Abstract
Skin ageing is a global process resulting from alterations in the level of many skin components. These modifications affect both the quality of the dermal extracellular matrix (ECM) and epidermis cohesion, inducing wrinkles and skin fragility, respectively. Proteoglycans (PGs) take a strategic part in dermis and epidermis homeostasis. The lumican plays an important role during the collagen fibre formation in the dermal ECM. In addition, syndecan are strongly implicated in keratinocyte activation and cohesion. The evolution of lumican and syndecan-1 levels with ageing was studied and a decrease in the synthesis of these two PGs was observed. Skin cohesion is also mainly dependent on the quality of the dermo-epidermal junction (DEJ). Basal keratinocytes of the epidermis adhere to this junction via specific structures: the hemidesmosomes. One of its components, collagen XVII is very important for the cohesion between the epidermis and dermis.

Lumican, syndecan-1 and collagen XVII represent original important targets for cosmetology in fighting against ageing of the main skin layers. Two synthetic acetyl-tetrapeptides (Acetyl Tetrapeptide-9 and Acetyl Tetrapeptide-11) have been selected by exploratory research and evaluated in vitro on different original models. Acetyl Tetrapeptide-9 stimulates in vitro the synthesis of lumican and collagen I to increase the quality of dermal ECM. Acetyl Tetrapeptide-11 boosts the synthesis of both syndecan-1 and collagen XVII in keratinocytes to reinforce global skin cohesion.

Introduction
Cosmeceuticals or so-called ‘doctor’ brands have recently enjoyed increasing appeal to consumers because they offer anti-ageing benefits without the need to undergo invasive procedures. To achieve this, a growing number of active ingredients is being incorporated into skincare products.

This trend holds out the possibility of sustainable growth for cosmeceutical products but also poses challenges to suppliers of active ingredients. Cosmetics manufacturers are seeking ever more effective and innovative cosmetic ingredients, such as enzymes, amino acids and peptides, with identified and specific mechanisms of action and demonstrable benefits that perfectly fit into the growing cosmeceutical trend.

Small proteoglycans in the skin: new targets to fight against skin ageing
The phenomenon of skin ageing and the identification of its biological pathways remain a constant focus of many research programmes. We have recently undertaken a fundamental research study targeting one specific family of molecules, small proteoglycans.

Small proteoglycans are characterised by glycosaminoglycan chains fixed to a linear core protein by covalent bounds. They are present both in the epidermis and the dermis and act as biomechanical supports, tissue organiser and biological filters, thereby playing an important role in skin homeostasis. However, knowledge of their synthesis and structure during skin ageing has always been rather limited.

Our research programme has identified two specific proteoglycans – lumican and syndecan-1 - whose synthesis counteracts the ageing process in the skin and has been demonstrated to decrease with ageing.
**Active Ingredients**

Lumican is located in the dermis, where it is involved in the formation of collagen fibres and consequently in skin resistance to traction. Syndecan-1 occurs preferentially in the supra-basal layers of the epidermis and is involved in epidermal cohesion.

In order to identify ingredients with corresponding activity, skin-specific DNA arrays were used. This allowed us to screen selectively potential actives that would influence the expression of genes coding for the two identified proteoglycans but also growth factors and structural proteins involved in skin structure and homeostasis.

Based on these findings, we have developed two new pure tetrapeptides, Dermican™ (INCI name: Glycerin (and) Acetyl Tetrapeptide-9) and Syniorage (INCI name: Glycerin (and) Acetyl Tetrapeptide-11), which are designed to fight the skin ageing process. The efficacy of both was further demonstrated on cell cultures, skin models and finally in clinical studies.

**Dermincan™ at dermis level to optimize collagen fibre functionality**

Dermican™ has been singled out for its unique biological anti-ageing action, which is targeted at increased collagen network functionality via lumican synthesis. DNA array tests made it possible to evaluate the influence of Dermican™ on the general gene expression profile of dermal fibroblasts including lumican and the structural protein COL1A1 coding for collagen I.

Stimulation of lumican synthesis by aged human dermal fibroblasts showed that Dermican™ achieved increases of 66% and 115% over a control in the expression index, as measured in terms of the number of stained pixels by fluorescence intensity of the green channel in arbitrary unit, when used at 0.74 and 2.2 µg/ml respectively. IL-4 at 0.1 µg/ml scored a 73.6% increase over the control.

**Figure 1a** Lumican during skin ageing – Variation in dermal fibroblasts with age (Northern-blot)

**Figure 1b** Syndecan-1 during skin ageing – Variation in keratinocytes with age (Northern-blot)

**Figure 2** Stimulation of lumican synthesis by aged human dermal Fibroblasts: visualisation (lumican in green)
Likewise, in the expression of COL1A1 gene, 2.2 µg/ml Dermican™ showed a 22% increase over the control, while TGF-
B1 at 10 ng/ml showed a 125% increase. In the stimulation of collagen I synthesis, Dermican™ showed increases of 49% and
112% at 2.2 and 7.4 µg/ml respectively; TGF-B1 at 3 ng/ml showed a 65% increase. For proteins in the same test, the
increases were 4%, 5% and 20% respectively.

