Sustained and selective controlled release natural Nanosystem

NANO LPD ´S MULTIVITAMIN

Cod. HD0006

INTRODUCTION

Skin care has transitioned from a general dictum of cleansing, moisturizing, and sun protection to a science and art of combining new ingredients to provide the patient with the most advanced aspects of skin rejuvenation. There are new products coming to the market on a frequent basis, and sorting through their claims can be frustating and challenging for the consumer and physician alike. In addition, increasing scientific work has been conducted on older products to help elucidate their mechanisms of action and to confirm their effectiveness.

Wrinkles now have a greater social impact because people live longer. Science and hedonism overlap in the search for causes, treatments and prevention of wrinkles. The cosmetic approach to wrinkles includes :

- Cleansing
- Photo-protection
- Active ingredients

Active ingredients go well beyond simple moisturizers and exert a more complex activity in protecting skin from external injuries, nourishing it and removing its superficial layers. Transport systems and excipients are increasingly effective.

Ageing skin is characterized by fine lines, wrinkles, lengitines, despigmentation and increased coarseness. Topical preparations allow to combat these changes abound in the over-the-counter market. Some of the most popular ingredients used in these products are vitamin

It has been noticed that despite of the fact of showing very good and promising in-vitro results, once those have been moved into in vivo test, the obtained results have not been that promising. To a certain extent the above mentioned is because of the difficulty that the active ingredients show to get the target cells.

For all these reasons , INFINITEC have designed and developed a natural model of sustained and controlled release on the skin in order to improve the penetration of the active ingredients and make them able to come through the Stratum Corneum and reach the target cells, i.e. the fibroblast.

That is the reason whereby Infinitec have developed a unique technique based on the nanotechnology which allows us to get nanolipids of natural origin that entrap the active ingredients and release in a sustained and controlled way into the cellular medium.

The selected active ingredients have been chosen according to a proven efficacy when performing at the fibroblast level, and also because of their natural origin:

- Vitamin A
- Vitamin C
- Vitamin E
- Vitamin F

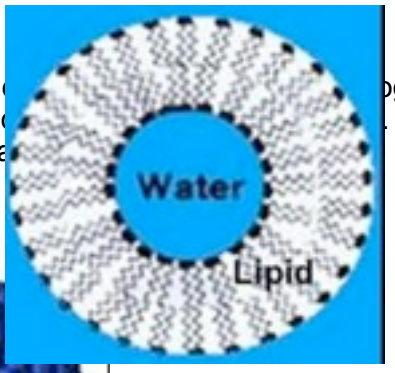
Therefore, summarizing, we can confirm that a new state of the art technology has been developed, which allows us to obtain a sustained release natural nanosystem that incorporates natural active ingredients too.

NANOLIPIDS, A NATURAL TECHNOLOGY

Nano LPD´s. Definition

The Nano LPD´s consist of vesicles, mainly made of phospholipids of natural origin. These phospholipids are organized in bilayers allowing the integration of active ingredients within its structure:

Phospholip Micela Phos Nano LPD´s



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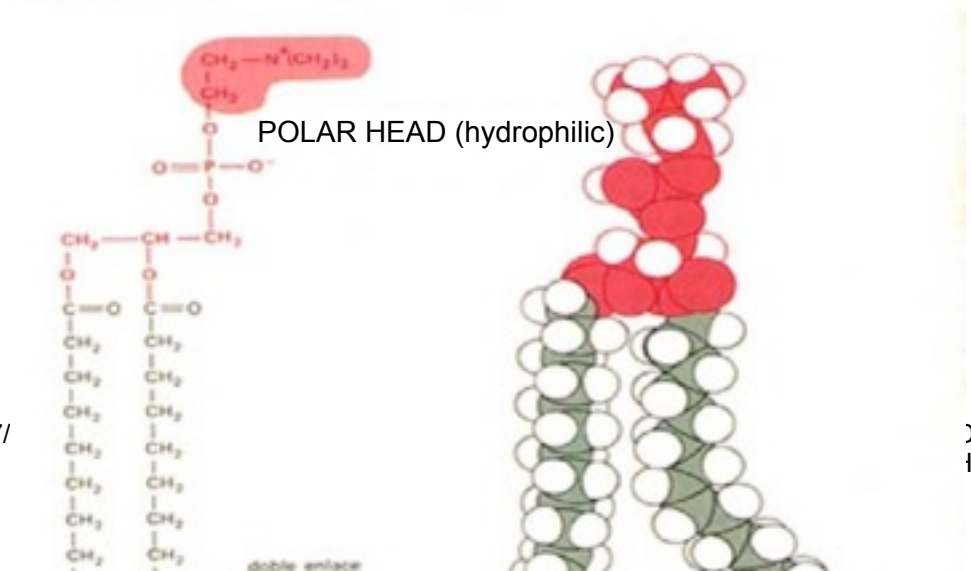
Nano LPD´s (diameter < 250 nm)

Nano LPD´s. Composition

Nano LPD´s are mainly made of natural origin phospholipids. These compounds show two distinc parts to the molecule, this is the reason they can display a dual personality. They have a non-polar tail (lipophilic) and a polar head (hydrophilic).

Structure of the phospholipids that form the Nano LPD´s :



NONPOLAR TAIL (lipophilic)

Like surfactants, phospholipids show an amphiphilic behaviour which is responsible of the capabalbilty to create micelles, bilayers and vesicles, and also to gather in the interface.

