EL-M061127

Clinical Trial Report

Clinical Efficacy Evaluation of Three Moisturizing Products in Improvement of Moisture Content of the Skin

November 27, 2006

Ellead Skin Research Center Co., Ltd.

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Clinical Efficacy Evaluation of Three Moisturizing Products in Improvement of Moisture Content of the Skin

Study Number :	**EL-M061127**
Sponsor :	**Bioland Co., Ltd.** **39-4 Songjeong, Byungchon, Cheonan, Chungnam, Korea**
Clinical Research Facility :	**Ellead Skin Research Center** **272-1, Seohyun-dong, Bundang-gu, Seongnam, Korea**
Study Dates :	**September 12, 2006 ~ November 27, 2006**
Investigator :	**Tae-Kee Moon, M.D.** ***Dermatologist***

November 27, 2006

Ellead Skin Research Center Co., Ltd.

Contents

Study Result Summary

Title of Study	Clinical Efficacy Evaluation of Three Moisturizing Products in Improvement of Moisture Content of the Skin			
Research Facility	Ellead Skin Research Center	Duration of Study	September 12, 2006 ~ November 27, 2006	
Methodology	Study Date	September 12, 2006 ~ November 3, 2006	Subjects	12 healthy women and men
	Study Details	1. Lasting Test for Moisturizing Effects : ● Subjects: 12 healthy women and men ● Procedures : Amounts of 2㎕/㎠ on each test areas were applied and after 9 minutes 30 seconds, purified water were applied. 30 seconds after applying purified water, Kim-Wipes was used to remove moisture and test areas were measured 5 times in 1 minute interval at 0, 1, 2, 3, 4, 5, 6, 7 and 8 minutes with Corneometer (CM825 Courage & Khazaka, Germany) 2. Evaluation Based on Corneometer measurement, skin hydration improvement rate of three products was compared with baseline and at each time point.		
Results	Clinical trials of three moisturizing products were requested by Bioland Co., Ltd. and all three moisturizing Products showed longer lasting moisturizing effects compare to baseline up to 8 minutes in time and after an application 8 minutes base, Oligo HA 0.01% showed highest improvement rate of moisture content of skin and the product which showed the longest lasting moisturizing effects Moisture content (%) was HA 0.01.			
Annexing	Report			

STUDY RESULT REFERRAL

1. Test Article: Three skin care products

2. Duration of Study: September 12, 2006 ~ November 27, 2006

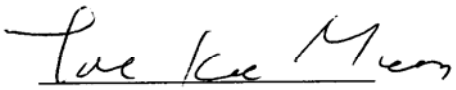
3. Method: Measurement of the moisture content using non-invasive instrument

4. Result:

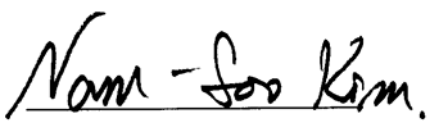
Clinical trials of three moisturizing products were requested by Bioland Co., Ltd. and all three moisturizing Products showed longer lasting moisturizing effects compare to baseline up to 8 minutes in time and after an application 8 minutes base, Oligo HA 0.01% showed highest improvement rate of moisture content of skin and the product which showed the longest lasting moisturizing effects Moisture content (%) was HA 0.01.

Research Facility: Ellead Skin Research Center

Research Coordinator: Tae-Kee Moon, M.D.
/Sub-Investigator



Clinical Investigator: Nam-Soo Kim, Ph.D., M.D.



Research Facility Information

Title of Study: Clinical Efficacy Evaluation of Three Moisturizing Products in Improvement of Moisture Content of the Skin

Sponsor

Name: Bioland Co., Ltd.

Address: 39-4 Songjeong, Byungchon, Cheonan, Chungnam, Korea

Study Monitor: Chang Sung Han

Chief Researcher

Research facility

Name: Ellead Skin Research Center Co., Ltd.

Address: 272-1, Seohyun-dong, Bundang-gu, Seongnam, Korea

Investigator: Tae-Kee Moon, M.D.

