

ANTI-CELLULITE & STRETCH MARKS CLINICAL STUDIES

ACTIPORINE – Cellulite Innovation

CELLULITE: IMPORTANCE OF MITOCHONDRIAL HOMEOSTASIS - A UNIQUE ACTION MECHANISM

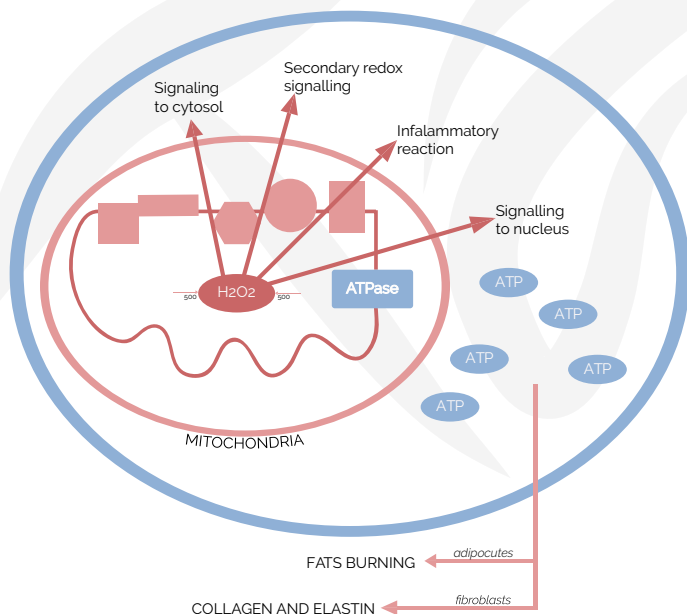
By maintaining mitochondrial homeostasis in adipocytes and fibroblasts, Actiporine 8G promotes not only the elimination of fats but also the synthesis of collagen, to smooth out dimples. Its mechanism of action is unique. It targets mitochondrial Aquaporin 8 which is responsible for transporting H₂O₂ from the inside of mitochondria to the outside. By freeing mitochondria of this toxic substance, Actiporine 8G maintains their physical and functional integrity and restarts the original cellular activity of adipocytes, i.e. lipolysis as well as collagen synthesis by the fibroblasts. Dimples are therefore reduced and smoothed out.

ROLE OF MITOCHONDRIA IN ANTI-CELLULITE STRATEGIES

Cellulite is mainly due to 2 phenomena:

- increase in fats storage into adipocytes and
- distortion of the collagen fibres which surround the adipocytes.

Smoothing out cellulite consists in deflating the adipocytes by activating lipolysis and firming the underlying tissues by stimulating collagen synthesis.



These two processes are connected to the integrity, vitality and thus energy reserves of the adipocytes and fibroblasts. However the only source of energy available to the cells is ATP, which is synthesized by the mitochondria.

IMPORTANCE OF MITOCHONDRIAL HOMEOSTASIS IN ANTI-CELLULITE STRATEGIES

It is now well known that mitochondrial DNA, due to its closeness to reactive oxygen species, and mainly H₂O₂, is continually subject to damages and mutations leading to mitochondrial malfunction and loss of cellular vitality. However H₂O₂ is a messenger of vital importance in a large number of metabolic reactions.

Therefore it is important to remove H₂O₂ from the mitochondria without, however, completely eliminating it.

It is necessary to ensure a balance (or mitochondrial homeostasis), in the fibroblasts and adipocytes so that they function correctly.

THE ROLE OF AQUAPORINE 8 IN MITOCHONDRIAL HOMEOSTASIS

Aquaporins belong to a large family of cellular channels with members in all kingdoms of life, and known as efficient water channels. They can be found in the cellular membrane, mitochondrial membrane or cytosol.

