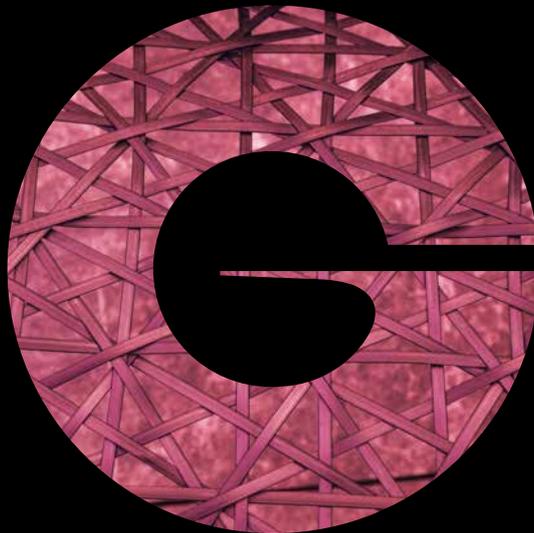


Active Beauty  
Tightenyl™  
The skin matrix Bio-lifter

Crafted by white technology



## Focus on the product

### The extracellular matrix: the architecture of youth

The skin extracellular matrix (ECM) is crucial in the dermis and physically supports the dermis-epidermis junction. It is composed of fundamental macromolecules: polysaccharides (e.g. glycosaminoglycans such as hyaluronic acid and Sulfated GAGs), proteins (e.g. collagens and elastin), and hybrid protein-sugar molecules: proteoglycans.

In baby skin, the extracellular matrix is perfectly organised, dense, compact and filled with these macromolecules. When aged, the visible appearance of the skin's surface is directly linked to the quality of the ECM: if one or several structural pillar is missing or disorganised, then the epidermis starts to wrinkle and skin loses its visible youth.

### S-GAGs and proteoglycans are key for ECM organisation

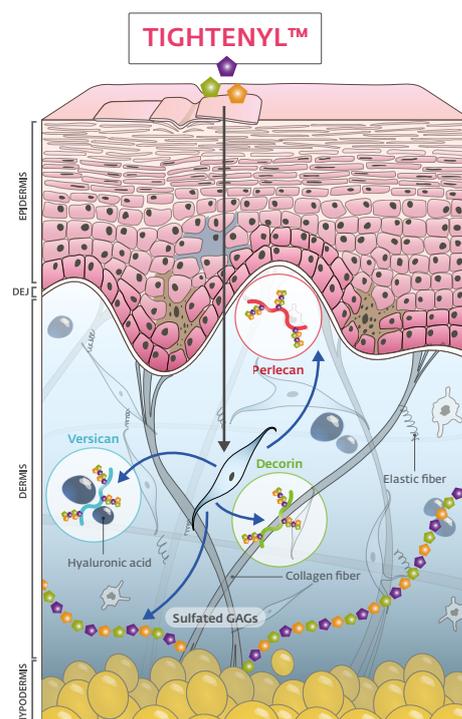
Two major sulfated glycosaminoglycans (S-GAGs) play a key role in the maintenance of the ECM infrastructure: chondroitin sulfate and heparan sulfate. These hydrophilic sugar polymers capture water in the dermis and maintain its bio-mechanic properties. As opposite to hyaluronic acid, S-GAGs, attached to core protein, have a fundamental structural function to organise and maintain the architecture of the dermis<sup>1</sup>:

- ▶ **Versican** (a core protein with a chondroitin sulfate side chain) creates connections between hyaluronic acid and elastic fibers to enable the skin viscoelasticity (suppleness of the skin).<sup>2</sup>
- ▶ **Perlecan** (a core protein with heparan sulfate side chain) binds to and cross-links many ECM components and cell surface molecules. It plays a key role in the anchorage of the epidermis in the dermis as a major component of the dermis-epidermis junction network (strength of the skin).<sup>3</sup>
- ▶ **Decorin** (a core protein with a chondroitin sulfate side chains) mainly binds to type I collagen fibers and controls the collagen fibers structure (firmness of the skin).<sup>4</sup>