Research Coordinator

Tel: 82-031-709-9070 / Fax: 82-031-703-9071

Establishment:

This institution conducts *in vivo* clinical trials for the efficacy evaluation of cosmetics in sunscreen, moisturization, elasticity, fine lines, etc. and developmental researches in related area.

Facilities & equipments:

Solar simulator Measuring System, Multi-port Solar simulator 601-300W, 3D-600 Meter, Solar UV Simulator (Oriel), Smartmeter GRP-1, Digital Camera, Skin-Visiometer SV 600, Visioscan VC98, IL1700 Radiometer, Camscope (Model DCS-105), Corneometer CM 825, Chemical Balance, Mexameter MX18, Chromameter CR-300, Cutometer 580MPA, Tewameter TM300, Sebumeter 810PC, Skin pH meter pH900PC, Thermohygrostat, Sensor for Room Condition SRC 100, Laboratories, Micropipittes & Pistone pipettes

Information on The Test Article

A) Name of Product : HA 0.01 %, Oligo HA 0.01%, Trehalose 0.01%

B) Identification Code : EL-M061127

C) Safety and Specification :

The requesting organization is responsible for the properties and safety of test article.

Duration of The Study

This study was conducted in the order of volunteer recruitment, evaluation of the safety and the efficacy of the test article, data analysis, and framing a report.
Duration of the study is as follows:

September 12, 2006 ~November 27, 2006

(Intake date: September 12, 2006 ~ November 3, 2006)

Research Facility

Ellead Co,. Ltd.

(Tel: 031-709-9070/6358, Fax: 031-703-9071, www.ellead.com)

Objective

Skin is a surrounding barrier of body, which protects the internal organs from the external environment. Total area of skin differs from individuals, but its average value is about 1.5-2.0 ㎡, 1.6 ㎡ for adult male, and 1.4 ㎡ for adult female, and these values are approximately 7-fold of that of children. The average thickness of skin is about 2-2.2 ㎜ and thickness except hypodermics is about 1.4 ㎜. The thinnest part out of body is eyelid and the thickest parts are the palm of the hands and foots. Skin is composed of the epidermis, dermis, and hypodermics. The thickness of hypodermics is determined by the amount of hypodermal fat and differs from a site, age, race, and nutrition. Because epidermis is the first visible region of skin surface, it is highly connected with cosmetics. Epidermis is composed of stratum corneum, stratum lucidum, stratum granulosum, stratum spinusum, and stratum basale. The stratum corneum was the final cell layer generated from division of keratinocyte. About 20 layers of thin corneous cells are overlapped to form stratum corneum, which is mainly composed of keratin and protein. The moisture of stratum corneum is protected by the amino acid called to NMF (Natural Moisturizing Factor) and its metabolite (pyrrolidone carboxylic acid: high hygroscopic metabolite of glutamine) and skin lipid.

Skin lipid is composed of both sebum secreted from sebaceous glands and lipid from derived epidermis, and 0.4~0.05 ㎎/㎠ of lipids are always maintained in human skin.

The main function of skin lipid is endowing moisture and softness to stratum corneum by prohibiting evaporation of moisture from skin, protecting invasion of bacteria or harmful materials from the external environment, and keeping moisture of internal body.

Subject Selection

The 12 subjects (12 healthy women) were recruited by the research facility and screened to pass inclusion/exclusion criteria by the principal Investigator. Any subject who was entered into the study by meeting its selection criteria should have signed the informed consent form for indicating her voluntary agreement to taking part in the study, and have kept the information she gain during the study confidential.