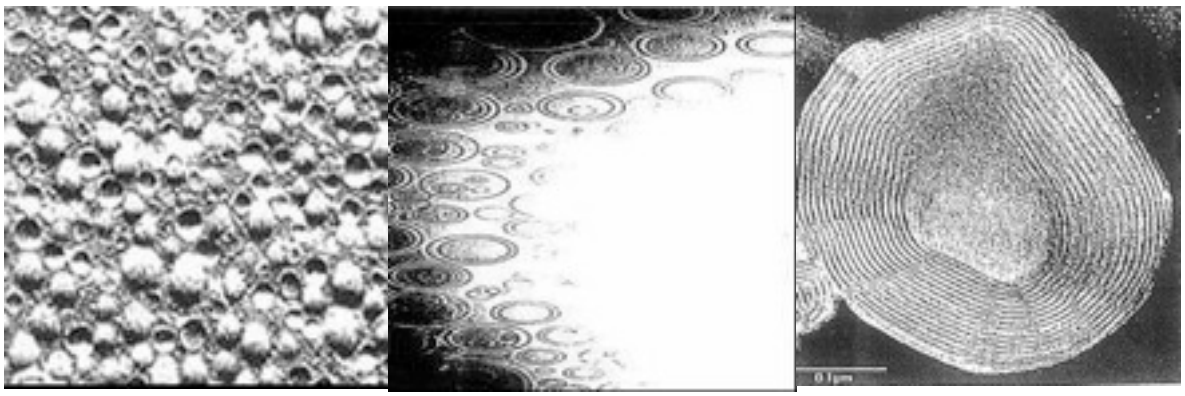
Nano LPD´s. Clasification

There are tow ways to classify Nano LPD´s. The first one is according to their size:

- Small , with diameters lower than 100nm
- Big, with diameters bigger than 100nm.

Using laser technology we are able to discriminate our Nano LPD´s isolating the ones whose diameters are lower than 250 nm.

A second classification :
Based on the number of bilayers that the vesicle can contain, i.e. this classification is based on the stratification:

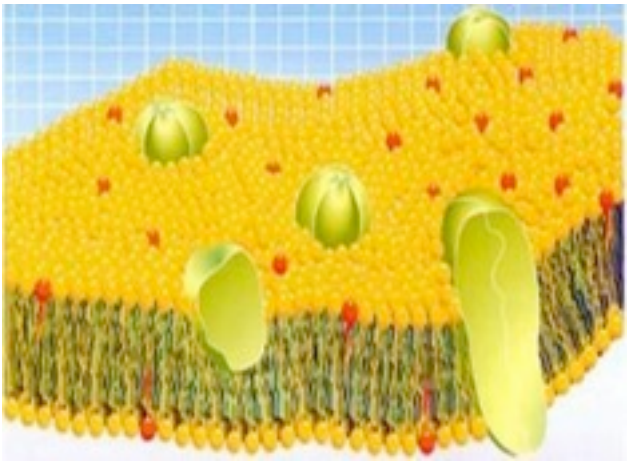


Unilamelars Nano LPD´s Oligolamelars Nano LPD´s Multilamelars Nano LPD´s

Nano LPD´s. Uses and advantages

Within the main advantages that can be obtained using Nano LPD´s are the following ones:

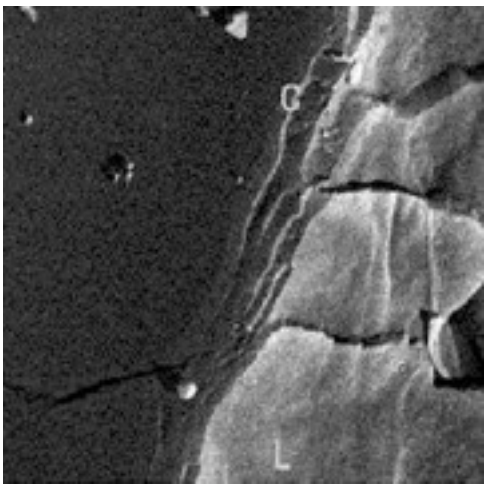
- They are **natural** delivery systems of active ingredients. Basically, they are lipidic nanostructures of phospholipidic origin.
- They are **selective** and **controlled** released systems.
- They are structural **analogues** of the **cellular membranes** that are also made of phospholipids.

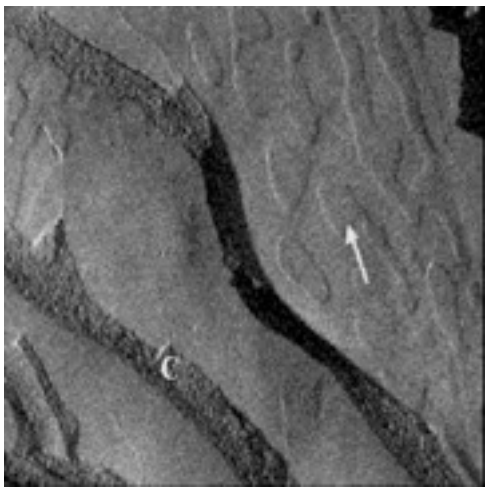


Cellular Membrane

Nano LPD´s

- **They increase the efficacy** and decrease the non desired side effects of the active ingredients (toxicity). Efficacy of the Nano LPD´s, as well as capacity of penetration at the Stratum Corneum (SC):