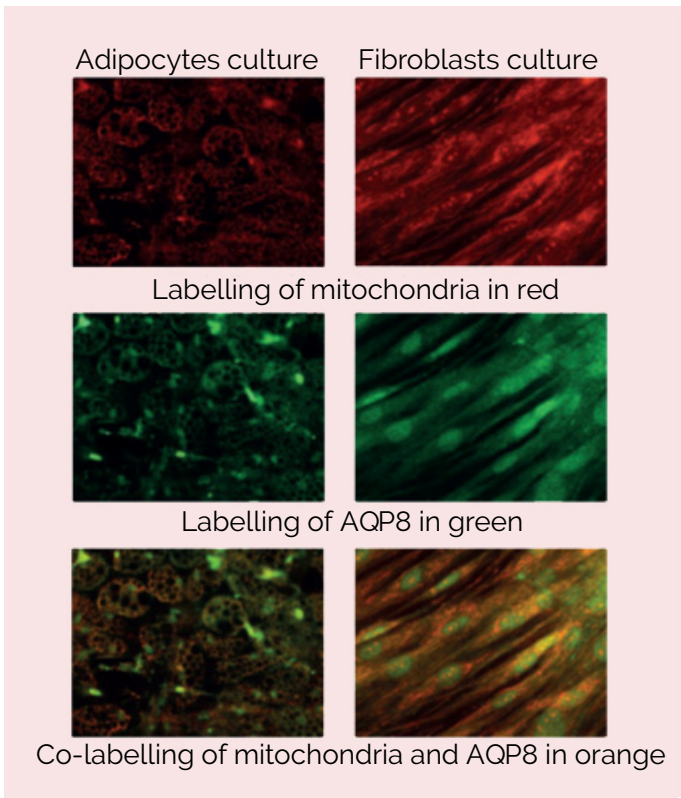
Aquaporine 8 (AQP8) has been discovered in keratinocytes in 2008 by Codif International. It is now well known in cosmetics for its hydration function. Recently, Biernet and al discovered a new function of AQP8 in transporting H₂O₂ from matrix mitochondria to the cytosol.

Carrying on this work, Codif International then localised AQP8 in adipocyte and fibroblast mitochondria and made the connection between AQP8 and mitochondrial homeostasis.

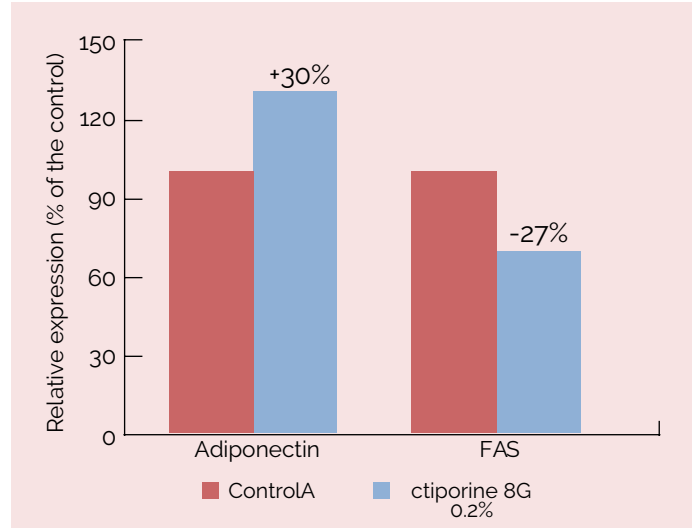
DEMONSTRATION: EVIDENCE OF AQP8 SYNTHESIS IN MITOCHONDRIA OF HUMAN ADIPOCYTES AND HUMAN FIBROBLASTS

Results: The colabelling of mitochondria and AQP8 put in evidence the synthesis of AQP8 into mitochondria of human adipocytes and human fibroblasts.

The stimulation of AQP8 synthesis should be translated by a strengthening of mitochondrial homeostasis, and therefore a reactivation of lipolysis by adipocytes as well as a reactivation of collagen synthesis by fibroblasts.