### Tightenyl™: bio-precursors for matrix rejuvenation

Tightenyl™ is a rational combination of bioavailable S-GAGs precursors: N-acetyl-glucosamine-6-phosphate, glucuronic acid, and magnesium sulfate in equimolar ratio. Once applied on the skin, these precursors help skin cells to relaunch the entire synthesis pathway of proteoglycans and their specific S-GAGs. Tightenyl™ shows very fast clinical results:

- ▶ In only 2 weeks, firmness, tonicity, viscoelasticity and plasticity are improved by more than 100% in average, showing the high power of restructuration of Tightenyl™.
- ▶ After 3 months, the dermis is perfectly reorganised and becomes the equivalent of a baby skin, from the inner skin to the visible level.



<sup>1</sup>Experimental Dermatology, 20, 2011 Jan., 445-456

<sup>2</sup>Journal of biological chemistry Vol. 277, No. 6, 2002 Feb., pp. 4565-4572, 2002

<sup>3</sup>Journal of biological chemistry Vol. 287, No. 22, May 2012, pp. 18700 -18709

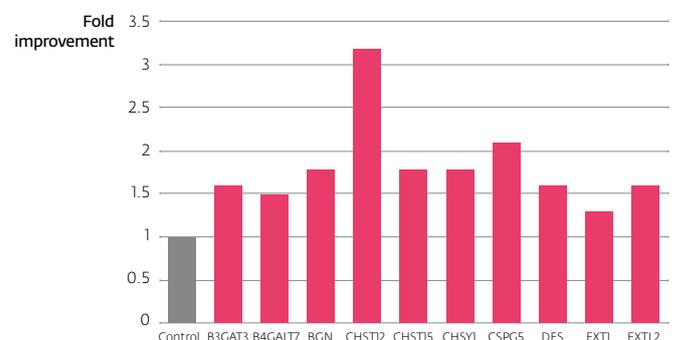
<sup>4</sup>Plosone, Vol.8, Issue 8, August 2013

# Biological activity

## Activation of the S-GAGs synthesis pathway (*transcriptomic*)

Human normal dermal fibroblasts from a 45 year old donor were incubated with Tightlyl™ at 0.6% and 1.2% over 18 hours. The mRNA expression of genes involved in S-GAGs and proteoglycans was quantified by q-RT-PCR.

Genes	% Tightlyl	Synthesis role
B3GAT3	1.2	Synthesis of heparan sulfate
B4GALT7	0.6	Synthesis of chondroitin sulfate
BGN	0.6	Synthesis of biglycan/decorin
CHST12	0.6	Secondary sulfate transfer on chondroitin sulfate
CHST15	0.6	Primary sulfate transfer on chondroitin sulfate
CHSY1	0.6	Chondroitin sulfate polymerase
CSPG5	0.6	Proteoglycan synthesis
DES	0.6	Dermatan sulfate epimerase
EXT1	0.6	Synthesis of heparan sulfate
EXTL2	1.2	Synthesis of heparan sulfate



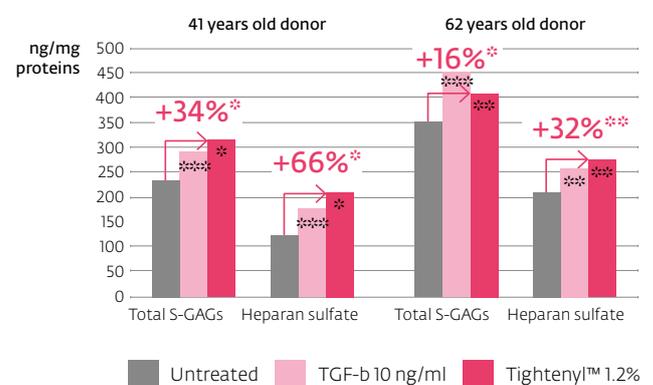
**Results:** Tightlyl™ significantly stimulates the gene expression of 10 essential enzymes involved in the synthesis of sulfated glycosaminoglycans and proteoglycans by fibroblasts ( $p < 0.05$ ).