Damaged Skin. Low lipid content
Stratum Corneum

Skin treated with Nano LPD´s
Regenerated Stratum Corneum

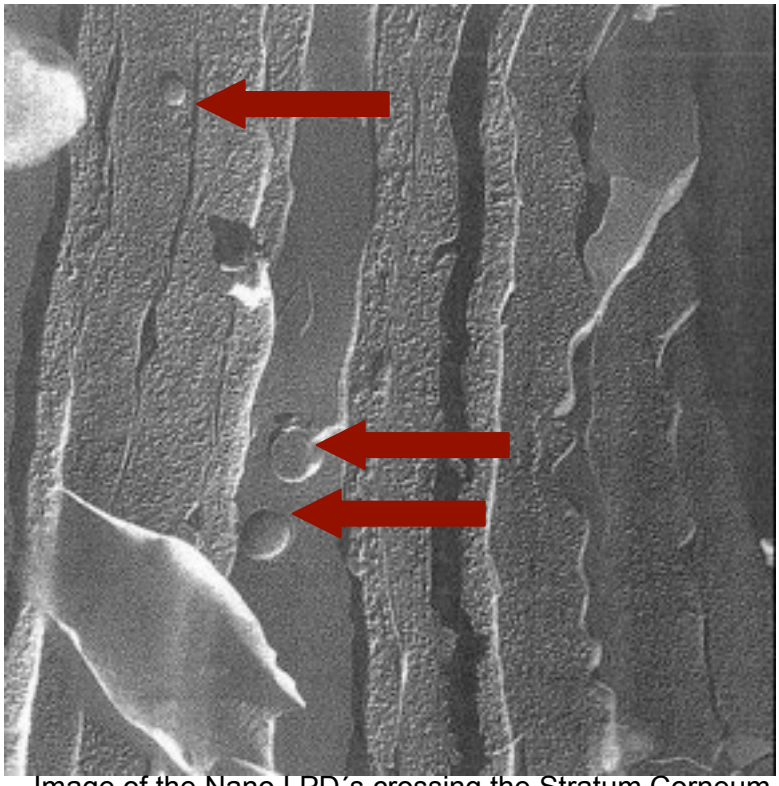


Image of the Nano LPD´s crossing the Stratum Corneum

Nano LPD´s.

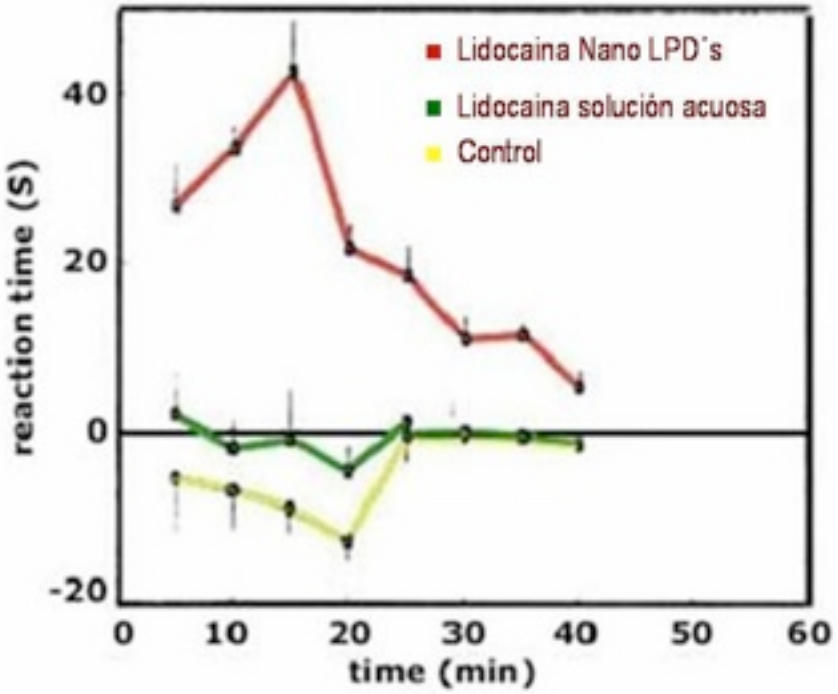
Incorporating the active ingredients into the Nano LPD´s the following advantages are obtained:

- Better bioavailability of the active ingredient.
- Stabilization of the Active Ingredient
- Introduction of alternative administration ways of a given active ingredient

Example

In order to notice a better efficacy of the Nano LPD´s we show the following case where lidocaine has been applied topically through different vectors. We can notice that an absorption, penetration and diffusion of the active ingredient

improvement of the analgesic activity is obtained compared to another vector as it is shown at the below chart.



Nano Interaction LPD´s – Active Ingredients

Due to the structure and composition of the Nano lipidic bilayer, LPD´s can incorporate both hydrophilic and lipophilic actives in the following way:

- Hydrophilic active ingredients within the vesicle.
- Lipophilic active ingredients between the layers.

WRINKLES

Wrinkles, as a sign of skin ageing, have an important social impact, especially because of longer lifetimes and more frequent social relationships; consequently, they are an important factor influencing our way of communication.

Nevertheless, the scientific interest of dermatologists and cosmetologists overlaps the merely hedonistic problem to find both cause and treatment/prevention opportunities.

Wrinkles represent the more evident outcome of cutaneous ageing. Their onset is linked to a variety of events, resulting from both chrono-and photo-ageing, among which are : dermo-epidermal junction thinning, due to decrease of laminine and loss of collagen, GAG and subcutaneous fat; in turn, gravity and muscle/articular movements play an important role. Both intrinsic (hormones, racial and genetic factors, oxidative stress, systemic disease) and extrinsic (temperature, air pollution, smoke, alcohol) factors worsen skin condition.

Often, the term wrinkle is misused and it is difficult to establish classification, histological correspondence and pathogenesis in a unique way. In fact, people call wrinkles all skin features different from a baby´s perfectly smooth skin : expression lines, more or less deep micro-relief furrows, articulation and mimic-muscle lines, laxity folds and in general all lines or folds recognizable on a no longer young face and body.