Anti-cellulite preventive effect

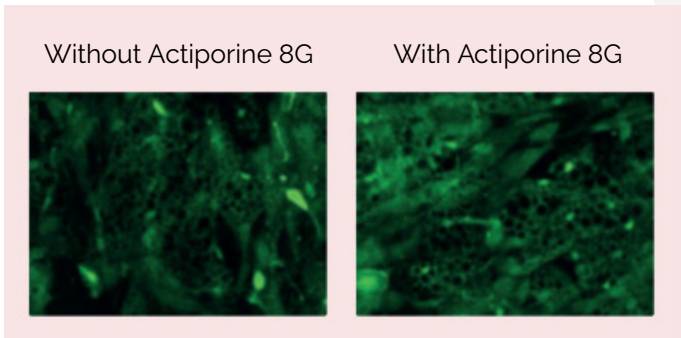


Actiporine controls fats storage and the expansion of the adipose tissue

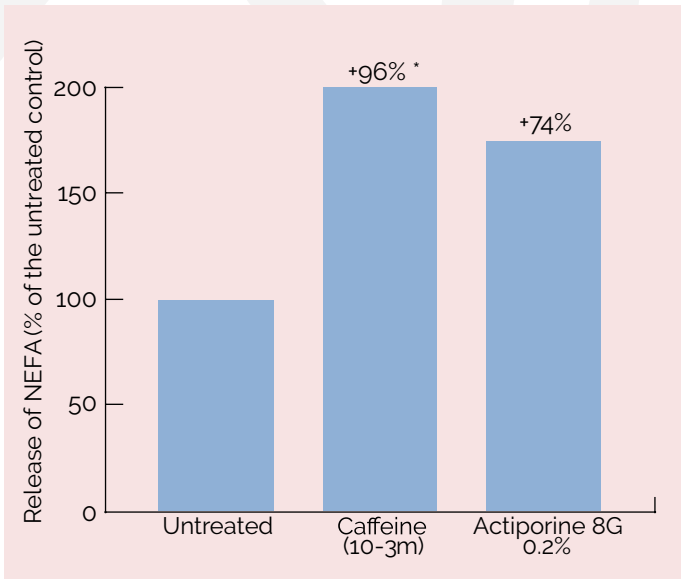
In addition to its lipolytic and firming effect, Actiporine 8G stimulates adiponectin synthesis by 30% (adipokin which controls the growth of adipose tissue), and inhibits fatty acid synthase (FAS) expression, the enzyme involved in fatty acid synthesis, by 27%.

IN VITRO TESTS: EFFECT OF ACTIPORINE ON ADIPOCYTES

Anti-cellulite healing effect

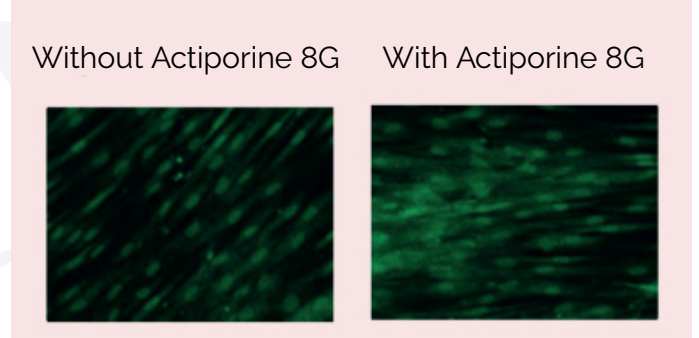


Results: Actiporine stimulates the synthesis of AQP8 into adipocytes by +50%

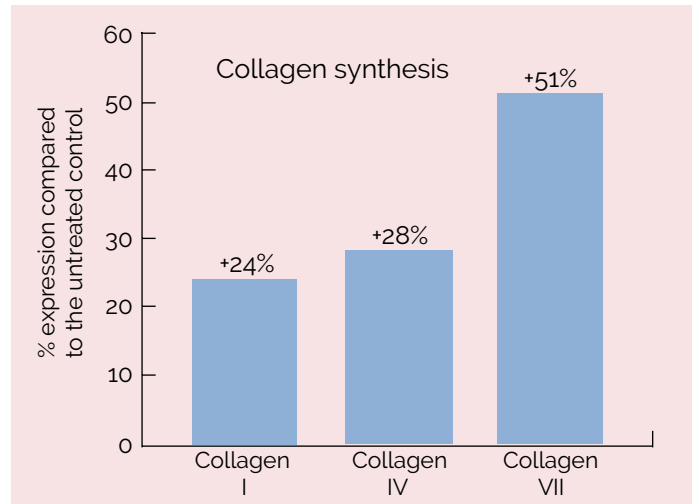


Results: Actiporine stimulates lipolysis by +74% into differentiated adipocytes

IN VITRO TESTS: EFFECT OF ACTIPORINE 8G ON FIBROBLASTS



Results: Actiporine stimulates AQP8 synthesis into fibroblasts by +58%



Results: Actiporine stimulates the synthesis of:

