- Final Report -

Animal Testing Report for Safety and Efficacy of Ultraskin

October 31, 2012

CHUNGANG University Industry-Academic Cooperation Foundation

CONES 全球医疗美容设备运营服务商 超能元泉

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CONES™ 超能元泰 全球医疗美容设备运营服务商

1. Test Overview

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<tr><td>Serial number</td><td>20120034</td><td>Exam date of receipt</td><td>Oct. 15, 2012</td></tr>
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<tr><td rowspan="2">The laboratory</td><td>Chuang University Industry-Academic Cooperation Foundation</td><td>Date of test</td><td>Oct. 22, 2012 - Nov. 3, 2012</td></tr>
<tr><td>Address</td><td colspan="2">Chungang Univ., Heukseok-dong, Dongjak-gu, Seoul, Korea</td></tr>
<tr><td rowspan="2">The sponsor</td><td colspan="2">WONTECH Co., Ltd,</td><td>Phone</td><td>Tel. 042) 934-6800
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<tr><td>Address</td><td colspan="3">WON TECH Co., Ltd. 64 Techno 8-ro Yuseong-gu, Daejeon, Korea</td></tr>
<tr><td>Purpose of the test and test content</td><td colspan="4">▣ Purpose of the test
This test is performed to determine the safety and efficacy of the device, Ultraskin (WONTECH Co., Ltd, Korea), by using micro-pigs (Micro-pig®).
▣ Test content
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<tr><th>Exam Name</th><th>Test animals (number of objects)</th><th>Test Method</th><th>Testing Device</th></tr>
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	Ultraskin, ultrasound equipment was irradiated to back side of Micro-pig with various Cartridge settings to observe any damage of skin tissue or adverse effects with visual evaluation, photos, and Dermoscope photos. H&E and NBTC staining confirmed any infection or necrosis reactions. Microscope measurement was used from skin surface to dermal tissue in order to determine depth of coagulation point. ▣ **Test Result** Skin surface test, skin biopsy, and NBTC test of Micro-pig showed no adverse reactions, and the ultrasound coagulation points of different cartridge settings were identical to the indicated depths.
Declaration	This test was performed according to the use and maintenance regulations of Chungang University College of Medicine and complied with Animal Ethics Committee's revised rules. **Principal Research Officer Beomjun Kim**

2. Test Content

2-1. Research Title

Animal testing report on safety and efficacy of Ultraskin

2-2. Research Purpose and Background

This test was to evaluate the safety and efficacy of Ultraskin's (WONTECH Co., Ltd. Daejeon, Korea) ultrasound by using Micro-pig®, which is similar to human skin structure and is proven to be suitable as animal skin test material.

2-3. Materials and Methods

1) Test System

(1) Type: Micro-pig®

(2) Supply source: Medi Kinetics Co, Ltd, Gyeonggi, Korea

(3) Why this test system was chosen

: skin absorption, allergic reactions, and dermal administration of Micro-pig® are similar to human's and there are less body hair than other species so it is easy to conduct tests. Also, epithelial skin structure is similar to human's, so it is known to be suitable for skin tests.

(4) Weight range: 18~23kg

2) Environment

This test was performed in sterilized SPF Micro-pig ® GLP facility with the temperature of 22±3 ℃, relative humidity 50±10 %, cleanliness 1,500, ventilation 15 T/HR, internal pressure 3mmHg, air velocity 5cm/sec, and noise level 10dB.

3) Test Group Formations & Dosage Settings

(1) Test group: 1 subject, Micro-pig®

(2) Anesthesia Method

- General anesthesia.

: injection of Zoletil 50 and Narcoxyl 2 that are diluted in fixed ratio

- Local anesthesia

: Isofluorane : Oxygen = 2.5: 2.5 (Choongwae Medical)

(3) Animal Test Method

- By using Ultraskin's 2 types of ultrasound Cartridges (4MHz and 7 MHz), ultrasound was radiated 4.5mm deep to the pig's back skin with 4MHz Cartridge and it was also radiated 3mm deep with 7MHz Cartridge. After radiating ultrasound, the accuracy of whether the coagulation point occured exactly at the target point was evaluated by measuring the penetration depth of skin tissue with Folliscope magnifier (LeedM, Seoul, Korea).

