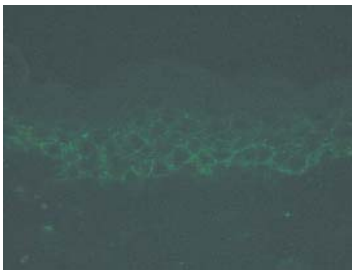
Amiporine® 10 mg/ml



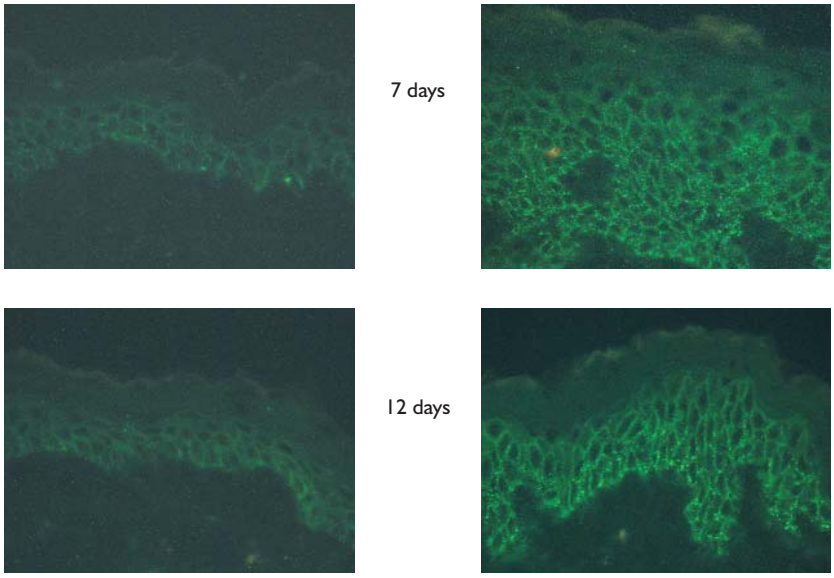
Amiporine® 15 mg/ml

Amiporine® stimulates the epidermis water channels

2 - Aquaporins expression observed on skin cells cultures: effect of a cream containing Amiporine®



Control



Aquaporins expression is widely stimulated in skin cells treated with the cream containing Amiporine®. An increasing thickness of the epidermis is observed: the skin is more tonic and renews easier.

3 - Consequences of Amiporine® activity on skin metabolism: communication between epidermis cells is optimal

Re-establishing water flow in the epidermis allows the skin to restore its suppleness and beauty. This unequalled effect of Amiporine® is the result of a general improvement in cells metabolism. As an example, the communication between keratinocytes was studied.

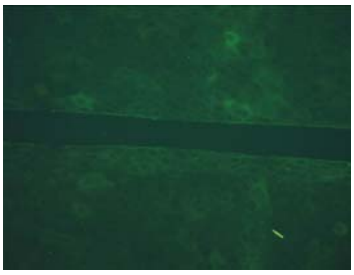
Analytical method

The following test was performed using a specific fluorescent tracer: Lucifer Yellow, that penetrates in cells if they have first been artificially cut.

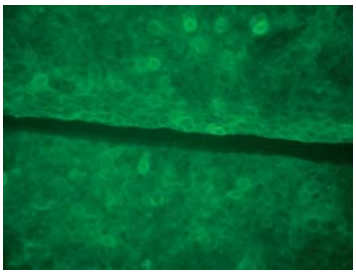
When entered in a cell, Lucifer Yellow can diffuse in neighbouring cells using gap junctions. These are involved in the skin tissue organisation and coherence since they favour cells communication.

The following pictures were taken using a fluorescent microscope. In this manner, the effect of Amiporine® on cell communication via gap junction was visualised.

By controlling water flow, Amiporine® favours cell communication: Amiporine® is an essential way to fight against ageing.



Control keratinocytes



Keratinocytes treated with Amiporine®

4 - Amiporine® effect on collagen I and III and matrix collgenase synthesis by human dermal fibroblasts

Amiporine® offers a natural face-lift effect to mature skin and maintains a optimal moisturising in younger skin.

Analytical method

In order to demonstrate the Amiporine® activity on human dermal fibroblasts via the epidermis, keratinocytes were previously incubated with different doses of the active. The medium obtained was then used for fibroblasts culture.

This experiment demonstrates that Amiporine® also acts on fibroblasts via the epidermis cells. The active stimulates collagen I and III synthesis and decreases collagenase synthesis. On the opposite of collagen that build the dermis matrix, collagenase damages it.

- **Keratinocytes treatment**

  Human keratinocytes were cultured and incubated overnight with a standard culture medium. Supernatants were then eliminated and replaced by a new medium (control) or medium containing Amiporine®.

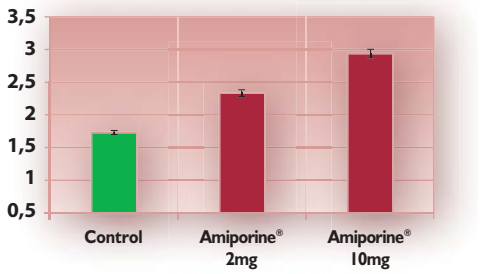
  After a 6-hour incubation, supernatants were eliminated, cells were rinced and again incubated with a new medium during 24 hours. After this period, supernatants were centrifuged and filtered for fibroblasts culture.