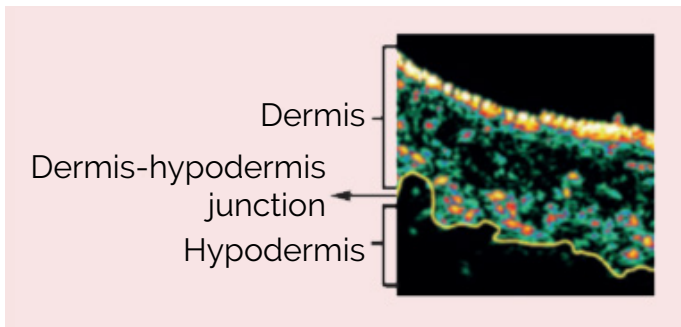
- Collagen I: +24%
- Collagen IV: +28%
- Collagen VII: +51%

By stimulating the synthesis of AQP8 into adipocytes, Actiporine 8G maintains mitochondrial homeostasis and reactivates lipolysis by adipocytes. This action allows the decrease of adipocytes volume. Meanwhile, the stimulation of adiponectine synthesis as well as the inhibition of fatty acids synthase lead to the control of adipose tissue expansion and avoid the reappearance of capitons.

By stimulating AQP8 synthesis by fibroblasts, Actiporine 8G maintains mitochondrial homeostasis and reactivates the synthesis of collagen I, IV and VII. This action firms dermis and smoothes out cellulite.

REVEAL – CLINICAL TESTS

Echographic analysis of the length of the dermis-hypodermis junction and fats inclusions into the dermis



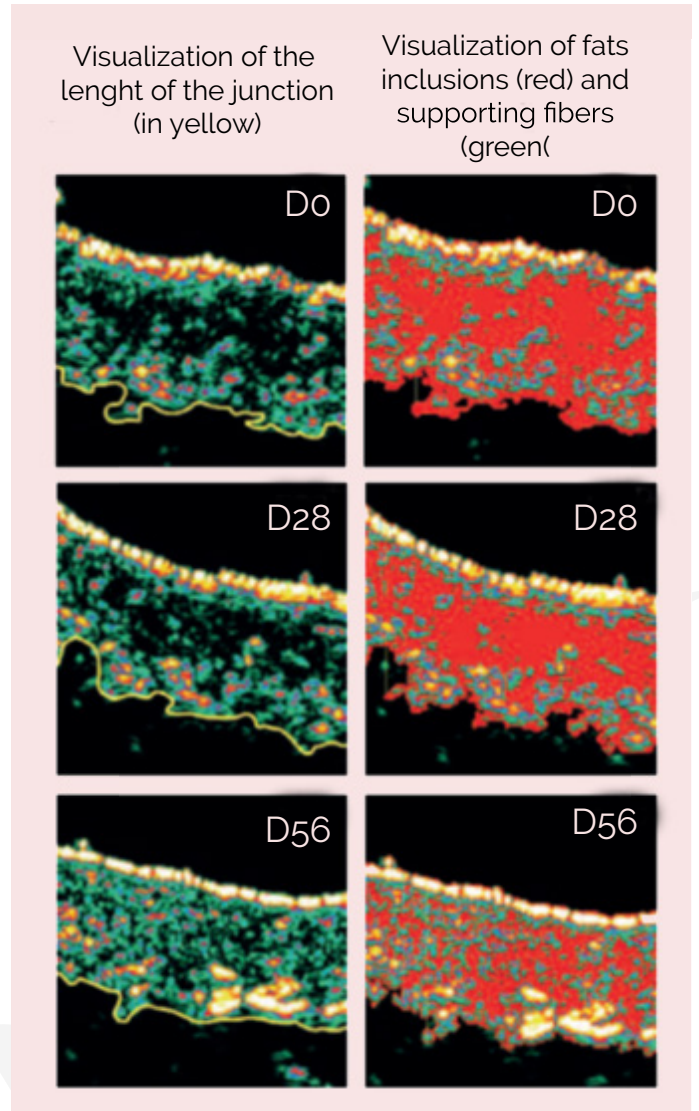
When they grow, adipocytes distort and enlarge the dermis-hypodermis junction and invade the dermis by forming fatty inclusions. Therefore an effective lipolytic and firming action must be achieved by reducing the length of the junction as well as by reducing the number of fatty inclusions in the dermis, promoting instead an increase in the support fibres.