However, wrinkles deriving from skin texture, or micro-relief, modification affect women more than all other wrinkles as signs of ageing in the common mind. Actually, young skin micro-relief is made of many lines, superficial and tidily arranged, whereas old/ageing skin is featured by less deep and untidy lines.

Even if skin ageing is well and widely described in the literature, a precise definition of the word wrinkle does not exist. The only concrete attempt to precisely define the term wrinkle can be attributed to Griffiths : an extension of the skin perpendicular to the axis of the wrinkle leaves a marked line representing the bottom of the wrinkle.

Photo-protection is the second step of a proper cosmetological approach to wrinkles treatment. More than even before, our generation enjoys the luxury of travel and leisure time for outdoor sports, markedly increasing our exposure to solar radiation. Exposure is increased at high altitudes and with reflection from surfaces covered with snow, sand, water or concrete. Our skin suffers the greatest damage – both acutely (with erythema and sunburn) and chronically (with photo-ageing and skin cancer).

Certainly, sunscreens are absolutely essential for protection, but they are not enough. The most significant inherent limitations are inadequate application (too little, too infrequently) and incomplete spectral protection.

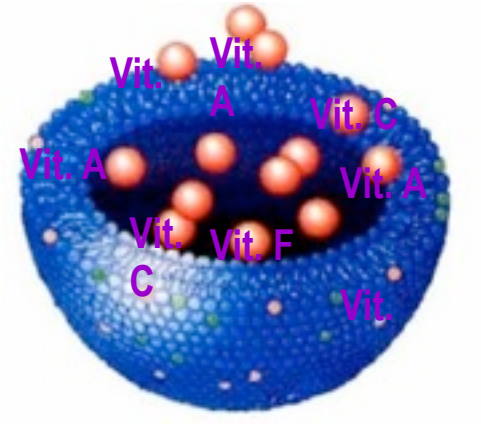
Because the skin naturally uses nutritional antioxidants to protect itself from photo-damage, sun protection can be enhanced with effective formulations of topical antioxidants. The challenge is to create stable formulations that give effective transcutaneous absorption of the active form.

NANO LPD´S MULTIVITAMIN

Composition and mechanism of action

Nano LPD´s Multivitamin is a controlled released natural system based on phospholipids, with a particle diameter lower than 250 nm. That allows a perfect penetration through the stratum corneum of the following active ingredients:

- Vitamin A
- Vitamin C
- Vitamin E
- Vitamin F



Nano LPD´s Multivitamin

VITAMINS AND THEIR DERIVATIVES	
Vitamin A	Normalize keratinization
	Down regulate sebum production in acne
	Reverse and treat photo-damage
	Striae
Vitamin C	Antioxidant
	Regulates collagen synthesis
	Formation of stratum corneum barrier lipids
	Regenerates Vitamin E
	Provides photo-protection (in combination with Vit. E)
Vitamin E	Membrane antioxidant
	Protects against oxidative damage
	Provides photo-protection (in combination with Vit. C)
Vitamin F	Cellular regeneration of the membranes and tissues

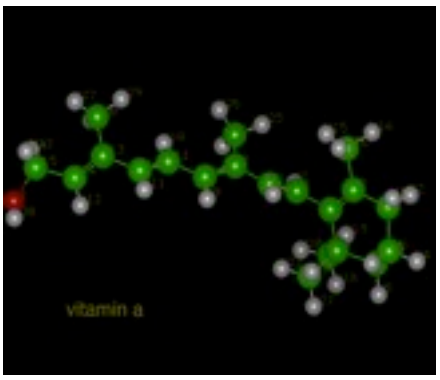
Vitamin A

Naturally occurring Vitamin A and its analogous (retinoids) have a major role in the differentiation and function of epithelial tissue. These compounds also have been used at pharmacologic levels for the treatment of several dermatologic diseases such as acne, photo-damage, and disorders of keratinization including psoriasis. More recently, retinol and retinoic acid have been found useful in the treatment of striae and cellulite.

The actions of retinoids are mediated by their nuclear receptors, which belong to the hormone nuclear receptor super-family. The activated receptors induce or supress the

transcription of target genes on ligand binding. These receptors fall into two classes, the retinoic acid receptors (RARs) and the retinoid x receptor (RXRs). All-trans-and 9-cis retinoic acid bind to and activate the RARs. Only 9-cis retinoic acid binds to and activates the RXRs. Human skin expresses predominantly RAR-γ and RXR-$\propto$. Under physiological conditions all-trans retinoic acid is the primary ligand mediating RAR´s regulation of gene expression in human skin.

Retinoic acid (directly) and retinol (indirectly) have unique roles in the repair of photo-damage. Retinoids repair photo-damage by a restorative mechanism and by limiting the progression of existing damage.



Retinoic acid blocks the UV induction of the matrix metalloproteinases, a family of enzymes responsible for the breakdown of collagen, the major constituent of the dermis.

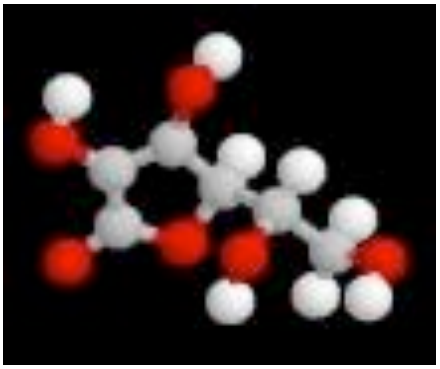
Retinoic acid also stimulates keratinocyte and fibroblast proliferation. Stimulated fibroblasts produce more collagen, thereby plumping up the dermis. More collagen gives the dermis greater thickness and resistance to trauma. Enhanced keratonocyte proliferation results in shedding of mature keratinocytes, resulting in smoother skin-surface texture. Enhanced collagen production also might explain the effacement of fine lines and wrinkles. Retinoids also inhibit UV-induced pigmentation, which results in a lightening of sun-induced age spots and overall uneven pigmentation.