- **Fibroblasts treatment**

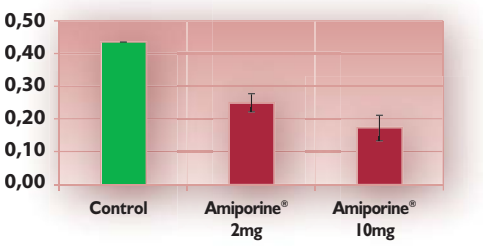
  Fibroblasts were cultured with keratinocytes supernatants.

  After a 48 hours incubation, supernatants were filtered and freeze at -20°C. The measurement of collagenase using ELISA dosage was then performed.

  The measurement of collagen I and III was performed by using a semi-quantitative method of fluorescent immuno-labelling : after the labelling of collagens I and III, cells were lysed and fluorescent supernatants were then quantified with a spectrofluorimeter (Fluoroscan).



Collagen I and III synthesis by fibroblasts cultured in a keratinocytes conditioned medium



Collagenase synthesis by fibroblasts cultured in a keratinocytes conditioned medium

Amiporine® increases the collagen production and decreases the collagenase production, leading to a limitative effect of skin ageing.

5 - Hormonal variation and aquaporins

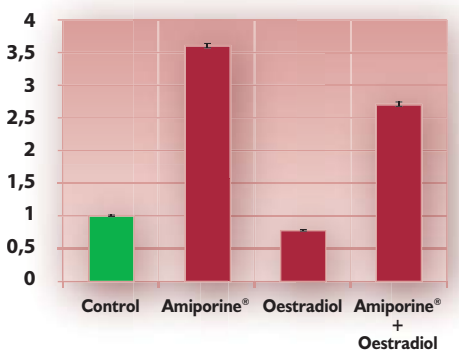
By protecting cells from the variations caused by frequent hormonal modifications, Amiporine protects the skin water capital and improves its metabolism: the skin is flooded with youth.

All their life long, women are subjected to several hormonal variations, particularly during menstrual cycle and on pregnancy.

These changes widely alter cells behaviour and can lead to a dysfunction affecting the general aspect of the skin.

Our research team performed a test which demonstrate that an increase in a sporadic hormonal concentration such as the estradiol, can alter aquaporins expression. This experiment was realised thanks to a flow spectrofluorimeter: this high-performance device allows to label the cells with a specific fluorescent antibody and to analyse cells precisely, one by one.

The results obtained prove that Amiporine® is able to reverse the estradiol effects on aquaporins expression and even to stimulate it despite the hormone presence.



Amiporine® limits the effects of sporadic and intense modifications in hormonal concentration on aquaporins expression by skin cells.

The added value of glycerine as a cosmetic active solvent

The glycerine gives its moisturising action to the active

The Amiporine® solvent is glycerine which is a polysaccharide perfectly tolerated by the skin and which contributes to its preservation and firmness.

Indeed, disposable scientific bibliography demonstrates that glycerine favours the epidermis barrier healing and increases the stratum corneum hydration.

These effects are partly caused by the aquaporin 3, one of the few proteins permeable both to water and other solutions such as glycerine or urea.

In this way, Amiporine® favours aquaporins activity in the skin, helps to reinforce the epidermis, and protect it against external aggressions.

Conclusion

Entirely natural, without preservative and stabilised in glycerine, Amiporine® is the first moisturising and restructuring active recommended in all cosmetic products.

Shaking up traditional ideas on skin moisturising, A.M.I. revolutionises the cosmetic skin care world by stimulating the skin metabolism with the more essential life element: water.

Amiporine® acts on the biological and chemical foundations, by favouring water flow in the skin.

Perfectly moisturised and boosted, the skin reveals its brightness and shows a young, sooth and restful aspect.

Safety

Alban Muller International has signed the UNITIS chart and therefore guaranties the Amiporine safety thank to the following toxicological tests:

Local toxicity

in accordance with the methods described in the Official Journal of French Republic dated 30th December 1999.

- Primary skin irritation (24 hours occlusive patch test)
- Ocular irritation (diffusion on agarose gel)

Allergenicity

- Sensitisation test according to the RIPT method (Baran et Maibach, 1994) on 50 volunteers

Systemic Toxicity

- Mutagenesis: Ames test
- Acute Toxicity: cytotoxicity of *Punica granatum* extract

Cosmetic uses

Technical information

Solubility ..... water soluble

Usage level ..... 2 to 4 %

Degradability ..... biodegradable

Formulation

Amiporine® can readily be incorporated in an oil-in-water emulsion or pre-diluted in water before being added in a cream or a gel.

Amiporine® should be cold-incorporated at the end of the formulation process (temperature between 30 and 40°C).

Compatibility

Amiporine® has a good compatibility with:
- most of preservatives;
- all kinds of emulsifiers;
- all kinds of perfumes.

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