

Laboratoire BIO-EC

Centre de REcherches Biologiques et d'EXperimentations Cutanées

Evaluation of an ionizing system on the penetration of a collagen solution into skin explants maintained ex vivo.

STUDY 08E1661

Tested product:

-Iono-Pen system

-Pentapharm collagen treatment solution, batch 41028301/800686

Study report submitted by BIO-EC on December 29, 2008
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STUDY 08E1506 according to quotation D07-366

Study Sponsor:

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OBJECTIVE OF THE STUDY

The purpose of this study was to investigate the ability of an ionizing system, **iono-Pen**, to promote the penetration of a collagen solution into human skin explants maintained in survival conditions.

The activity was evaluated by observing the general morphology and, more specifically, by tracking collagen traces within the stratum corneum and epidermis after Masson's trichrome staining.

EXPERIMENTAL PROCEDURE

1. Tested Products

- Iono-Pen system
- Pentapharm collagen treatment solution, batch 41028301/800686

2. Treatment

An abdominal skin flap (defatted) obtained from a 36-year-old woman (reference P636AB36) was used. Two areas of similar surface were prepared.

One vial of collagen treatment (Pentapharm), batch 41028301/800686, was reconstituted with 2 mL of cosmetic vial solvent (products supplied by the sponsor).

- **1 mL** was applied topically to one area and spread over the entire surface using a finger cot.
- The other area was treated with the same amount of product administered using the ionizing system.

The application time was identical for both treatments.

3. Preparation of Explants

Nine explants were prepared as follows:

- Three control explants were taken from the untreated area.
- Three explants were prepared from the area treated with conventional topical application of the collagen treatment.
- Three explants were prepared from the area treated with the ionizing system.

The explants were distributed into three groups:

- **T**: Untreated area – 3 explants
- **P1**: Area treated without ionization – 3 explants
- **P2**: Area treated with ionization – 3 explants

After treatment, explants were maintained in survival for 30 minutes in **BEM medium (BIO-EC's Explants Medium)** at 37°C in a humidified atmosphere enriched with 5% CO₂.

4. Sampling for Histology

Thirty minutes after the end of application, each explant was cut into two halves:

- One half was fixed in standard Bouin's solution
- The other half was frozen at –80°C

5. Histological Processing

After 48 hours of fixation in Bouin's solution, samples were dehydrated and embedded in paraffin using a Leica 1020 dehydration processor. They were embedded according to operating procedure MO-H-153 using a Leica EG 1160 embedding station.

Sections of 5 μm thickness were prepared according to procedure MO-H-173 using a Minot-type microtome (Leica RM 2125) and mounted on Superfrost® histological glass slides.

6. Microscopic Observations

Microscopic observations were performed using light microscopy with a Leica DMLB microscope at 63× magnification.

Images were captured using a Sony DXC 390P tri-CCD camera and stored using Leica IM1000 data archiving software.

6.1 General Morphology

General morphology was assessed on paraffin sections after Masson's trichrome staining (Goldner variant) according to procedure MO-H-157. Collagen fibers appear green when stained with light green.

RESULTS

A. General Morphology

At T0 (baseline):

Control skin (T):

The stratum corneum is moderately thick, slightly laminated, and clearly keratinized at the surface (red structure), with very slight keratinization at its base. The stratum lucidum (zone at the base of the stratum corneum) is clear and shows no particular staining.

The epidermis presents 4 to 5 cellular layers with good morphology. The dermo-epidermal junction has moderate relief. The papillary dermis shows fairly thick collagen fibers forming a dense network and is well cellularized.

At 30 minutes after treatment:

Skin treated by conventional topical application of collagen solution (P1):

The stratum corneum is fairly thick, well laminated, and clearly keratinized at the surface, with very slight keratinization at its base. At the level of the stratum lucidum, a slight green staining is observed fairly regularly. The rest of the skin structure remains unchanged.