Damage to surface and dermis of skin and safety evaluation of the ultrasound irradiation was confirmed by using dermatologists' visual evaluation, photos (D3000,

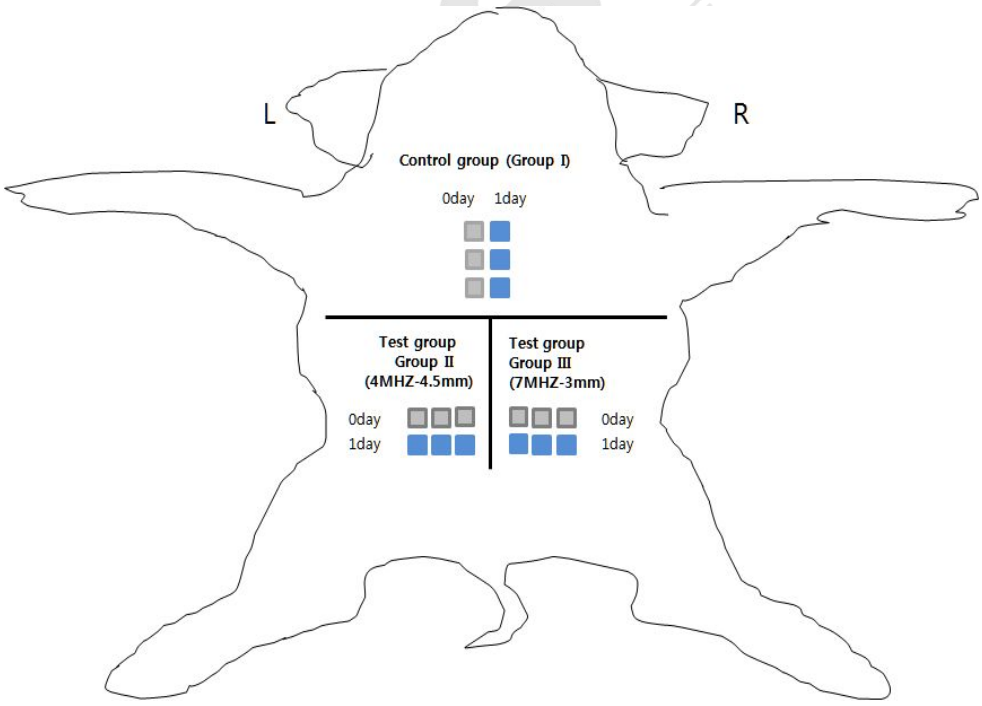
Nikon, Tokyo, Japan), Dermoscope skin polarization photos (DermLite Pro, California, USA), H&E (Hematoxylin Eosin staining) and NBTC (nitroblue tetrazolium chloride) test through pathologic skin test.

(4) Test Conditions

- Test group was divided into 3 groups and safety of the skin tissue and accuracy of ultrasound irradiation was evaluated at 0 day and 1 day after Ultraskin ‘s ultrasound was irradiated. (see Table 1 and Figure 1)

Test Group	Test Conditions
Group I	Control (Non-treated group)
Group II	4MHz – 4.5mm
Group III	7MHz – 3mm

(Table 1. Test group classification according to test conditions)



(Figure 1. Ultrasound Irradiation Fractogram)

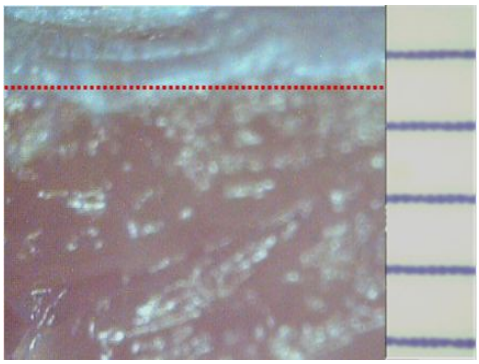
(5) Test Methods

- Visual Evaluation: visual evaluations of dermatologists
- Mechanical Evaluation: Dermascope, Folliscope, digital camera photos
- Structural Evaluation: H&E staining, NBTC staining