## Activation of cellular S-GAGs production (*in vitro*)

Human normal fibroblasts from 41 and 62 year old donors were incubated over 3 days with 1.2% of Tightlyl™. The total amount of S-GAGs and N-sulfated GAGs was quantified using specific kit assays.

**Results:** Tightlyl™ significantly triggers the production of sulfated glycosaminoglycans by +34% ( $p < 0.05$ ) and +16% ( $p < 0.05$ ), respectively, in mature and very mature fibroblasts.

It also significantly reactivates the production of N-sulfated glycosaminoglycans (mainly heparan sulfate) in both cell types [+66% ( $p < 0.05$ ) and +32% ( $p < 0.01$ ) respectively].



\*\*\*  $p < 0.001$  Student's t-test

\*\*  $p < 0.01$  Student's t-test

\*  $p < 0.05$  Student's t-test

# Biological activity

## Higher efficacy than retinol (*ex vivo*)

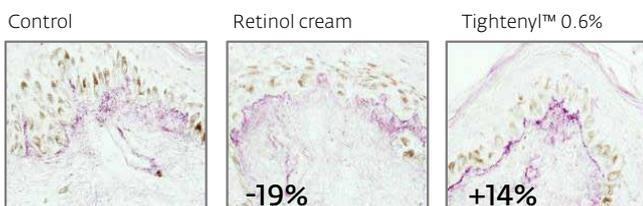
The action of Tightenyl™ on S-GAGs and proteoglycans production was evaluated on 41 year old human skin explants. Skin explants were topically treated either with a water solution (control), or a commercial cream containing retinol (benchmark) or a water solution containing Tightenyl™ at 0.6% following this schedule:

	Day 0	Day 1	Day 2	Day 5	Day 6	Day 7	Day 8
Water	X	X	X	X	X	X	X
Retinol cream	X	-	X	X	-	X	X
Tightenyl™	X	X	X	X	X	X	X

Explants were then sampled and analysed by staining (analysis of skin morphology), and by immuno-staining to enable the image quantification of S-GAGs and proteoglycans.

### 1. Expression of chondroitin sulfate and heparan sulfate

#### Day 8 - Chondroitin sulfate



#### Day 8 - Heparan sulfate



**Results:** Tightenyl™ at 0.6% significantly **increases heparan sulfate and chondroitin sulfate synthesis** in the dermis by **+14% in only one week**, while the commercial retinol cream inhibits chondroitin sulfate production (-19%) and slightly stimulates heparan sulfate production (+8%).

# p<0.01 Student's t-test

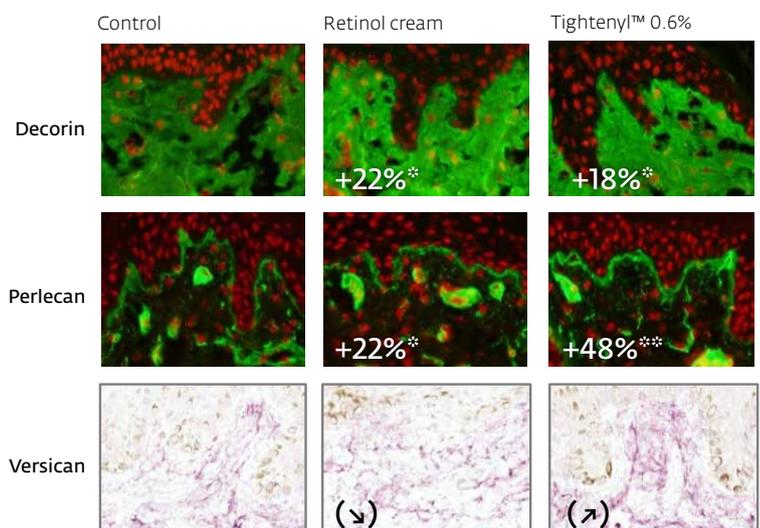
### 2. Expression of decorin, perlecan and versican

**Results:** Tightenyl™ at 0.6% significantly **stimulates the synthesis of decorin (+18%) in 8 days** at almost the same level than the retinol cream.