A. Inclusion Criteria

1. 20-55 years old females in good general health
2. Has a fair skin without any skin disorder (e.g. atopic dermatitis, etc.) or any concurrent illness defined as any pre-existing or incurred illness condition, or pathology
3. Willing and able to follow all study directions, accept skin examination and commit to all follow-up visits for the duration of the study
4. Must have read and signed the informed consent form prior to start of the study
5. Do not use any other moisturizing product (e.g. moisturizing body cleanser, etc.) because this will interfere with the results and might make your skin irritated

B. Exclusion Criteria

1. With history of allergy to cosmetic products or skin cleansers
2. Has a history of a disease/condition or a concurrent illness that could interfere with the outcome of the study
3. Currently or within the last 6 months using oral retinoids or steroids

4. Currently or within the last 3 months undergoing dermatologist treatments or procedures
5. Currently involved or participated in any skin care clinical investigation within the last 3 months
6. Pregnant or nursing female

C. Exclusion Criteria during the study period

1. Withdrawn from the study by requesting of subject, but being not related to test product application
2. Reported any adverse experience defined as any unwanted sign or symptom, which in any way may be related to test product
3. Severe unexpected adverse events, which are associated with the use of this test product
4. Lost from the study without clear giving reasons why subject do not wish to continue if any subject fails to return for any scheduled examination

D. Restrictions of Subject

1. The products need to be used as instructed in order for the results to be meaningful.
2. Subjects will not be allowed to use any other moisturizing product that functions similarly as the test product assigned to them.

E. Management of Subjects

1. Good Clinical Practice (GCP)

Both Sponsor and Investigator have intended to observe the GCP, the fundamental spirit of the Helsinki Accords, and the domestic regulations in doing, assessing and recording the study.

2. Informed Consent Information

Investigator will get subject to see detailed written information in the above study and understand the nature of the study, why and how it will be performed and the possible risks. When subject have read the information provided and subject are interested, subject will be asked to sign the attached consent form prior to study entry. Additionally, the subjects will be considered dependable and able to read, understand and follow instructions.

- The objective and methods of the study
- Any unexpected skin reaction or sign
- Does not receive any disadvantage by consenting to the study
- May stop the study at any time after sent to con the study
- Proper compensation and medical care having followed correctly the instructions given for the occurrence of any serious problem or adverse event
- Maintained the Confidentiality of the subject
- Needed to protect human rights of a subject

3. Adverse Events

The Sponsor, Bioland Co., Ltd. has a responsibility for medical treatment and compensation if any serious problem occurs as a direct result of the subject’s

participation in the study, having followed correctly the instructions given.

In the event of such adverse reaction must involve following

- Investigator in charge must be compliant of the study protocol
- The occurrence must not be of the researcher's intention, or damage
- Severe unexpected adverse events, which are associated with the use of this product, must be reported to the sponsor. Bioland Co., Ltd.

No compensation will be provided in the case of

- Adverse reaction from using any article that is not supplied by Bioland Co., Ltd. or the study conducted is not requested and sponsored by Bioland Co., Ltd.
- No beneficial effect from the test article of participating the study
- Damage provoked from not following the testing procedure agreement
- Damage resulted from inattention or negligence on the part of subjects

F. Compensation

Compensation to the subjects will be given immediately after successful completion of the study.

If the subject in conjunction incurs medical expenses with severe clinical symptoms judged to be definitely related to test material use by the principal investigator or study physician, provided that the study instructions given have been followed correctly, they will be billed separately to the Sponsor, Bioland Co., Ltd. If a

physician is engaged by the Sponsor to examine any problem or to participate in any observation beyond scheduled visits, his fees will also be billed separately to the Sponsor.

G.. Confidentiality

The subjects' names and their participation in the study will remain confidential. Subjects will likewise be asked to keep any information she gains during the study confidential. Subject's personal information will not be used for any promotional or advertising purpose without her prior consent.

Any information of the study requested by Food and Drug Administration or for research purposes will be released preserving the subjects' anonymity.

H. Responsibilities of Subject

The below are to be followed for safety of subjects and the study instructions.

- The direction for application of the test article, restrictions, and scheduled visit must be kept as instructed.
- In case of any unexpected or unusual sign symptom, subject should report to principal investigator.
- Reasons for subject removal from the investigation may include occurrence of significant protocol violation or non-compliance. Subject lost from the study will be classified as dropouts by the exclusion criteria as mentioned above.

I. Right to Withdraw

Participation of the subjects is purely voluntary. Subjects may stop the study at any time without receiving any disadvantage, and do not have to tell Investigator the reasons if subjects wish and understand that these reasons will be documented.