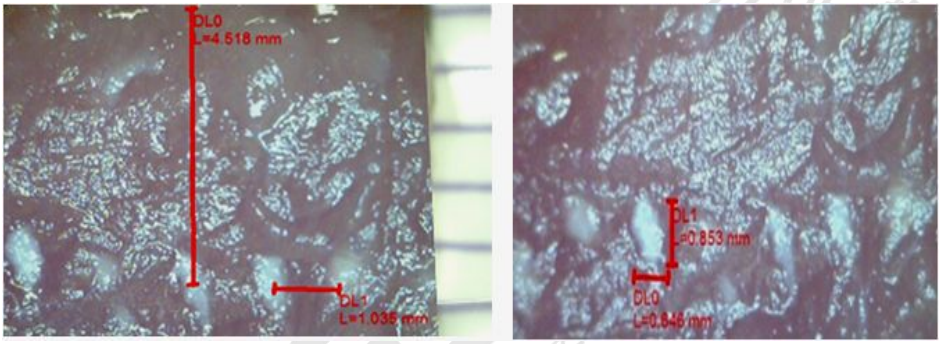
3. Test Results

1) Evaluation of Irradiation Depth Accuracy to Determine Efficacy

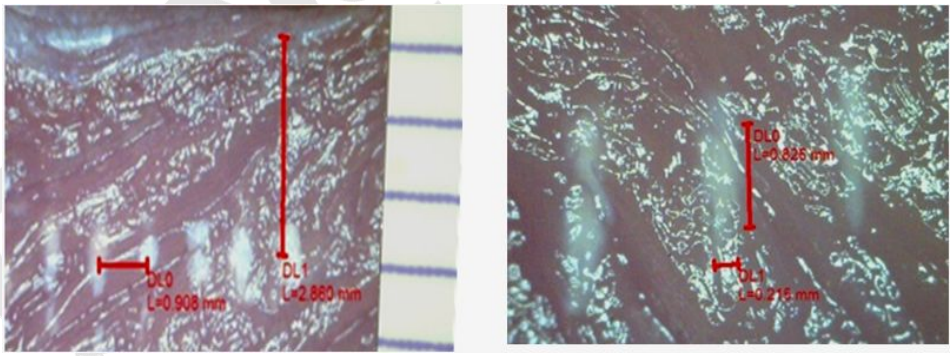
(1) The key element of the efficacy evaluation is whether ultrasound is radiated to the targeted point with the exact penetration depth that was intended in order to achieve wrinkle improvements by using ultrasound. In order to evaluate whether Ultraskin's ultrasound reaches to the target points accurately per Cartridge's penetration depth of 3mm or 4.5mm, Micro-pig ®'s skin was divided into three zones and 18 of 1cm x 1cm square fields were prepared in each zones. After ultrasound was radiated to 6 places per field, targeted coagulation point's penetration depth and focus size were measured from skin surface to dermis with Folliscope. When test setting was 4 MHz, the power was 1J and the time duration was 20ms; when setting was 7 MHz, the power was 1J and time was set to 30ms, and the test results were observed of coagulation points at 4.5mm and 3mm with 4 MHz and 7 MHz Cartridges. Except for the control group, test group was tested under 2 different conditions (4MHz Cartridge - 4.5mm penetration target condition, 7MHz Cartridge - 3mm penetration target condition), and both of these 2 conditions showed that targeted penetration points reached to exact coagulation points. The penetration depth was measured with Folliscope and the digital image processing software and tape measure were also used, and the digital measurement and tape measure's measurement results were identical. (See Figure 2A-2E)



(Figure 2A. Skin coagulation point was not observed in the enlarged photo of skin from untreated control group (Group I). Red dotted line indicates boundaries of the skin surface.)



(Figure 2B. Group II test result photo. 30 times enlarged photo to see the penetration depth and focus size after 4MHz Cartridge with 4.5mm penetration aim was irradiated.)