It **increases twice better than the retinol cream the production of perlecan (+48%)**, and slightly **promotes the production of versican** whereas the retinol cream inhibits its production.

\*\* p<0.01 Student's t-test

\* p<0.05 Student's t-test



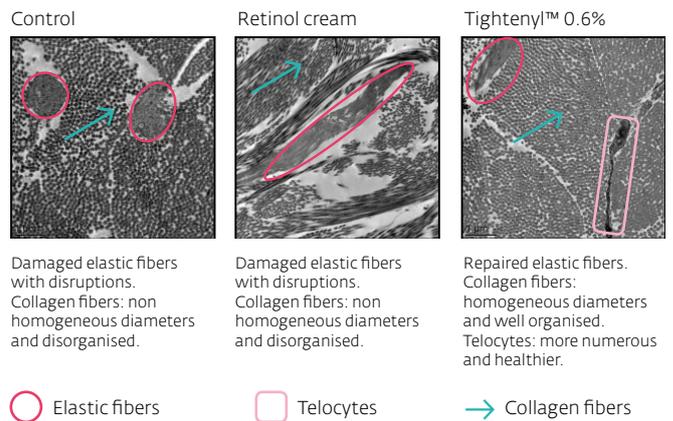
# Biological activity

## Improvement of the dermis infrastructure (*ex vivo*)

### 1. Transmission electron microscopy

Skin explants were analysed by electronic microscopy to observe the ultrastructure of the extracellular matrix.

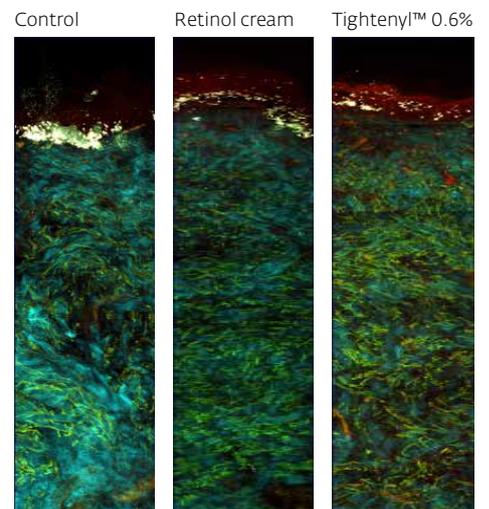
**Results:** After 8 days of treatment of the skin explants with Tightenyl™ at 0.6%, a visible effect can be seen on the skin fibers: elastic fibers look repaired and compact, collagen fibers show homogeneous diameters and a proper organisation, and telocytes (novel cells identified and playing a key role in skin tissue homeostasis) are more numerous and look healthier.



### 2. Biphoton microscopy

The skin explants were analysed by means of biphoton microscopy to observe the organisation of extracellular matrix components. The intense green color is linked to elastin fibers autofluorescence, and the blue color is linked to collagen fibers detection.

**Results:** Tightenyl™ at 0.6% enables a visible reorganisation of the skin fibers: both elastic fibers and collagen are more compact and well organised, than in the untreated conditions. The intensity of elastic fibers fluorescence is better than with the retinol cream.

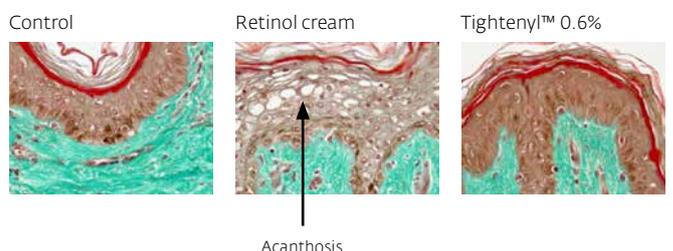


## Improvement of skin morphology (*ex vivo*)

Skin explants were stained using trichrome Masson technic to observe the evolution of the skin morphology after a Retinol cream or Tightenyl™ at 0.6% treatment.