Skin treated with collagen solution using the ionizing system (P2):

The stratum corneum is moderately thick and moderately laminated, with clear surface keratinization and very slight basal keratinization. At the level of the stratum lucidum, a moderate and fairly regular green staining is observed. The remainder of the skin structure is unchanged.

DISCUSSION

General Morphology

At T0:

The observed morphology corresponds to normal skin architecture, with no collagen staining in the stratum lucidum or deeper layers.

At 30 minutes:

- In explants treated with conventional topical application, the stratum lucidum shows slight green staining, indicating limited collagen penetration. No staining is observed in deeper epidermal layers.
- In explants treated with the same collagen solution using the ionizing system, the stratum lucidum shows more pronounced green staining. No staining is observed in deeper living epidermal layers.

CONCLUSION

Under these experimental conditions:

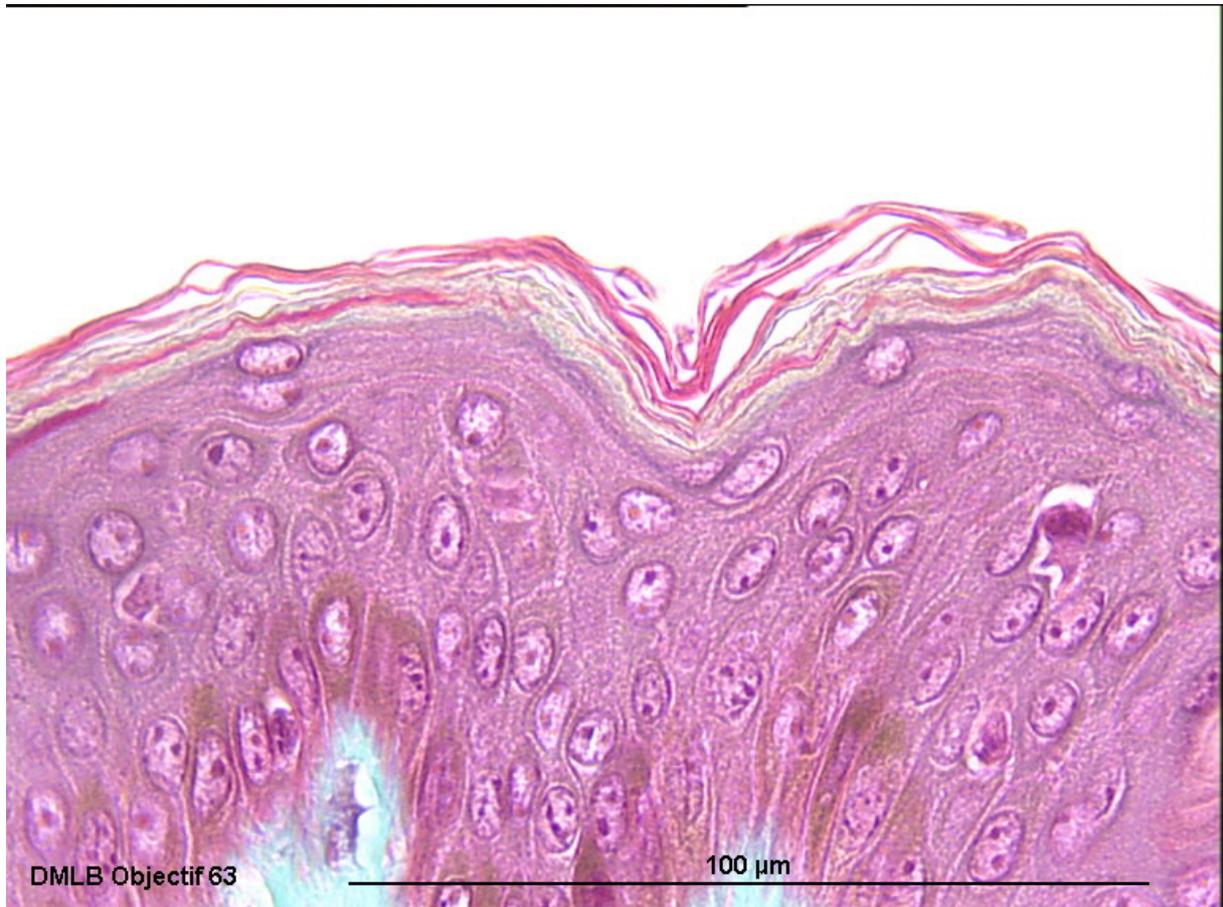
- The ionizing system increases the penetration of collagen applied in solution to the surface of the stratum corneum.
- This penetration appears limited to the base of the stratum corneum and is not observed in the underlying living epidermal layers.

