

# Improvement of dermal extracellular matrix structure and composition after treatment with Imedeen Time Perfection™ in an *in-vitro* skin equivalent.

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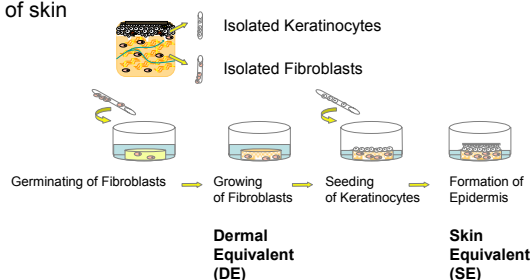
**SUMMARY:** Reconstructed skin equivalent (SE) was used to test the effects of Imedeen Time Perfection (ITP) active ingredients on structure and composition of dermal extracellular matrix (ECM). Histological results showed that ITP had a strong positive effect on the dermal reconstruction, stimulated fibroblast colonisation, proliferation and ECM synthesis. The immunohistological results showed that the stimulation concerned especially the type I collagen, the major component of the ECM and fibrillin, the major component of microfibrils.

## INTRODUCTION

Imedeen Time Perfection (ITP, produced by Ferrosan A/S, Denmark) is a skincare nutritional supplement containing BioMarine Complex, vitamin C, grape seed extract and tomato extract providing 10% lycopene.

Skin equivalent (SE) cultured in Laboratoire des Substituts Cutanes was used to test effects of ITP ingredients on structure and composition of dermal extracellular matrix.

### Generation of skin equivalent



## METHODS

Preparation of skin equivalent (SE): SE was cultured according to standard laboratory method [1,2]. A porous dermal sponge (chitosan-cross-linked -collagen-GAG matrix) was seeded with living human fibroblasts at density of  $2 \times 10^5$  cells/cm<sup>2</sup>. After 3 weeks of culture in fibroblast medium, human keratinocytes at density of  $2 \times 10^5$  cells/cm<sup>2</sup> were seeded on the top of the dermal equivalent. After 7 days of submerged culture in keratinocyte medium, the SE was lifted to air-liquid interface and cultured for 14 days. Culture medium was refreshed every second day.

Application of ITP ingredients: Medium in treated samples was supplemented with ITP ingredients: BioMarine Complex (70 µg/ml), grape seed extract (10µg/ml), tomato extract (10 µg/ml) and vitamin C (6 µg/ml). The supplementation was from first medium change and during the entire culture period of 6 weeks. Control SE without added ingredients were cultured in parallel. All ingredients except for tomato extract were well water soluble. Tomato extract was solubilized in tetrahydrofuran (THF) before adding to the medium. Same final THF concentration was added to medium of control SE.

Tissue harvesting: The samples were collected after 6 weeks in culture.

Histology: Three samples of each SE were fixed in formalin and embedded in paraffin. Sections were stained with hematoxylin-phloxin-safran.

Immunohistochemistry: Three samples of each SE were embedded in OCT Tissue-Tek. Frozen sections were blocked in phosphate buffer saline with BSA. The antibodies were directed against components of extracellular matrix: elastin, fibrillin, collagen type I and III.

## RESULTS

### Skin equivalent

After 6 weeks of incubation under standard culture conditions, the skin equivalent (SE) showed a very close resemblance to native skin both morphologically (Figure 1) and regarding expression of characteristic biomarkers. Both had been demonstrated previously<sup>1,2</sup>.

### Improvement of dermal structure after treatment with Imedeen Time Perfection

ITP active ingredients had a strong positive effect on the dermal reconstruction by stimulating fibroblast colonisation, proliferation and deposition of extracellular matrix components in the porous structure of dermal substrate (Figure 2) resulting in more *in-vivo*-like structure as compared to control SE (Figure 1).

Figure 2 shows a densely packed dermis filled with newly synthesized extracellular matrix components (light pink extracellular material) and a large number of fibroblasts (dark dots).

Also deep parts of dermis were colonized by fibroblasts and filled completely with extracellular components which is not commonly seen under standard culture conditions.

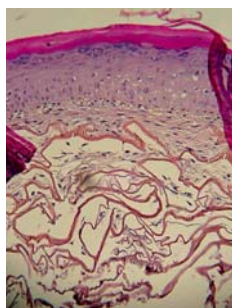


Figure 1: Skin equivalent

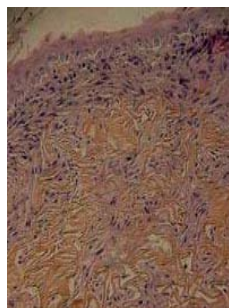


Figure 2: Skin equivalent treated with ITP ingredients

### Increase of collagen type I and fibrillin after treatment with Imedeen Time Perfection

Immunohistochemical staining demonstrated a larger amount of collagen type I, the major component of extracellular matrix, and fibrillin, the major component of microfibrils of elastic tissue necessary for elastin deposition, present in ITP treated skin substitute (Figures 3 and 4) as compared to control cultures (Figures 5 and 6).

Collagen III did not show any differences between treated and control samples.

Elastin was not expressed neither in ITP treated nor control cultures under the experimental conditions. This was due to relatively short culture period of 6 weeks. Under standard conditions it typically takes 8 to 10 weeks before elastin can be detected. Absence of elastin shows that the speed of maturation of the SE was not affected in ITP treated cultures.

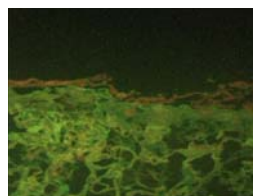


Figure 3: Collagen I (green) in SE treated with ITP

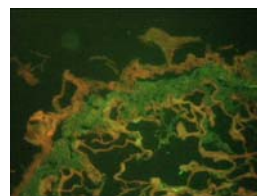


Figure 4: Collagen I (green) in control SE

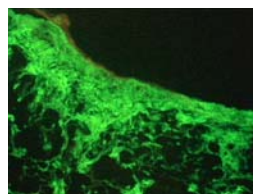


Figure 5: Fibrillin (green) in SE treated with ITP

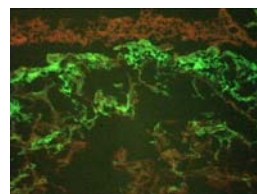


Figure 6: Fibrillin (green) in control SE

## REFERENCES

[1] Saintigny G, Bonnard M, Damour O, Collombel C. Acta. Derm. Venereol, 1993, 73:175-180; [2] Sahuc F, Nakazawa K, Bethod F, Collombel C, Damour O. Wound Rep Reg, 1996, 4:93-102