**Results:** A clear improvement of skin compactness with visual improvement of cell cohesion and a better dermis-epidermis junction is observed with Tightenyl™ after 8 days. A significant irritation of the skin is seen with the retinol cream (acanthosis: accumulation of water in skin keratinocytes).

In one week, the dermis is more compact, the skin extracellular matrix is fully rejuvenated. VISIBLE BETTER RESULTS THAN WITH RETINOL.



# Efficacy

## Fast improvement of mechanical properties (clinical)

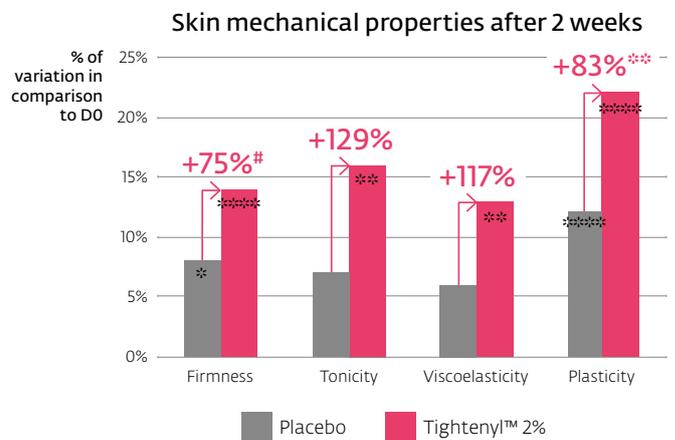
The clinical efficacy of Tightenyl™ was evaluated in a single blind test *versus* placebo under dermatological control. Twenty four women volunteers aged 47 to 65 years old participated in the study, 50% of them being menopausal. The volunteers applied twice a day the placebo on one side of their face, and a cream containing 2% of Tightenyl™ on the other side. Bio-mechanical properties of the skin were evaluated on each side of their face after 2 weeks.

**Results:** Tightenyl™ at 2% gives in 14 days a considerable improvement of the skin mechanical properties vs placebo:

- ▶ +75% of skin firmness (Uf)
- ▶ +129% of skin tonicity (Ur)
- ▶ +117% of skin viscoelasticity (Uv/Ue)
- ▶ +83% of skin plasticity (Uv)

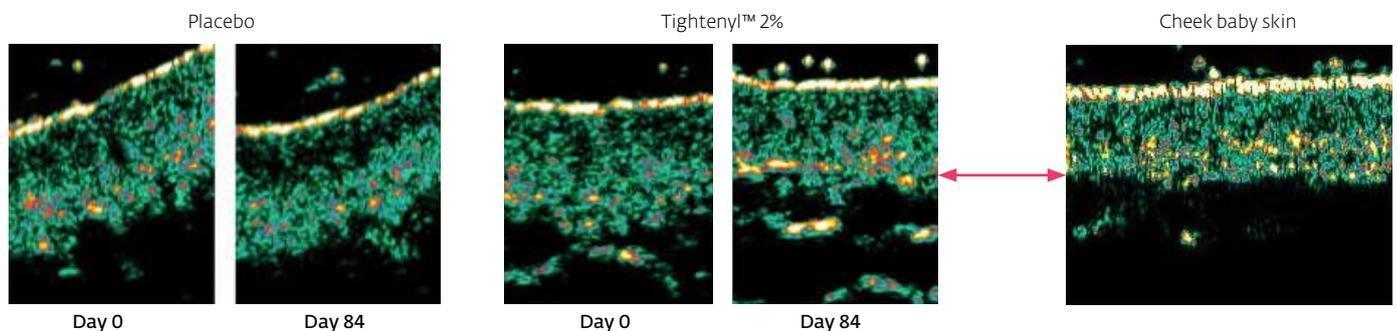
The skin recovers its **youth properties in a 2 weeks' time.**

\*\*\*\* p<0.001 ANOVA  
\*\* p<0.01 ANOVA  
\* p<0.05 ANOVA  
# p<0.1 ANOVA



## Visible improvement of skin density (clinical)

The direct impact of Tightenyl™ on skin matrix redensification by reorganisation of the ECM, was evaluated by skin echography of the volunteers. In a parallel the cheek of a baby was analysed by echography as a benchmark.