Experimental Design

A. Volunteers

Before being entered into the study, the subjects were recruited and pre-screened by the Investigator for the criteria indicated in the subject selection section. The research agency recruited the 12 subjects for the study by conducting via phone interview of the applicants who registered on-line at the internet site of Ellead, and Yonsei-Monet dermatology to take part in the study. Subjects should have read and signed the informed consent form prior to start of the study. The study has been a double blind, randomized study.

B. Procedures

The study evaluated the efficacy of Three moisturizing products requested by Bioland Co., Ltd., in improvement of moisture content of the skin. Subjects were evaluated using the non-invasive instruments, Corneometer® CM 825 (Courage & Khazaka, Germany; a probe will be pressed on the skin to measure moisture content).

All evaluations were done in a climate-controlled facility at a temperature of 23± 2 °C and a relative humidity of 45 ± 5 %. Subjects were asked to arrive 30 minutes before the evaluation time in order to adjust to the conditions.

1. Lasting Test for Moisturizing Effects

The 12 subjects (12 healthy women) were directed to take a shower in the morning without using any soaps or moisturizing body cleanser and to visit Ellead Skin Research Center Co.,Ltd. to wash their right and left forearm areas (from wrist to elbow) under

running water. Total 3 perfect squares of 2cm on each side with 1.5cm distance between each perfect square were drawn on testing areas (on flexure part of forearm), 5cm distance from subject's wrist. 3 Test areas were randomly selected on either right or left forearm of each subject.

After the climate-controlled facility standby, test areas were measured 5 times with Corneometer and the average of 3 measures excluding the maximum and the minimum measurement was calculated (baseline).

Amounts of 2㎕/㎠ on each test areas were applied and after 9 minutes 30 seconds, purified water were applied. 30 seconds after applying purified water, Kim-Wipes was used to remove moisture and test areas were measured 5 times in 1 minute interval at 0, 1, 2, 3, 4, 5, 6, 7 and 8 minutes with Corneometer also the average of 3 measures excluding the maximum and the minimum measurement was calculated.
No washing off or rubbing on test areas was allowed.

Evaluation

A. Instrumental Evaluations

Based on Corneometer measurement, improvement rate of skin moisturzation was calculated by comparison with baseline and at each time point(0, 1, 2, 3, 4, 5, 6, 7 and 8 minutes) results.

Results

A. Subject's Characteristics

Table 1. Subject's Characteristics

No.	Name	Age	Sex
1	KEN	37	F
2	KJO	38	F
3	KKH	46	F
4	BLM	23	F
5	SMN	21	F
6	KEK	41	F
7	PKS	49	F
8	PHO	41	F
9	SYS	53	F
10	YNS	48	F
11	LJS	26	M
12	ICY	39	F
Average		39	
S.D. *		10	

*: represent the standard deviation from mean

Participants in the study were 12 healthy adult women age 21-53 years (39 as average) who had signed an informed consent. Separately the age group was as follows:

Table 2. The age bracket of subjects

	Twenty	Thirty	Forty	Fifty
No.	3	3	5	1
%	25	25	42	8

B. Results

(1) Corneometer measurement results

Each test site applied test samples were evaluated at baseline and at 0, 1, 2, 3, 4, 5, 6, 7 and 8 minutes using Corneometer® CM 825.

When the skin's moisture content increases, the measured value(hydration level) should also rise; if the skin's moisture content decreases, however, the measured value should also decrease.

Table 3. Corneometer measurement results of three moisturizing products

Average	HA 0.01%	Oligo HA 0.01%	Trehalose 0.01%
Bef	28.1	25.2	29.4
0min	72.3	66.5	76.2
1min	52.4	46.6	51.5
2min	49.4	43.9	48.4
3min	47.1	42.0	46.3
4min	45.6	40.9	45.0
5min	45.1	40.4	44.3
6min	43.7	39.8	43.6
7min	43.1	38.8	43.3
8min	42.4	38.2	43.1

Table 4. Skin moisturization rate of elevation (%)* of three moisturizing products