Vitamin C

Vitamin C has a great reducing potential and reacts with many reactive oxygen and nitrogen species in vitro. Ascorbate effectively quenches singlet oxygen, superoxide, hydroxyl and water-soluble peroxyl radicals, and hypochlorous acid.

The electron-donation capacity of ascorbate is used quite often by at least eight human enzymes for which it is a cofactor. Three participate in the hydroxylation of proline and lysine in collagen biosynthesis, two in the synthesis of carnitine, two in catecholamine and hormone biosynthesis, and one in the metabolism of tyrosine.

Ascorbate concentration in total skin ranges from 0.4 to 1 mg/100 g. Of wet-tissue weight. Ascorbate is distributed in all layers of the skin. In humans ascorbate appears to be more concentrated in epidermis (3.8 μmol/g) than in dermis (0.7 μmol/g). Upon exposure to a variety of stressors including UV light and ozone, ascorbate concentration in skin decreases. Topical application of ascorbate provides photo-protection and prevents inflammation and UVB-induced immunosuppression.



The activity of ascorbate on collagen synthesis has been investigated extensively. Ascorbate seems to play a role in regulating collagen type I and II gene transcriptions. This regulation is independent of its role as a cofactor for lysyl and propyl hydroxylases.

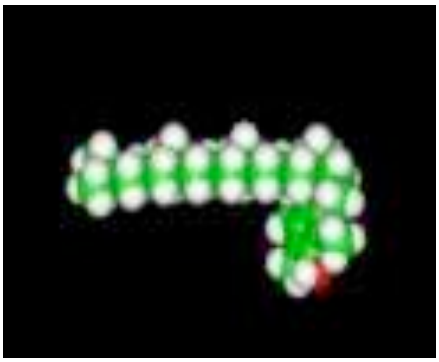
More recently, the role of ascorbate in the formation of stratum-corneum barrier lipids has been discovered. Ascorbate, not α-tocopherol, normalizes epiermal lipid profiles (in particular glucosphingolipids and ceramides) in reconstructed epidermis.

Vitamin E
Vitamin E comprises eight naturally occurring forms of a fat-soluble antioxidant that is present in plasma, membranes, and tissues. Their functions as chain-breacking antioxidants come from their ability to rapidly scavenge lipid-peroxyl radicals before they can react with other lipids, thereby ending the propagation of lipid peroxidation in membranes.

In addition, vitamin E exhibits scavenging activities against a wide spectrum of free radicals including singlet oxygen, superoxide, and hydroxyl radicals.

In human, vitamin E is the most abundant lipid-soluble antioxidant. Like vitamin C, α-tocopherol concentration is higher in the epidermis (31 nmol/g) than in dermis (16nmol/g). Vitamin E concentrations in the stratum corneum of exposed skin areas such as the forehead and the cheek are 20-fold higher than those in unexposed areas such as the upper arm.

α-tocopherol and γ-tocopherol are reduced (by 45% and 35%, respectively) in human stratum corneum exposed to a suberythemal dose of UV light. Another environmental stressor, ozone, diminish α-tocopherol in the upper layers (i.e., stratum corneum and upper epidermis). That depletion correlated with a large increase in malondialdehyde in the same layers.



After topical applications of free α-tocopherol in various models, investigators found that it had protective effects on UV-induced oxidative damage, immunosuppression and depletion of Langerhans cell, skin carcinogenesis, and erythema.

Vitamin F
Vitamin F - Glyceric Ester is a mixture of biologically active, esterified, polyunsaturate fatty acids. The ester form of this vitamin is used because it is more stable to oxidisation.

The polyunsaturated fatty acids which are in the majority, have been identified and then quantified by means of gas chromatography, with the following results:

Linoleic Acid $C_{18:2}$	50.0 – 57.0 %
Linolenic Acid $C_{18:3}$	0.7 – 1.2 %
Arachidonic Acid $C_{20:4}$	0.2 – 0.4 %

Vitamin F - Glyceric Ester intervenes in the constitution of the cellular membranes and is a coassistant in enzyme synthesis. The polyunsaturate fatty acids in vitamin F can not be synthesised by the body and must be brought in from without.

The components of Vitamin F are polyunsaturate fatty acids which intervene in cellular regeneration of the membranes and tissues. The components also intervene in the defence mechanisms as they are precursors in the synthesis of the elements which carry out such functions.

The application of Vitamin F - Glyceric Ester in cosmetics is based on its ability to modify such states of the skin as dryness, rashes and peeling. The polyunsaturate fatty acids have envigorating properties and improve the look of the cutis, therefore, this product is also applied to eliminate small folds and wrinkles.