(Figure 2C. Group III test result photo. 30 times enlarged photo to see the penetration depth and focus size after 7MHz Cartridge with 3mm penetration aim was irradiated.)

(Figure 2. Photo of ultrasound target coagulation point's penetration depth and focus size. Coagulation points were observed under 2 conditions at exact penetration depth through Folliscope. Red line indicates infiltration areas and coagulation focus sizes)

2) Skin Safety Evaluation

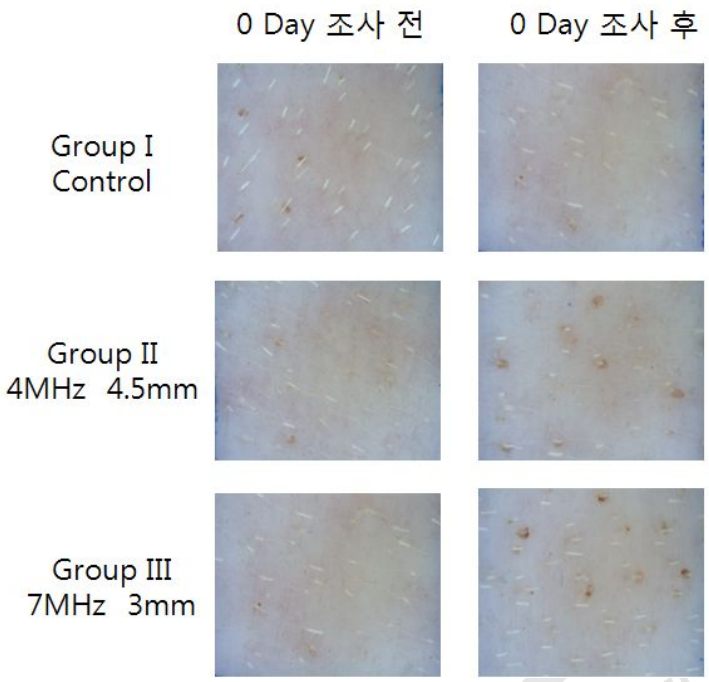
(1) Visual Evaluation and Measurement of Skin Surface

- Evaluation was conducted before irradiation, immediately after irradiation (Day 0) and 1 day after irradiation (Day 1).
- In all 3 areas that ultrasound was irradiated, no adverse skin reactions including surface damages, blisters, erythema, or heat damages were discovered from the dermatologist's visual evaluation and photos of skin surface.
- Comparison of before and after of irradiation with dermascope (See Figure 3)

(i) Evaluation Results of before and after ultrasound irradiation (Day 0 result)

: After Micro-pig® was put under anesthesia, 4MHz – 4.5mm, 7MHz – 3mm were irradiated, respectively, to the pig's back area per ultrasound's frequency and penetration depth of the target focus by using Ultraskin Cartridges. Dermoscope was used to observe the changes of the skin surface before and after ultrasound irradiation.

The skin surface was observed before Ultraskin was irradiated, was observed again after it was irradiated then compared to the before pictures, and after comparing the images of before and after the irradiation, under the 2 irradiation conditions, no change was observed on the skin surface, and no adverse reactions such as skin burn was observed.



(Figure 3. Dermoscope pictures of before and after ultrasound irradiation to evaluate adverse reactions. No adverse reaction of the skin surface was observed compared to the before pictures under either of 2 irradiation conditions.)

(ii) Evaluation of 24 hours after ultrasound irradiation (Day 1 result)

: Skin damage and adverse reaction under total of 2 kinds of irradiation conditions were evaluated 24 hours after ultrasound was irradiated, and same as the result after Day 0, no skin burn, adverse reactions, or skin surface damage was observed (Figure 4).

(Figure 4. 24 hours after irradiation (Day 1) Dermoscope picture showed no adverse reaction from the skin surface or skin burns. As a result, no skin damage was found from the ultrasound irradiation with 2 different ultrasound irradiation settings.)