General morphology



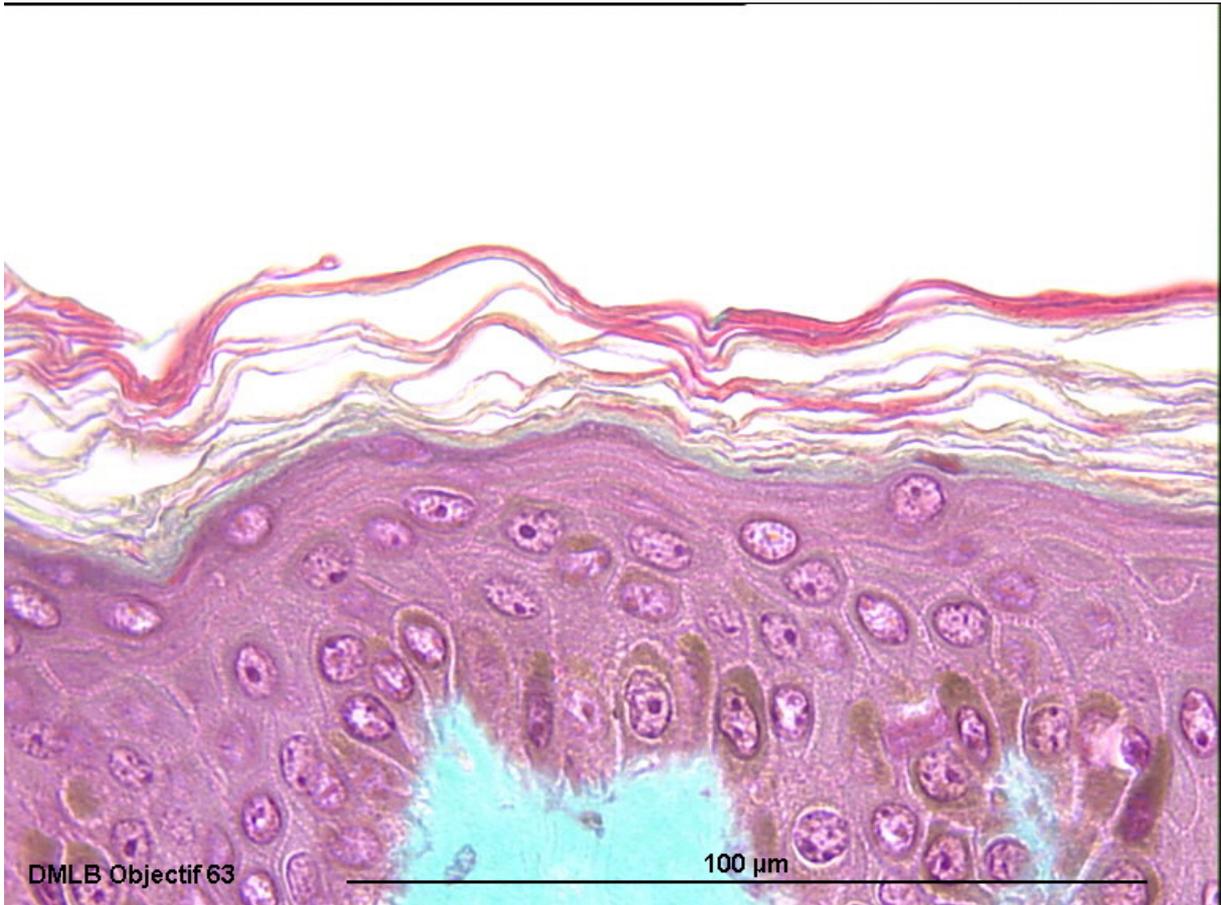
Untreated control explants

General morphology



Untreated control explants