COSMETIC APPLICATIONS

- Anti-ageing agent
- Antioxidant
- Prevention of photo-damage

DOSAGE

- 3% - 5% of Nano LPD´s Multivitamin

EFFICACY TESTS

Fibroblast outgrowth

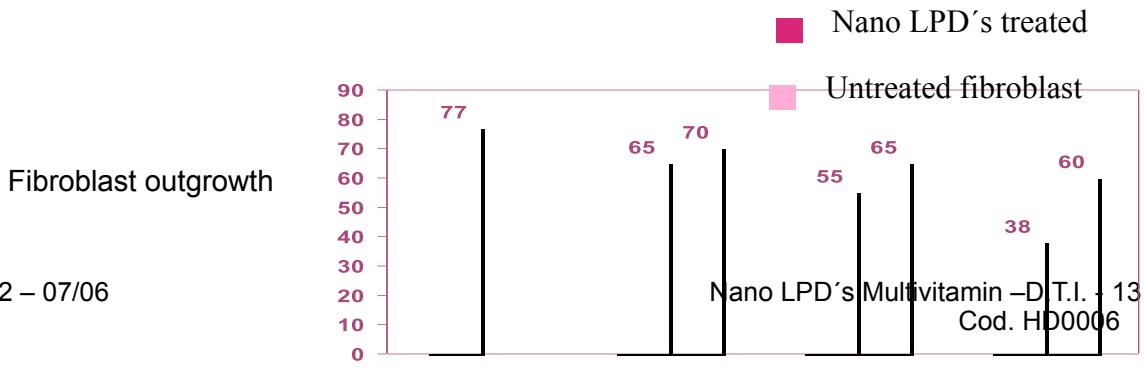
Objective
Evaluate the capability of Nano LPD´s Multivitamin to increase fibroblast growth potential

Materials and method
The study population consist of 50 individuals grouped according to age as follows : 18-29, 30-45, 46-60 and 80 years and older.

Skin biopsies were cut into small fragments (15-20 fragments per biopsy) and each fragment placed in a well of a 48-well dish. The fragments were incubated for up to 1 month in Dulbecco´s modified minimal essential method containing nonessential amino acids and 10% fetal bovine serum at 37^0C in a humidified atmosphere containing 5% CO_2. The number of tissue fragments from which fibroblasts were isolated was determined, expressed as a percentage of the total number of tissue fragment incubated. We have previously shown that isolation of fibroblasts from tissue fragments can be used as reliable means for quantification growth potential of fibroblasts within the tissue (Varanai et al., 1994).

Results
Fibroblast outgrowth from skin fragments was used as a measure of fibroblast growth potential within the tissue. Fibroblast outgrowth declined with increasing age. Fibroblast growth potential is increased with Nano LPD´s Multivitamin treatment at 1%

The following graph shows the obtained results :



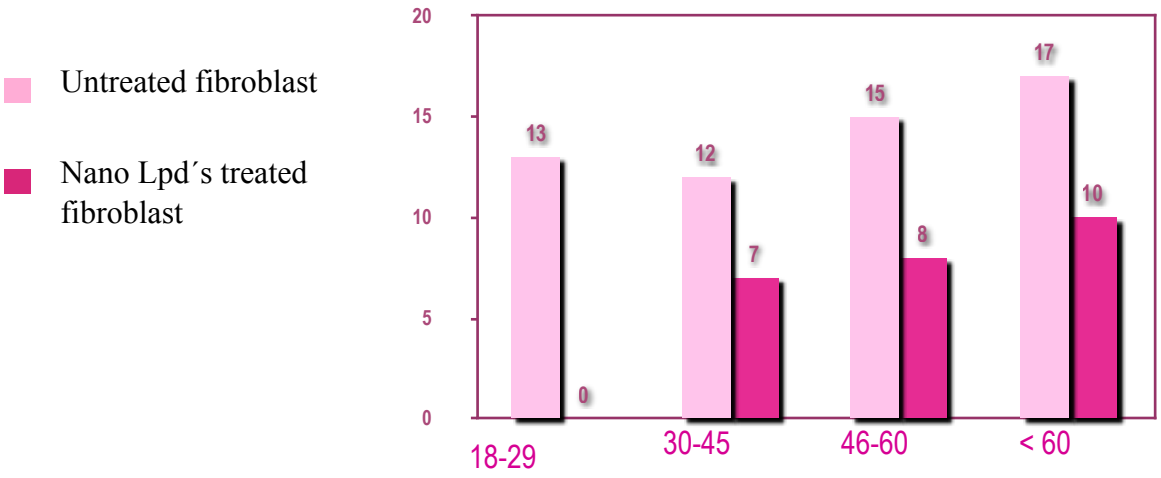
MMP assays 18-29 30-45 16-60 > 60

Objective
To evaluate the capability of Nano LPD´s Multivitamin to inhibit MMP concentration.