(2) Histopathological evaluation

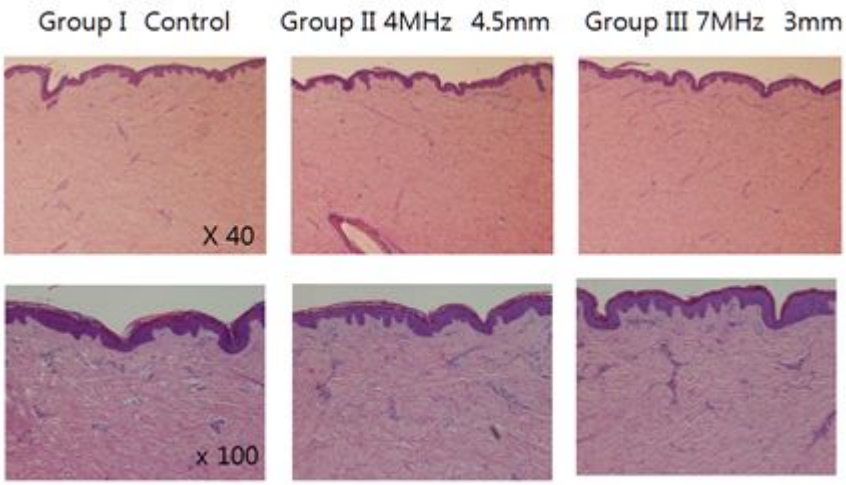
(i) Hematoxylin & Eosin (H&E) Staining Result

By using Ultraskin, depending on the frequency and focus depth of ultrasound, 4MHz - 4.5mm, 7MHz - 3mm, respectively were irradiated. Then, by using 4mm punch biopsy, histophatological evaluation was conducted. Collected skin tissue was dipped in a 10% formalin fixative and made into paraffin blocks, then H&E staining was conducted, and no skin irritation or adverse reaction was found.

From all areas where ultrasound was irradiated, it was confirmed that no parakeratinized reaction or skin surface damage was found, and compared to the control group, infiltration of inflammatory cells or dermal necrosis was not found. (Figure 5A). The same histopathological test was conducted again after 24 hours

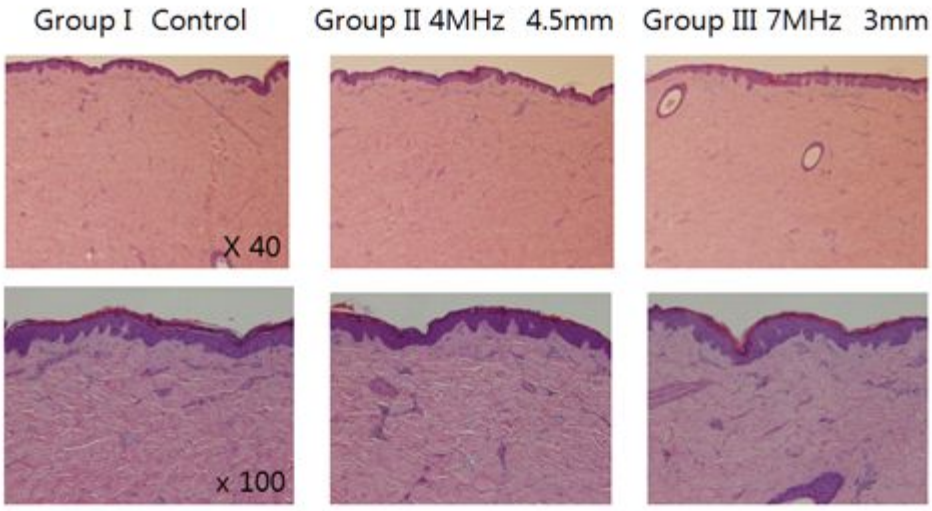
(Day 1), and same as Day 0, no skin surface damage or adverse reaction in dermis was found. (Figure 5B).

[0 Day after Irradiation]



(Figure 5A. From the skin biopsy performed immediately after ultrasound was irradiated, compared to the control group, it was confirmed that no abnormal keratinization of epidermis, or abnormal inflammation or tissue necrosis of dermis were found under 2 ultrasound conditions.)