Thus, lumican is directly involved in the synthesis of the
collagen fibres (fibrillogenesis) and consequently in the
organisation of the collagen bundles, resulting in tighter and
firmer skin. Several in vitro tests have demonstrated
Dermican™’s capacity to boost the synthesis of lumican and
collagen I.

Dermican™’s efficacy and tolerability were demonstrated in a
clinical study with 17 female volunteers aged between 45 and
55. After four months of treatment, it induced an increase in
skin thickness and an improvement of skin firmness.

By acting both on the structure and mechanical properties of
skin, it confirmed its anti-ageing efficacy, leading to a firmer and
plumper skin. With Dermican™, we are moving to the next
generation of anti-ageing products. Beyond collagen synthesis,
we also ensure that newly synthesised fibres are fully functional.

Based on its mechanism of action, this active ingredient is also
suitable for other applications related to firmer skin, and it is
thus distinctly possible that Dermican™ may improve stretch
marks as well.
### Active Ingredients

#### Syniorage at epidermis level, to restore the radiance and firmness of mature skin

With age, the epidermis loses its resistance and radiance, mainly due to reduced cohesion of the epidermal cells. As a result, skin becomes dry and slack and is easily damaged with even the lightest friction or shock. To prevent this, manufacturers of personal care products must design products to meet the specific needs of mature skin.

Syniorage specifically targets the two constituents of the epidermis that are primarily responsible for its cohesion: syndecan-1 and collagen XVII. The latter is a protein that occurs in the hemidesmosomes, a microscopic, rivet-like structure that affects the strength of the bond between the epidermis and the dermo-epidermal junction.

The overall effects of Syniorage were established by DNA array technology. This test made it possible to evaluate the influence of Syniorage on the general gene expression profile of human primary epidermal keratinocytes.

Several in vitro tests on keratinocytes demonstrated Syniorage’s ability to boost the synthesis of syndecan-1, reinforcing epidermal cohesion, and the production of collagen XVII protein, leading to an improvement of the adhesion between the epidermis the and dermo-epidermal junction.

Over five days, Syniorage achieved increases of 103% and 130% in the synthesis of human keratinocytes by comparison with a control, as measured by the number of stained pixels by fluorescence intensity of the green channel in arbitrary unit, when used at 0.87 and 2.6 µg/ml respectively. KGF at 0.01 µg/ml, meanwhile, scored a 66% increase over the control.

In the expression of the collagen XVII COL17A1 gene over three days, meanwhile, Syniorage achieved increases of 13% and 18% over a control when used 0.87 and 2.6 µg/ml respectively.
Active Ingredients

Increased significantly and the skin texture was refined, so that the subjects looked visibly younger.

Conclusion
For a comprehensive anti-ageing approach, the combination of Syniorage, specific for the epidermis, with Dermican™, which specifically targets ageing of the dermis, is an optimal choice of ingredients for various easy-to-use products in the growing market for cosmeceutical skin care products.

Co-Author’s Biography
Dr Gilles Pauly, medical doctor dermatologist and immuno-allergist, has been co-founder in 1982 and Research Director of the Research Center of Laboratoires Sérobiologiques, IRBD (research institute in biology and dermatology). He is co-author of more than 90 patents and 81 publications and scientific presentations in the cosmetic field. After the acquisition of Laboratoires serobiologiques by Cognis in 1999, one of the worldwide leader of specialty chemicals, Gilles Pauly was successively R&D Manager, and since 2005, Scientific Director of Laboratoires Serobiologiques. The R&D Department has developed during this period a strong know-how in:
1. In vitro technologies of cell cultures and molecular biology
2. ex-vivo/in vivo efficacy tests
3. Histology, histochemistry, immunohistochemistry with image analysis 2D and 3D
4. Phytochemistry
In order to develop new innovative concepts in skin and hair biology and new innovative actives, to contribute to make Laboratoires Serobiologiques a major player on the market of active ingredients for the cosmetic industry.

Figure 9 Stimulation of the expression of collagen XVII COL17A1 gene by human epidermal keratinocytes: visualisation at 3 days.

The efficacy and tolerability of Syniorage was proven in a clinical test on 19 female volunteers aged between 60 and 70. After a two-month course of treatment, the biomechanical firmness and elasticity of the superficial epidermal layers had increased significantly and the skin texture was refined, so that the subjects looked visibly younger.

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For further information please contact:
T4 International
New Pastures
539 London Road Boxmoor
Hertfordshire HP1 2RE
United Kingdom
Telephone: +44 (0)1442 249091/215681
Fax: +44 (0)1442 234936
E-mail: info@cosmeticsciencetechnology.com
Web site: http://www.cosmeticsciencetechnology.com