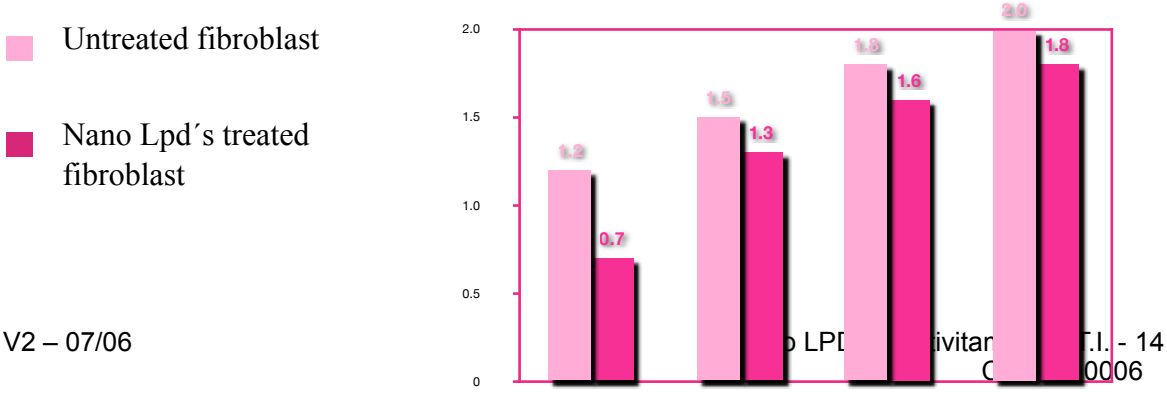
Materials and method
Skin samples were frozen in liquid nitrogen immediately after collection, and kept frozen at -80^{0}C until used for analyses. Skin samples were crushed under liquid nitrogen in mortar and pestle and homogenized in 20nM Tris (pH 7.6), 5mM $CaCl_2$. Insoluble material was removed by centrifugation and the supernatant used as the source of MMP. Collagenase enzyme levels were measured by hydrolysis of [^{3}H] labeled type I fibrillar collagen (Hu et al, 1978) after activation for 90 min with 1nM aminophenyl mercuric acetate. Western blot analysis with antibodies to interstitial collagenase (MMP-1) was performed as described (Fisher et al, 1996). Gelatinase levels (MMP-2; 72 kDa gelatinase and MMP-9; 92 kDa gelatinase) were measured by gelatin zymography and quantitated by scanning laser densitometry.

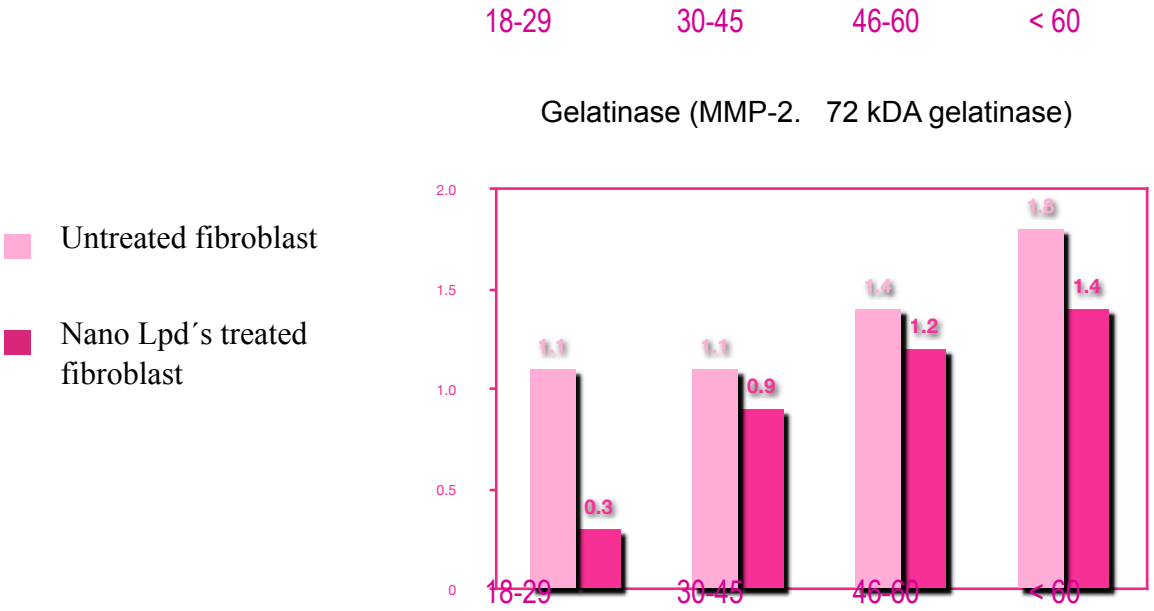
Results
These are the results obtained :

Collagenase (MMP-1. Interstitial collagenase)



Gelatinase (MMP-9. 92 kDA gelatinase)