[1 Day after Irradiation]



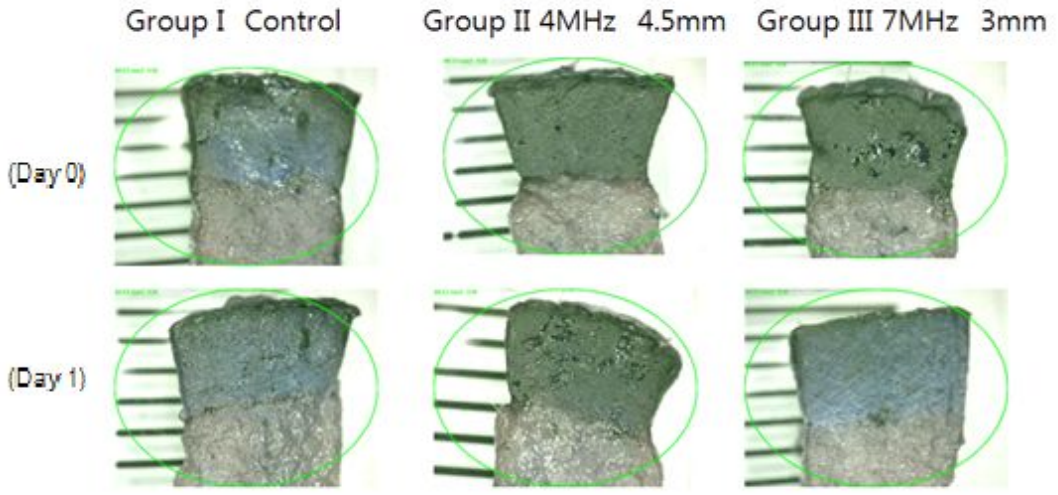
(Figure 5B. From the skin biopsy performed 24 hours after (Day 1) ultrasound was irradiated, compared to the control group, it was confirmed that no abnormal keratinization of epidermis, or abnormal inflammation or tissue necrosis of dermis were found under 2 ultrasound conditions.)

(ii) NBTC Staining Result

With Ultraskin, according to the frequency and focus depth of 4MHz – 4.5mm and 7MHz – 3mm were irradiated respectively. Skin tissues of the irradiated areas were biopsied by 4mm, then stored in freezer of -15 ℃ for about 2 hours. Then, by using surgical blade, the irradiated areas were cut perpendicularly by 2mm thick, then soaked in 500μM NBTC (nitroblue tetrazolium chloride, Sigma, USA) for 24 hours. After 24 hours, the tissues that were soaked in NBTC solution were placed in petri dish, then by using high-definition digital single-lens camera (Nikon D3000), pictures were taken to evaluate whether there was difference between control group and the ultrasound irradiated group. NBTC staining determines cell viability, so when stain shows up on frozen tissues, dark blue cytoplasmic pigment occurs due to the decrease of NBTC by NADH (nicotinamide adenine dinucleotide).

NADH immediately reduces cell death, and with this fundamental principle, cells with blue stains after NBTC staining indicate viability and areas with no blue stain indicate coagulation necrosis.

NBTC staining showed that dark blue cytoplasmic pigments occurred in peripheral areas including epidermis of ultrasound irradiated group and the positive control group, and same results were confirmed in all tissues both right after ultrasound irradiation (Day 0) and 24 hours after (Day 1). The mentioned results confirmed that all cell viabilities were normal in skin tissue areas where Ultraskin was irradiated using 4MHz – 4.5mm and 7MHz – 3mm (Figure 6).



(Figure 6. NBTC staining done to skin tissue sample immediately after ultrasound irradiation (Day 0) and 24 hours after (Day 1), compared to the control group, ultrasound irradiated groups under both conditions showed normal blue staining in skin tissue, and no tissue necrosis was observed.

4. Conclusion

It is found that when ultrasound frequency of 4MHz – 4.5mm and 7MHz – 3mm were irradiated to skin with Ultraskin, the penetration depths were implemented exactly. Also, under the skin evaluation, no damage on skin surface was found and no adverse infection reaction, inflammation, or tissue necrosis of the dermis was found. The skin safety evaluation result confirmed that the adverse reactions mentioned above was not found immediately or 24 hours after ultrasound was irradiated.

5. References

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