



Peptan Beauty *ex vivo* objectivation test

Peptan is a bioactive ingredient known to promote skin beauty. The efficacy of Peptan has already been demonstrated through *in vitro* trials and clinical studies. The aim of this study is to assess the influence of Peptan collagen peptides on several indicators of skin elasticity, moisture and tonicity. This was carried out by using a model of *ex vivo* human skin explants, maintained in survival, soaked in a solution containing Peptan. The results obtained showed that Peptan exhibits a positive action on the parameters assessed.

Introduction

In this study, we intended to explore further the activity of Peptan collagen peptides on skin structure by histological techniques through the examination of parameters such as the influence on the expression of collagen and acidic Glycosaminoglycans (GAGs). Acidic GAGs close in the epidermis and in the papillary dermis (upper layer of the dermis) are essentially composed of Hyaluronic acid which is involved in skin moisture. As for collagen, it is a component of the dermal fibrillar structure. *In vivo*, GAGs are involved in skin elasticity and moisture (1-2) and collagens are responsible for skin tonus (3).

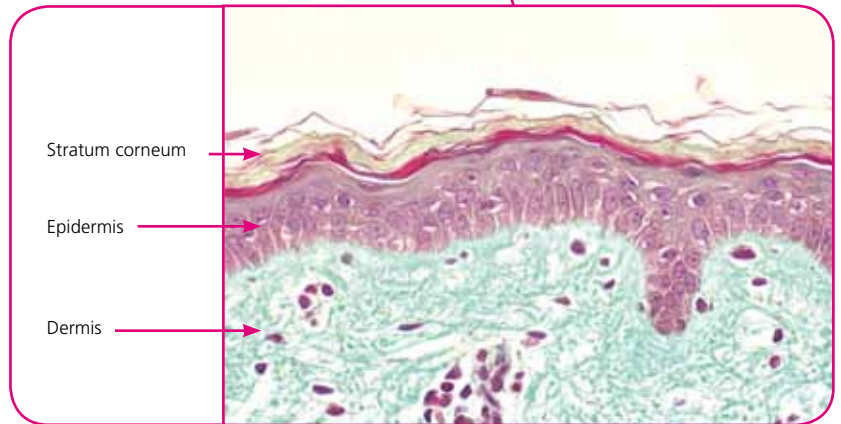


Figure 1: General morphology

1. Methods

The *ex vivo* test was performed, using a technique developed by Bio EC laboratory (France), on explants obtained from a 49-year-old Caucasian woman undergoing abdominoplasty. The prepared skin explants were kept in survival culture medium for 9 days (D9) and Peptan Fish collagen peptides (Peptan F) was mixed to the culture medium on D0 so as to obtain the final concentration required.

Skin explants were soaked in the medium to simulate oral ingestion. Only the lower part of the dermis was in the media (The epidermis was not in contact with the solution).



Figure 2: Skin explants in culture media

Batch	Designation	Nb explants	Sampling
D0	Control	3	D0
T	Untreated explants	3	D9
P1	Peptan F 0.01 mg.mL ⁻¹	3	D9
P2	Peptan F 0.1 mg.mL ⁻¹	3	D9
P3	Peptan F 1 mg.mL ⁻¹	3	D9

The cellular viability and general skin morphology were assessed after staining deparaffinised sections. The immunostaining of collagens was made on frozen sections with polyclonal anti-collagen antibody (specific to collagen I, III or VII). The visualisation of acidic GAGs was performed after staining of paraffinized sections with Alcian blue.

The following parameters were analysed:

- Total collagen surface occupied in the dermis
- Glycosaminoglycan (GAG) acids in the epidermis

In both case, the images were analyzed so as to quantify the results and were then statistically processed.

2. Results:

The explants were processed as described previously and the various parameters presented above were evaluated.

2.1 General morphology

The results show that after 9 days, **Peptan F induces an increase in epidermal thickness** as well as an **increase in collagen density in the papillary dermis** on the general morphology.

The increase in collagen density has been confirmed by additional measures described in the total surface occupied measures.

