

**IN VITRO EVALUATION OF PENETRATION
CAPABILITY OF A COSMETIC ACTIVE THROUGH IN
VITRO RECONSTRUCTED HUMAN SKIN**

BLOOMAGE FREDA BIOPHARM CO., LTD

**HA-Oligo degraded by hyaluronidase
(miniHA™)**

Farcoderm srl

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KEY PERSONNEL

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INTRODUCTION

Aim

The aim of this study was to investigate the penetration capability of a cosmetic active (Sodium Hyaluronate Oligosaccharides) through reconstructed human skin. In order to investigate the depth reached by the absorbed active, two parallel tests were performed:

- on reconstructed human epidermis
- on reconstructed human full thickness (epidermis + dermis)

Tested product was applied on the tissues surface in aqueous solution at 0.5% and the hyaluronic acid absorbed by the tissues during the time was evaluated by ELISA assay.

An absorption kinetics was constructed by the evaluation of the hyaluronic acid absorbed by epidermis and full thickness (epidermis + dermis) in the following experimental times:

- T30min, 30 minutes after product application
- T1h, 1 hour after product application
- T2h, 2 hours after product application
- T4h, 4 hours after product application
- T8h, 8 hours after product application
- T24h, 24 hours after product application

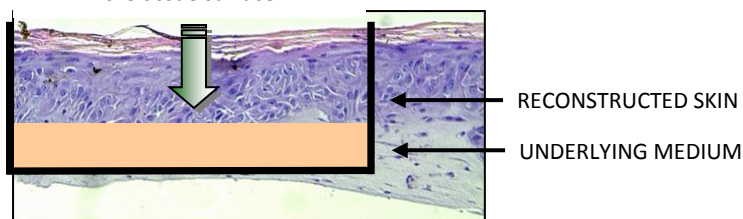
At the end of each experimental period, the treated tissues were washed, homogenated and used to determine the hyaluronic acid amount absorbed in their structure.

Tested product

BLOOMAGE FREDA BIOPHARM CO., LTD**HA-Oligo degraded by hyaluronidase
(miniHA™)****INCI:** Hydrolyzed Sodium Hyaluronate**MOLECULAR WEIGHT:** < 10,000 Da**APPEARANCE:** white powder almost taste-less and odor-less**SOLUBILITY:** Soluble in water forming a clear solution

Experimental system

Tested product is applied on
the tissue surface



The product, applied on the tissue surface, penetrates the tissue; a certain amount of the product is kept by the tissue structure.

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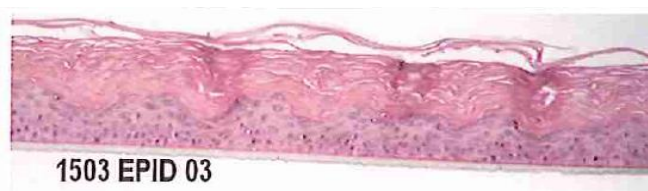
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MATERIALS AND METHODS

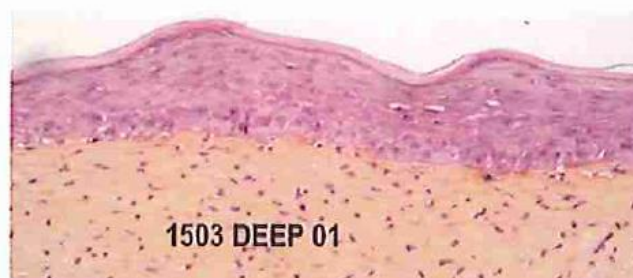
Tissues and Medium

Sterlab Reconstructed Human Epidermis and Sterlab Reconstructed Human Full Thickness were used in the test (STERLAB Batch n° 1503 EPID 03 and STERLAB Batch n° 1503 DEEP 01) as well as Maintenance medium (STERLAB Batch n° 1503-HC-520).

Human reconstructed epidermis: 0,5 cm² epidermis cultivated from human keratinocytes (EP.AJ 12), reconstituted by airlifted culture on insert polycarbonate filters 0,4 µm.



Human reconstructed full thickness: 0,5 cm² full thickness cultivated from human keratinocytes (EP.AJ 12) and collagen matrix with fibroblasts (FI. AA 29), reconstituted by airlifted culture on insert polycarbonate filters 0,4 µm.



Sample preparation and trial number

Tested products was diluted in phosphate buffer at 0.5% and the obtained solution was applied on the tissue surface. 2 units were used for each experimental condition.

Test procedure for penetration test

32 ul/cm² of solution were applied on 2 tissue units for 30 minutes (T30min), 1 hour (T1h), 2 hours (T2h), 4 hours (T4h), 8 hours (T8h), and 24 hours (T24h); at the end of exposition period treated tissues were washed with phosphate buffer and subjected to homogenization in order to dosage the hyaluronic acid absorbed.

Test procedure for hyaluronic acid dosage

Hyaluronic acid dosage was carried out by means of ELISA (Enzyme Linked Immunosorbent Assay, commercial kit). Basal amount of hyaluronic acid of untreated tissues was automatically subtracted during the test session.