Results after 28 days of treatment:

- Length of the junction: -11% and up to -46%
- Fats inclusion: -3% and up to -24%

Results after 56 days of treatment:

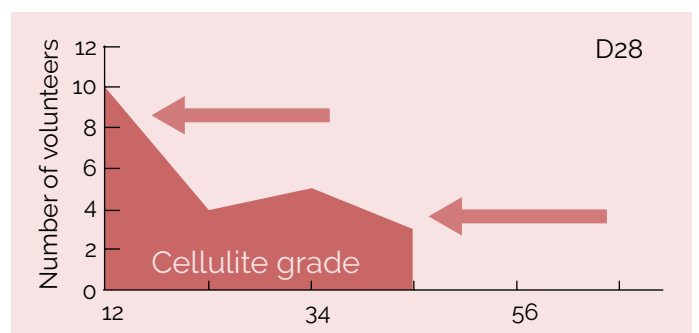
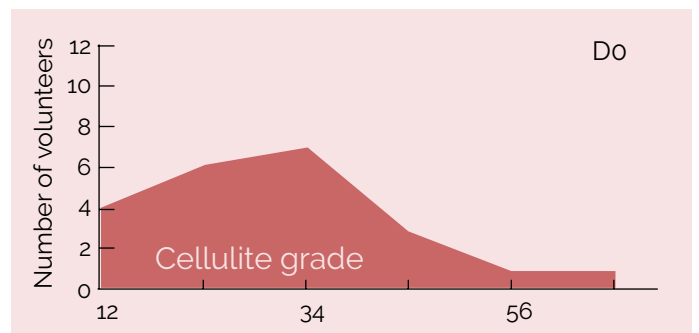
- Length of the junction: -18% and up to -64%
- Fats inclusion: -7% and up to -25%

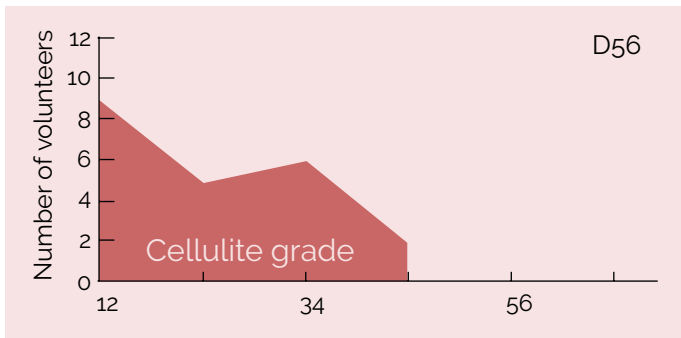


ANALYSIS OF CELLULITE GRADE

The impact on orange peel effect has been evaluated thanks to a visual scoring of cellulite on not pinched thighs, using 10 points scale.

Variation of cellulite grade during the treatment





Due to its unique action mechanism on AQP8, Actiporine 8G restores mitochondrial homeostasis in adipocytes and fibroblasts and reactivates lipolysis and collagen synthesis. Fatty inclusions are eliminated, the dermis regains its firmness and dimples are visibly smoothed out.

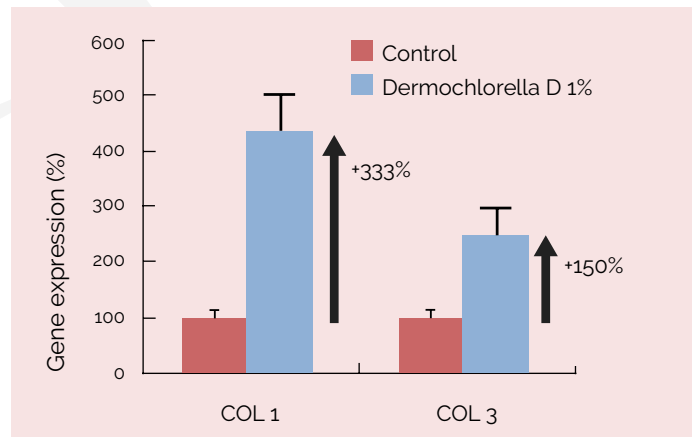
DERMOCHLORELLA – Skin restructuring

From the age of 20, our cells start to produce less collagen, elastin, sebum and then the skin dries out, becomes thinner and loses its elasticity. Wrinkles and stretch marks are the result of this lack of skin elasticity. Stretch marks appear when the elastic fibres of the dermis tear. Frequently they are located on the thighs, hips, breasts and buttocks due to a rapid weight gain or loss, pregnancy, puberty... Dermochlorella is an extract of a green microalgae *Chlorella vulgaris*, rich in peptides and amino acids, acts on all these elements of the dermal structure.