Rate of elevation (%)*	HA 0.01%	Oligo HA 0.01%	Trehalose 0.01%
0min	157.31	164.35	159.02
1min	86.56	85.21	75.07
2min	75.79	74.28	64.49
3min	67.59	66.89	57.22
4min	62.35	62.36	52.88
5min	60.47	60.49	50.42
6min	55.43	58.17	48.35
7min	53.46	53.97	47.12
8min	50.79	51.88	46.46

Table 5. Moisture content (%)** of three moisturizing products

Moisture content (%)	HA 0.01%	Oligo HA 0.01%	Trehalose 0.01%
0min	100.00	100.00	100.00
1min	55.03	51.85	47.21
2min	48.18	45.20	40.56
3min	42.96	40.70	35.99
4min	39.64	37.94	33.25
5min	38.44	36.80	31.71
6min	35.24	35.39	30.40
7min	33.98	32.84	29.63
8min	32.29	31.56	29.22

Fig. 1. Corneometer measurement results

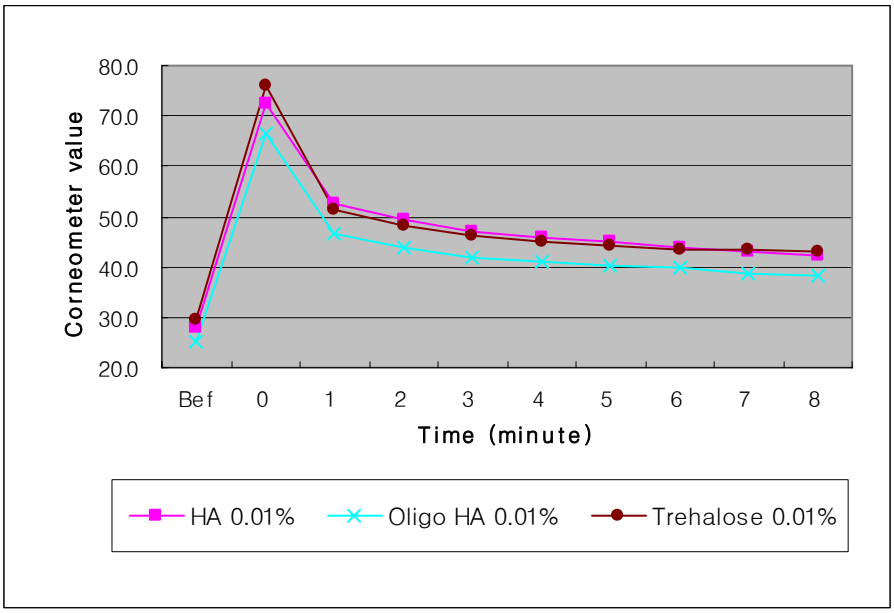


Fig. 2. Skin moisturization rate of elevation (%)*

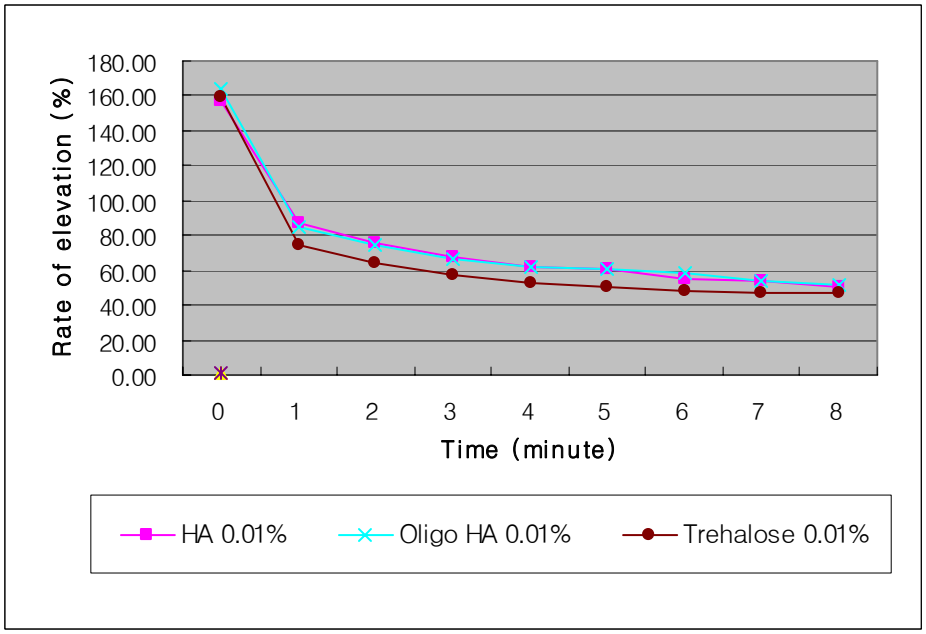
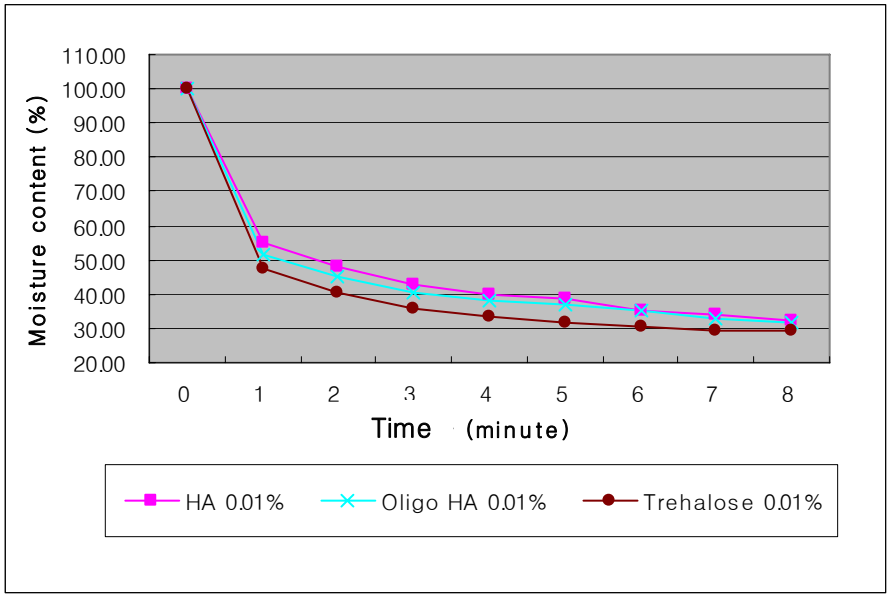


Fig. 3. Moisture content (%)** of three moisturizing products



Corneometer measurement results of Three Moisturizing Products are shown on Table 3.and Fig.1. Changes in moisture content of the skin with moisturizing products are shown on Fig.1.by evaluating rate changes based on the average at each time point. Also improvement rate of moisturizing effects of each moisturizing products at each time point against baseline are shown in Fig.2. Table 4. Improvement rate of moisturizing effects from three Moisturizing products were calculated using rate of elevation formula and the lasting effects of the products are displayed by Moisture content (%) formula and the results are described on Fig.3. Table 5.

$$\text{* Rate of Elevation (\%)} = \frac{[C_X - C_b]}{C_b} \times 100$$

$$\text{** Moisture content (\%)} = \frac{[C_X - C_b]}{[C_b - C_0]} \times 100$$

C_X : Measurement result of each hour

C_b : Measurement result of baseline

C_0 : Measurement result of 0 hour

Based at 8 minutes time point, Oligo HA 0.01% showed highest Improvement Rate of Moisture Content of Skin followed by HA 0.01% and Trehalose 0.01%.

Also, HA 0.01% shows highest Moisture content (%) followed by Oligo HA 0.01% and Trehalose 0.01%.

Conclusion and Summary

- As requested by Bioland Co., Ltd., Ellead Skin Research Center performed the clinical efficacy evaluation of three moisturizing products in improvement of moisture content of the skin with 12 panelists.

- Based at 8 minutes time poimt, Oligo HA 0.01% showed highest improvement rate of moisture content of skin and HA 0.01% shows highest Moisture content (%) .