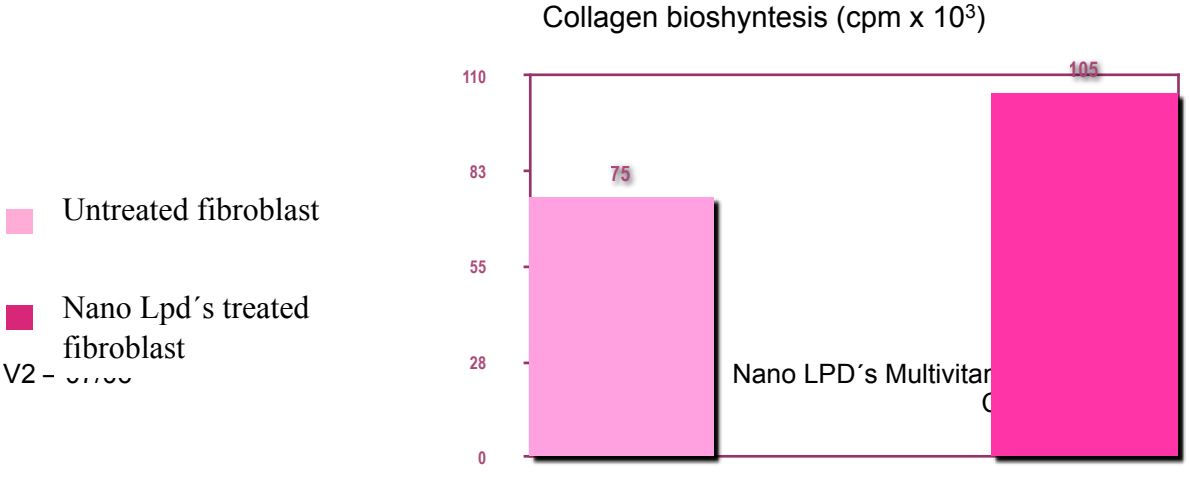


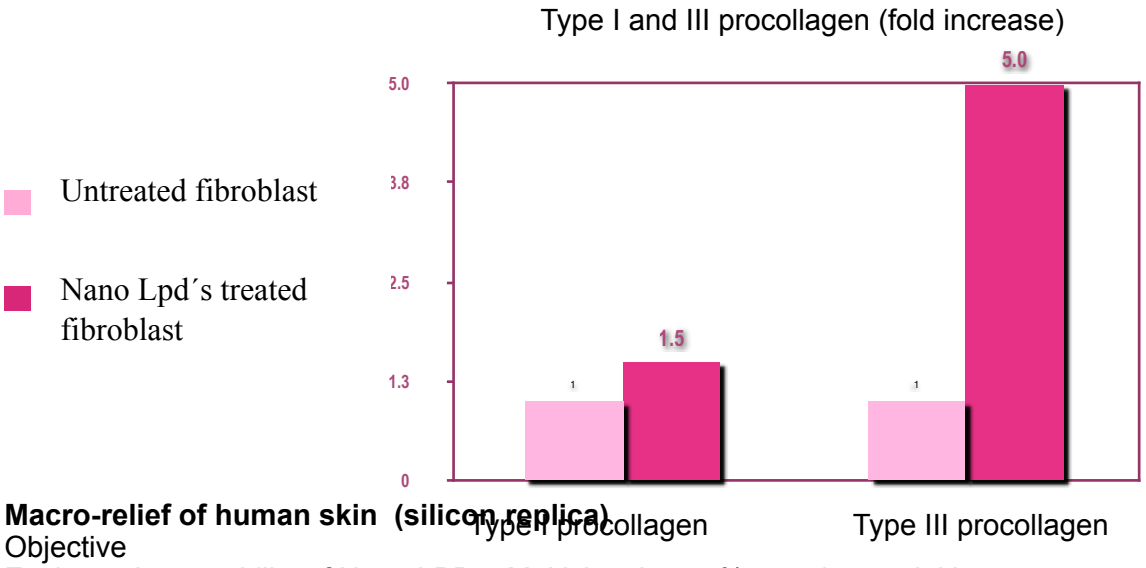
Collagen biosynthesis

Objective
Evaluate the capability of Nano LPD´s Multivitamin to increase collagen synthesis

Materials and method
Type I procollagen (∝1 chain) protein levels were assessed by western blot analysis and by immunohistology. Type III procollagen immunohistology (∝1 chain) was performed using an antibody from Chemicon Internacional (Temecula, CA). Total collagen biosynthesis by fresh skin samples was assessed by incorporation of [^{14}C]proline into pepsin-resistant, trichloroacetic acid (TCA)-precipitable material. Skin samples that had been freeze-thawed prior to incubation with [^{14}C]proline (to disrupt cells and thereby prevent collagen biosynthesis) served as control for nonspecific label incorporation. To measure type I procollagen biosynthesis specifically, fresh skin samples were incubated for 24 h in keratinocyte basal medium, supplemented with Ca^{2+} to a final concentration of 1,4mM. At the end of the incubation period, media were collected and analyzed for type I procollagen protein by enzyme-linked immunosorbent assay (ELISA).

Results
These are the results obtained :





Macro-relief of human skin (silicon replica)

Objective

Evaluate the capability of Nano LPD´s Multivitamin at 5% to reduce wrinkles

Methodology

It has been assessed the macro-rugosity of silicon replicas of the eye contour obtained from 15 volunteers. The treatment with the evaluated product has lasted four weeks and samples at time 0 and time 28 days have been measured.

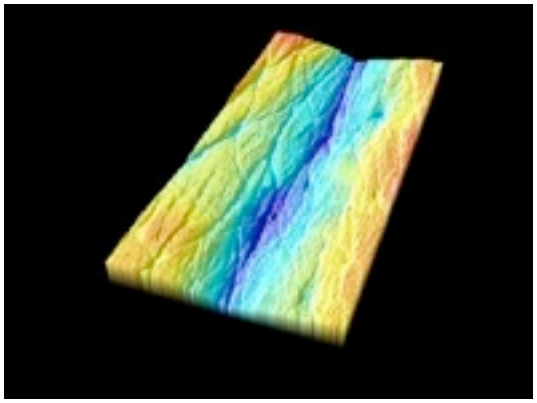
The measurement of the rugosity has been carried out through confocal microscopy.

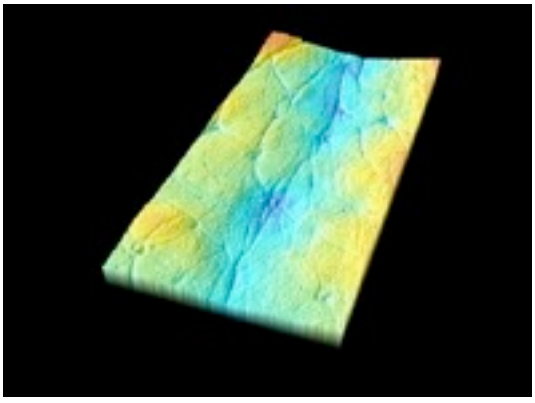
Results

The skin replicas below show the improvement of the wrinkle depth after 28 days of treatment using a cream containing 5% of Nano LPD´s Multivitain.

After this test, it can be concluded that as average Nano LPD´s Multivitamin reduced the depth of wrinkles up to 25%.

T0 **T28**





N=21	
Ra	RMS
-25,55	-25,45

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