The space surrounding the cells contains macromolecules, polysaccharides or glycosaminoglycans, fibrous protein, salts and water which as a whole are designated as the extracellular matrix, responsible for tissue cohesion. The main structure proteins are collagen and elastin. The extracellular matrix components are synthesized and secreted by cells such as the fibroblasts and degraded by enzymes called MMP (Matrix MetalloProteinase) whose activity is inhibited by endogenous antagonists called TIMP (Tissue Inhibitor of Metalloproteinase).

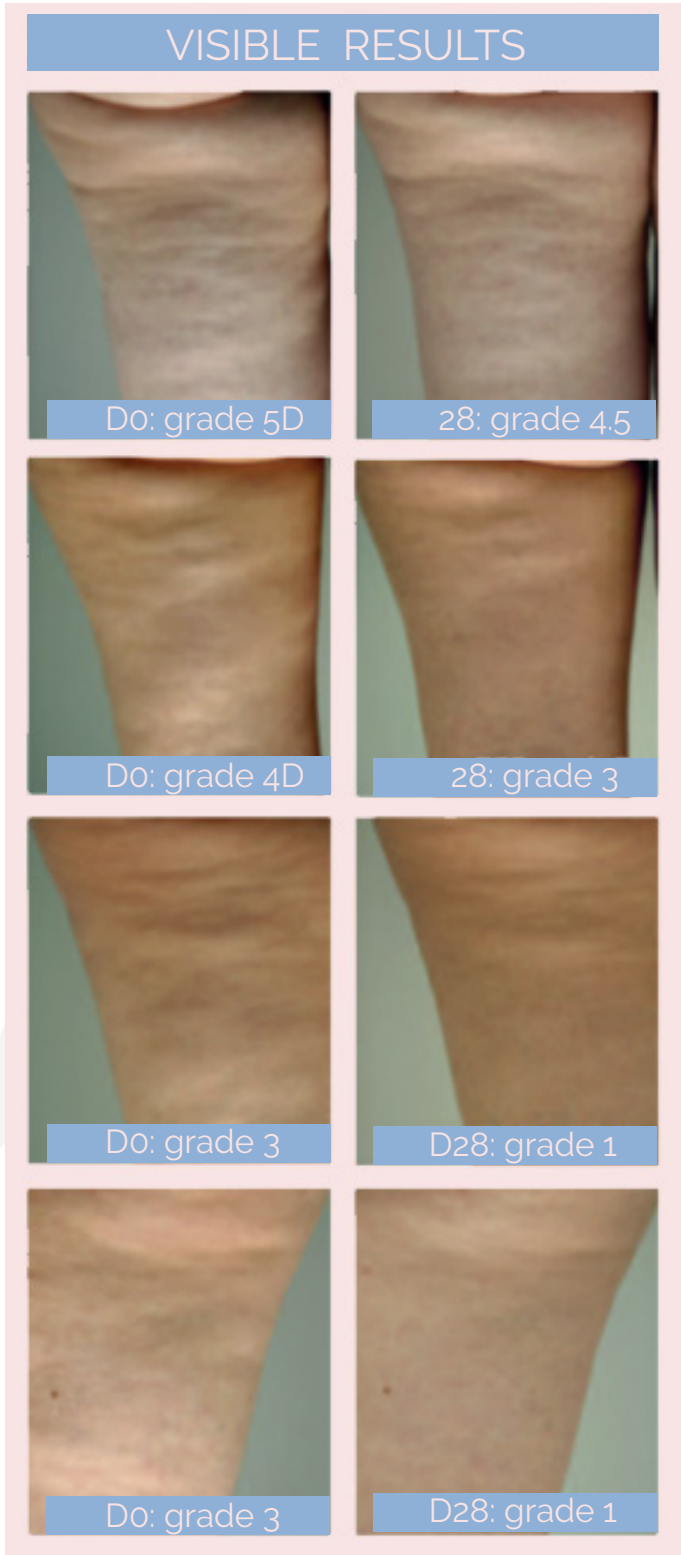
IN VITRO TEST: EFFECT ON COLLAGENS 1 AND 3

Collagen is the main fibre protein of the body which gives tissues their elasticity. Its role may be compared to that of a frame. It is composed of different types depending on their location and it is essential for the healing process.