**Results:** Tightenyl™ visibly improves the skin density after 3 months of treatment (dermis is compacted and organised, less black visible), ending up in a **quality of skin matrix almost equivalent to baby skin.**

On the placebo side, a significant degradation of the skin matrix is observed (more black), confirming the ageing process progress.

# Efficacy

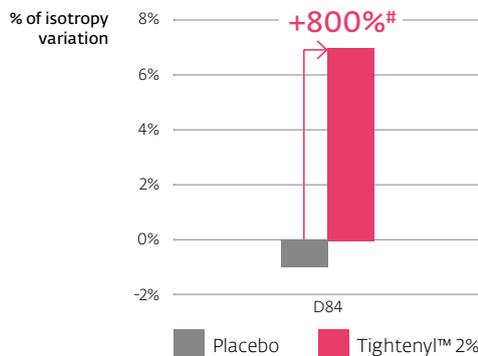
## Bio-lifting effect (clinical)

### 1. Skin isotropy

Skin isotropy is directly correlated with the quality of the skin matrix. It reflects the quality of the skin surface (micro-furrows and small wrinkles give an aged look to the face). Isotropy was measured with the PRIMOS 3D device on both sides of the volunteers' face at D84. Silicone replicas were made to visualise the skin surface, and full face pictures were taken with the Visia device in standardised conditions.

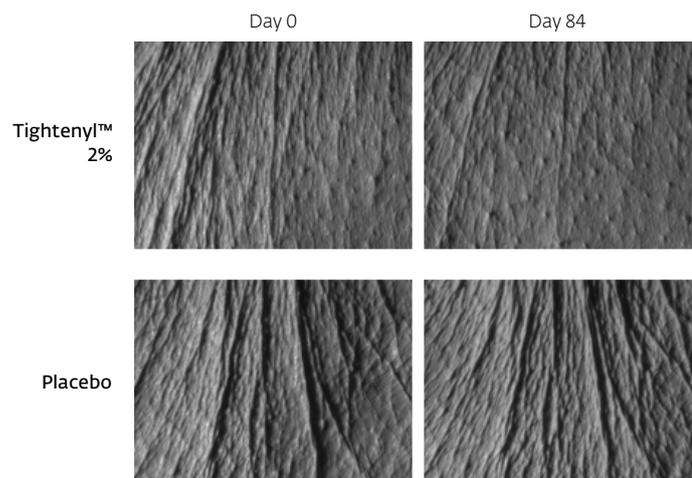
**Results:** Tightenyl™ increases by +800% the skin's isotropy: the rejuvenation of the skin matrix is clearly demonstrated on silicone replica.

# p<0.1 ANOVA



### 2. Outstanding silicone replica

**Results:** The skin is visibly smoother after 3 months by using Tightenyl™ 2%.



### 3. Best in class Bio-lifter (clinical)

**Results:** The results are outstanding by using 2% Tightenyl™ after 3 months. **The skin is visibly smoothed**, meaning that the **dermis structure is better organised**.



# Summary

## Technical information

Suggested INCI:	Glycerin (and) Water (and) Disodium Acetyl Glucosamine Phosphate (and) Sodium Glucuronate (and) Magnesium sulfate
Origin:	Biotechnology
Preservation:	Preservative free
Appearance:	Clear, light yellow liquid
Solubility:	Water soluble
Dosage:	0.6-2%
Processing:	Can be added at the end of the formulation process under stirring or homogenising. Can be heated for a short time with the water phase of formulation. Formulate at temperature below 50°C, and final pH below 6.0.

## Claims

Claims:	Skin lifting, skin sagging prevention, anti-ageing, skin firming, anti ageing products for sensitive skins
Applications:	Replacement of retinol, Anti ageing creams, Face lifting serums, Intensive treatments for facial rejuvenation, Night and day creams to firm the skin, Active anti- ageing sprays, Multifunctionnal creams (BB or CC creams), Dermocosmetic products.

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