2.2 Total collagen surface occupied

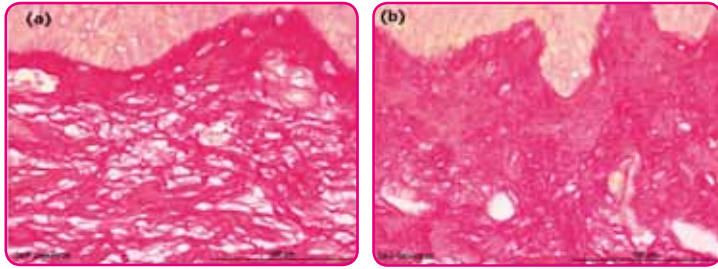
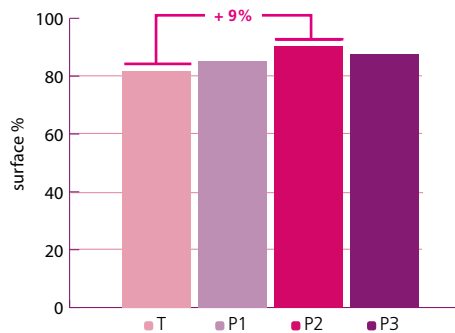


Figure 3: Total collagen surface occupied. (a) Untreated batch explants at D9. (b) Explants treated with Peptan F (0.1 mg.mL⁻¹).

Surface percentage occupied by total collagen in the papillary dermis



After 9 days of treatment, the product Peptan F at 0.1 mg.mL⁻¹ induces a **significant increase of 9%** ($p < 0.001$) in the surface percentage occupied by collagen in the papillary dermis. For Peptan F at 1 mg.mL⁻¹, there is significant increase of 5% ($p < 0.01$).

Comments from Bio EC laboratory: due to the high amount of collagen in the dermis, a 5%-increase corresponds to a good activity, comparable to the best products usually tested in similar conditions.

3. Conclusion:

In this study we have shown that **Peptan F exhibits a positive effect on skin structure**. Peptan does induce **cells to synthesise collagen and hyaluronic acid**, key components of the skin associated with **skin tonus, elasticity and moisture**. These results are fully in line with Peptan previous in vitro and clinical studies.

References

- 1 Carrino, DA., Sorrell, JM. and Caplan, AL. Age-related changes in the proteoglycans of human skin. Arch Biochem Biophys. 373(1), 91–101 (2000).
- 2 Willen, DM., Sorrel, JM., Lekan, CC., Davis, BR. And Caplan, AI. Patterns of Glycosaminoglycan/Proteoglycan Immunostaining in Human Skin During Aging. Journal of Investigative Dermatology 96, 968–974 (1991).
3. Shuster, S., Black, M. and McVitie, E. The influence of age and sex on skin thickness, skin collagen and density. British Journal of Dermatology 93(6), 639–643 (1975).

2.3 GAG acids:

Being the only GAG acid of the skin, the **hyaluronic acid represents approximately 70% of the GAG's**. The increase of the surface occupied by the Alcian blue is hence directly correlated to the stimulation of hyaluronic acid. After 9 days, for P2, Peptan F 0.1mg.mL⁻¹, Acidic GAGs are clear and quite regular in the inter-cellular spaces in the epidermis and very slightly in all the papillary dermis. They are clear in the fibroblasts. **Peptan induces a quite clear increase in the epidermis** (image analysis: **+745 %**, $p < 0.001$). After 9 days of treatment, the product P3, For Peptan F at 1 mg.mL⁻¹, induces a clear increase in the epidermis (image analysis: **+625 %**, $p < 0.001$).

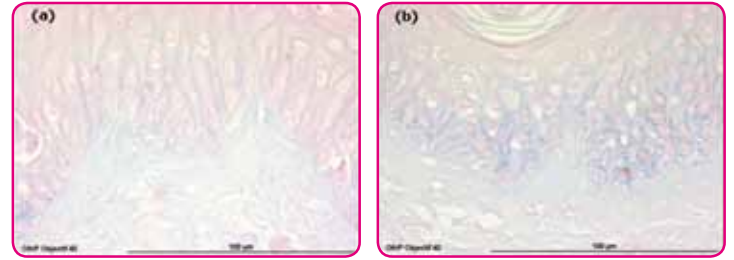
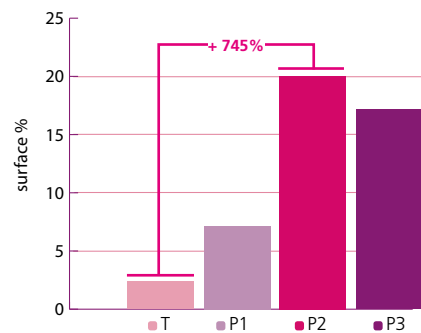


Figure 4: staining of acidic GAGs. (a) Untreated batch explants at D9. (b) Explants treated with Peptan F (0.1 mg.mL⁻¹).

Surface percentage occupied by acid GAGs in the basal layer



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