Results: Dermochlorella increases collagen in fibroblasts:

- Collagen I by +333%
- Collagen III by +150%

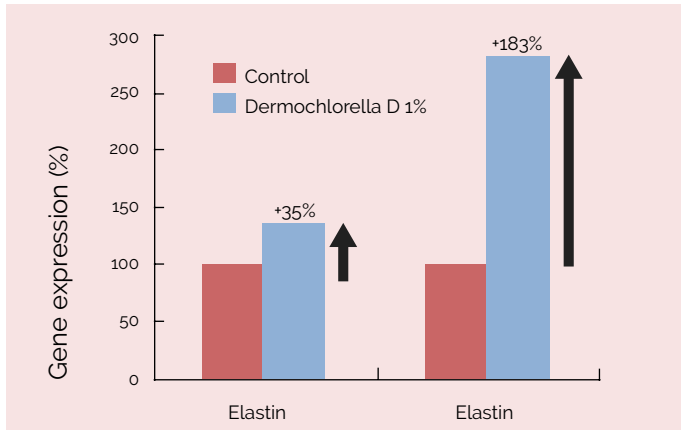


- Results after 28 days: Results after 56 days:
- -10% and up to -67%
 - -15% and up to -75%

Volunteers distribution with regards to their cellulite grade during the treatment

IN VITRO TEST: EFFECT ON ELASTIN AND ELAFIN

Elastin is a glycoprotein secreted by dermis cells which has elastic properties. Its synthesis decreases with age resulting in the appearance of stretch marks under the action of mechanical constraints. Elafin is a specific inhibitor of elastase, an enzyme responsible for elastin fibre degradation.

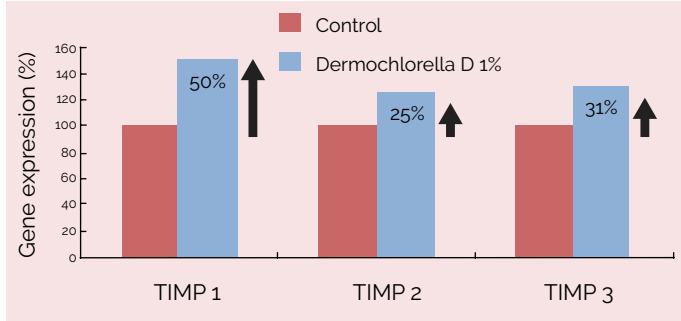


Results: Dermochlorella increases:

- Elastin by +35% in fibroblasts
- Elafin by +183 in keratinocytes

IN VITRO TEST: EFFECTS ON TIMPS EXPRESSION

TIMPs are capable of inhibiting all MMPs. Therefore, they play a key role in maintaining a balance between extracellular matrix formation and degradation in various physiological processes.



Results: Dermochlorella increases TIMPs in dermal cells:

- TIMP-1: +50%
- TIMP-2: +25%
- TIMP-3: +31%

THE DERMIS-EPIDERMIS JUNCTION: ATTACHMENT POINT BETWEEN THE EPIDERMIS AND THE DERMIS

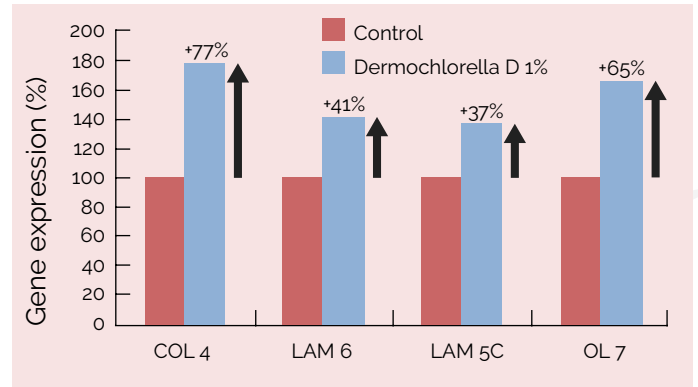
The skin contains the Dermis-Epidermis Junction (DEJ) or basal membrane which ensures an optimum cohesion between the dermis and the epidermis. Initially it is sinusoid in shape, and becomes flatter with age: the dermis is less well attached to the epidermis. It contains specific molecular components:

- collagen glycoproteins, the main components in the extracellular matrix: type IV collagen provides the mechanical stability of the basal membrane and type VII collagen is the major component of anchoring fibrils

- several types of laminins. They represent the major non-collagenic components. The isomers present are laminin 5 and 6. They have a major structural role with the formation of a network to which other collagenic or noncollagenic proteins bind

The degradation of the DEJ results in a reduction in the exchange surface between the epidermis and the dermis which can even result in the detachment of the epidermis with rubbing.

IN VITRO TEST: EFFECTS ON TIMPS EXPRESSION



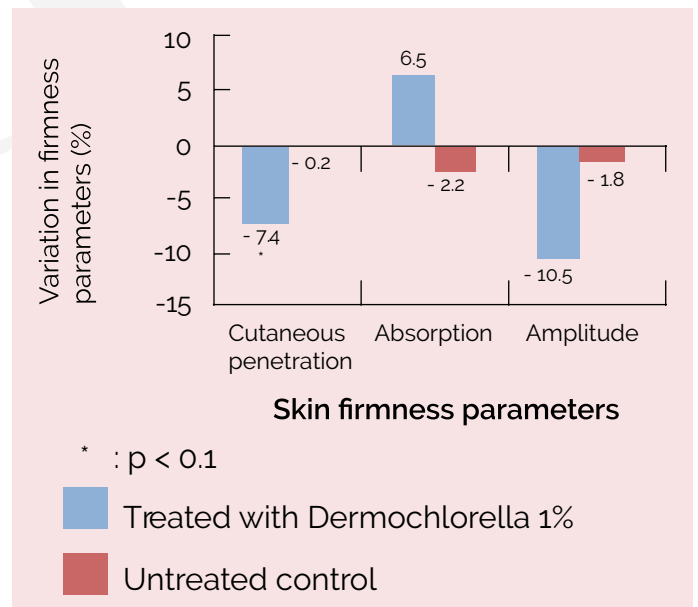
Results: In fibroblasts Dermochlorella increases:

- Collagen IV by +77%
- Laminin 6 by +41%

Results: In keratinocytes Dermochlorella increases:

- Laminin 5 by +37%
- Collagen VII by +65%

CLINICAL TEST: EFFECT ON SKIN FIRMNESS AND TONE



Results after 84 days of use:

- Cutaneous penetration: -7.4% and up to -26%

A decrease in this parameter indicates that the bead creates only a slight depression on the skin => the skin is firmer

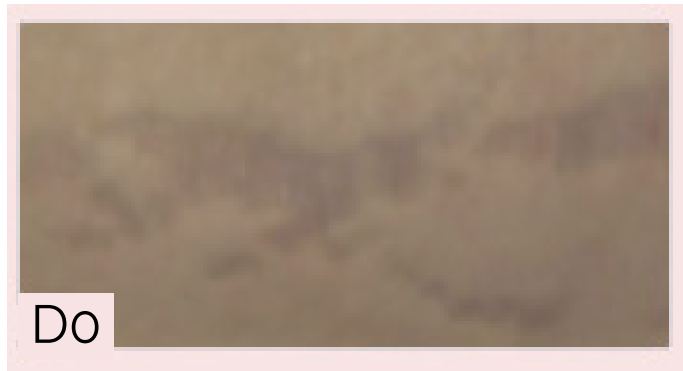
- Absorption of bounces by skin: +6.5% and up to +66%

An increase in this parameter indicates that the bounces stop sooner => the skin tone increased

- Amplitude of bounce: -10.5% and up to -46%

A decrease in this parameter indicates that the bead bounces less intensely => the firmness and tone of the skin increased.

CLINICAL TEST: ANTI-STRETCH MARKS EFFECT



Results after 84 days of use:

- Significantly decreases the color of stretch marks: -10.4% and up to -32%
- Decreases morphology of stretch marks: -2.9% and up to -7.9%

Dermochlorella is a restructuring active ingredient that allows the skin to find its tone and firmness again.

References:

1. Codif International products technical files
2. Biernert, G. P., and Moller, A. L. (2007) J. Biol. Chem 282, 1183-1191