General morphology



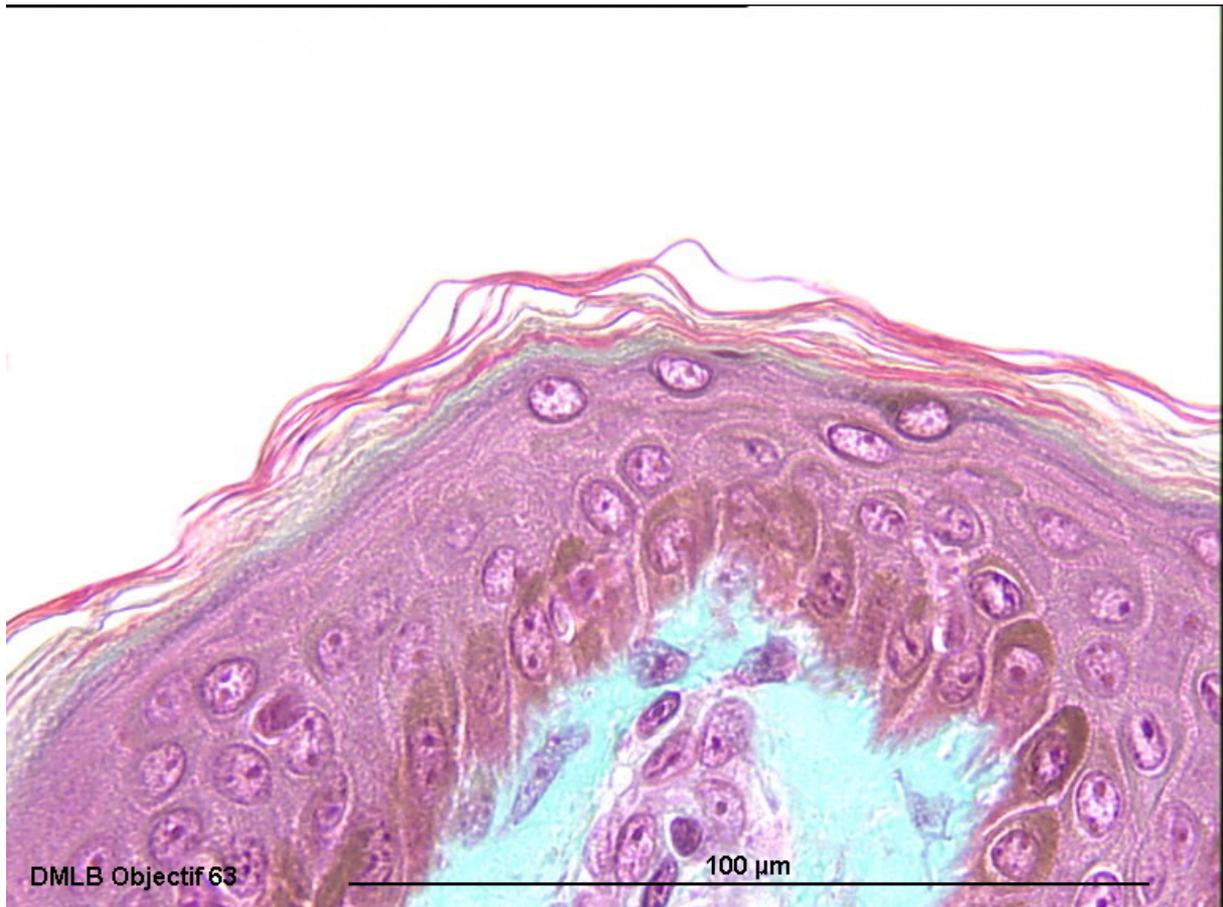
Explant + Pentapharm collagen treatment + massage