Result calculation

For each collected sample at different experimental times, hyaluronic acid content, expressed in ng, was calculated. Then, according to the hyaluronic acid quantity applied on the tissue, the percentage of product absorbed at each experimental time was calculated. Absorption into dermis is mathematically calculated by difference between the total absorption into full thickness skin and epidermis.

Results are expressed as mean data ± standard deviation.

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RESULTS

In the tables below the data obtained from the hyaluronic acid dosage in the epidermis and full thickness model treated with HA-Oligo degraded by hyaluronidase (miniHA™) are reported.

HA-Oligo degraded by hyaluronidase (mini HA™) into Human Reconstructed Epidermis

Experimental time	Hyaluronic acid (ng) (mean ± st. dev.)		% absorption
Applied quantity	80000		-
T30min	8889,3	± 270,5	11,1%
T1h	18672,4	± 284,1	23,3%
T2h	23836,6	± 1252,3	29,8%
T4h	26726,5	± 2178,7	33,4%
T8h	27175,7	± 1465,3	34,0%
T24h	29406,0	± 1138,6	36,8%

HA-Oligo degraded by hyaluronidase (mini HA™) into Human Reconstructed Full Thickness (epidermis + dermis)

Experimental time	Hyaluronic acid (ng)		% absorption
Applied quantity	80000		-
T30min	10122,4	± 1103,9	12,7%
T1h	28964,8	± 2820,1	36,2%
T2h	35359,3	± 3028,7	44,2%
T4h	42847,2	± 4024,3	53,6%
T8h	48564,9	± 5930,3	60,7%
T24h	55564,3	± 7737,5	69,5%

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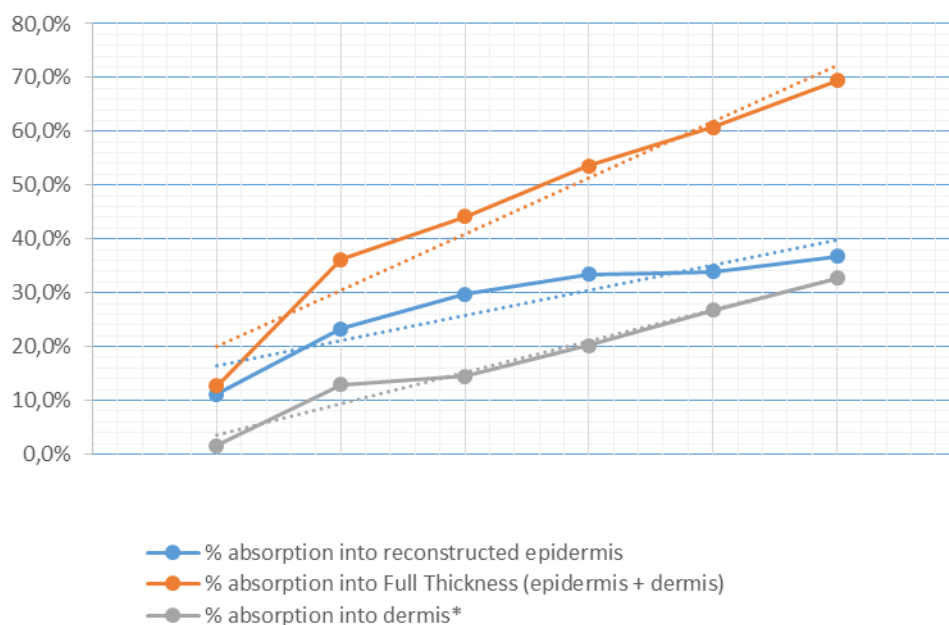
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HA-Oligo degraded by hyaluronidase (mini HA™) into dermis*

Experimental time	% absorption
T30min	1,5%
T1h	12,9%
T2h	14,4%
T4h	20,2%
T8h	26,7%
T24h	32,7%

T30min	1,5%
T1h	12,9%
T2h	14,4%
T4h	20,2%
T8h	26,7%
T24h	32,7%

HA absorption



* results mathematically calculated by subtraction of full thickness and epidermis absorption data

The results obtained in the penetration test on human in vitro reconstructed skin show that hyaluronic acid

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is capable to penetrate the tissues during the monitored experimental period.

According to obtained data, we can see that there is a quite constant time-dependent absorption gradient through the full thickness skin (epidermis + dermis), reaching 69.5% absorption.

The HA distributes through epidermis and dermis:

- The absorption have a quite linear pattern in the epidermis until 4 hours-exposition, while it reaches a plateau from 4 to 24 hours (maximum absorption potential is reached)
- The absorption have a quite linear pattern in the dermis (data mathematically calculated by subtraction of full thickness and epidermis experimentally obtained absorption data) during the monitored experimental time.
- At the end of monitored experimental time, HA equally distributes between epidermis and dermis.

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CONCLUSIONS

According to the applied and already described experimental protocol, collected data show that

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HA-Oligo degraded by hyaluronidase (miniHA™)

**crosses the skin just after 30 minutes application and
its penetration through epidermis and dermis has a time-depending trend.**

San Martino Siccomario – 8th April 2015

Experimenter

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Quality Control

Dr.ssa Angela MICHELOTTI

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