General morphology



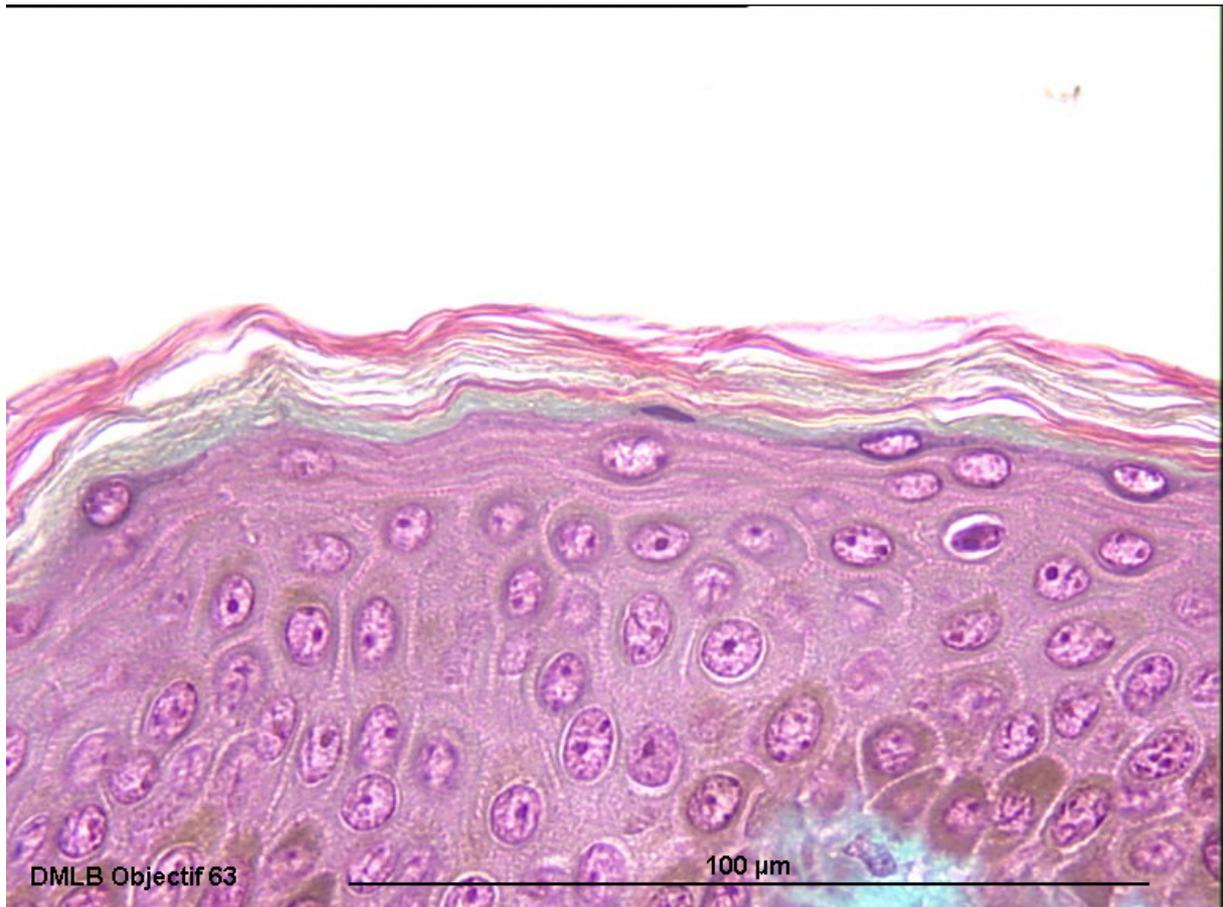
Explant + Pentapharm collagen treatment + massage

General morphology



Explant + Pentapharm collagen treatment + ionization + massage

General morphology



Explant + Pentapharm collagen treatment + ionization + massage