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Anti-inflammatory and draining effect of the Deep Oscillation® device tested clinically and on a model of human skin maintained in survival condition

The objective of this study was to evaluate the anti-inflammatory, toning and draining effect of the Deep Oscillation® device. The Deep Oscillation® device uses the forces of pulsed electrostatic attraction and friction to provoke oscillations that act on the epidermis, dermis, and subcutaneous layers of tissue. An *ex-vivo* study was first completed by using a model of skin maintained in survival condition. The draining and anti-inflammatory effects of the device were determined by pro-inflammatory cytokine assay and by histological analysis of capillary dilation. The analgesic effectiveness of the Deep Oscillation® was then evaluated by immunohistochemical analysis of TRPV1. To corroborate this data, a clinical study was conducted by selecting 20 subjects with periorbital bags or dark circles to undergo treatment with the device. Evaluations included photography, dermatological scores as well as ultrasound analyses. Using an *ex-vivo* model of human skin maintained in survival condition, the Deep Oscillation® device was effective in reducing inflammation with a significant reduction of dilated capillaries and IL8, while lowering sensory receptor levels. Clinically, the device was successful in reducing both dark circles and bags by an average of 40%.

Key words: Deep Oscillation®, inflammation, surviving skin, undereye bags and circles

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The Deep Oscillation®, developed by Physiomed, uses a therapeutic technology based on the combination of pulsed electrostatic attraction and friction to produce oscillations acting deeply on the tissues of the body from the epidermis down through the conjunctive and adipose layers and into the muscles. Hernández Tápanes *et al*. [1] were able to prove, by diagnostic ultrasound, penetration of the oscillations up to 8 cm in depth. Observed physiological effects of the device include edema resorption [2], stimulation of the wound healing process [3, 4] and strengthening of the cutaneous tissues [5]. The therapy also limits the production of inflammatory mediators, especially during treatment for chronic pain and more so during the treatment of fibromyalgia [6].

The objective of this study was to evaluate the anti-inflammatory, analgesic, and draining effect of the Deep Oscillation® device by *ex-vivo* [7] and clinical analyses. A model of skin maintained in survival condition aided in determining the draining, anti-inflammatory and analgesic efficacy of the Deep Oscillation®. The "Draining and Anti-Inflammatory Effect" program was first evaluated by calculating vascular dilation using digitalized images of dermal capillary diameters, as well as semi-quantitative scores for edema. A second program, "Anti-inflammatory Effect", was analyzed by calculating the reduced liberation of pro-inflammatory cytokines such as IL8. A third program, "Analgesic Effect", was evaluated immunohistochemically by measuring the reduction of TRPV1 receptors. Sensory receptors, TRP, are susceptible to mild pain due to environmental factors such as temperature and mechanical or chemical stresses and are identifiable at the surface of the keratinocytes. These sensory receptors have an increased expression following capsaicin, inflammatory reactions, burns or exposure to chemical agents.

A secondary component of the study clinically evaluated the improvement of 20 subjects exhibiting bags and dark circles under the eyes, following 10 sessions of the Deep Oscillation®. The study comprised dermatological scores aimed to benchmark the subjects' conditions and volume measurements, by ultrasound of the undereye bags. In addition, photography was also used to document modifications.

Methods

Human skin model maintained in survival condition

Human skin fragments were obtained from eight different female patients undergoing plastic surgery and were kept alive *ex vivo* by placement in inserts positioned in

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culture wells of Dulbecco Minimum Essential Medium, antibiotics and fetal bovine calf serum, while a porous membrane (3 μm) was fitted to allow for slow diffusion between the two compartments [7]. The models underwent two sessions of the Deep Oscillation® at D0 and D1, testing a set of 3 programs. The "draining program" consisted of three frequencies (120-180 Hz for three minutes; 10-30 Hz for three minutes; and 85 Hz for two minutes). The second program evaluated was the "Anti-inflammatory Effect", using two frequencies (98-160 Hz for three minutes; 85 Hz for 3 minutes). The third program, the "Analgesic Effect," consisted of three frequencies (98-160 Hz for three minutes; 5-28 Hz for three minutes, and 5-65 Hz for three minutes). The fragments of skin were next placed in organ culture in a humidified incubator at a warm atmosphere at 37 °C with 5% CO_2. The cultures were stopped four hours, 24 and 48 hours after the end of the second session of treatment by the Deep Oscillation®. The fragments of skin were fixed in formol liquid and embedded in paraffin for histological and immunohistochemical analyses. Supernatant in the culture was conserved for the cytokine assay.

Changes in vascular capillary diameters were evaluated by staining with hematoxylin-eosin, and dilation was evaluated by counting the number of dilated vessels among all of the vessels present within a histological slice. This number was projected to the total number of vessels in order to calculate the percentage of dilated vessels. In addition, a morphometric analysis of the area (μm^2) occupied by the lumen of the vessels was conducted in order to determine the average area (μm^2) occupied by the vessels in the dermis [8, 9]. A histological evaluation of edema served as a second analysis with the aid of semi-quantitative scores. A lack of edema received a score = 0, light edema = 1, moderate edema = 2 and finally, severe edema = 3.

A third analysis consisted of a pro-inflammatory cytokine assay based on the modification of cytokine secretions, such as IL8. An immunoassay technique using a spectrophotometric reading was used to measure the concentration of this cytokine (pg/ml) (Chemicon International Inc. assay kits). The assay, using fragments of skin with the same weight and size, was conducted using the culture supernatant.

The final analysis consisted of an immunohistochemical evaluation of the TRPV1 pain receptors within the epidermis (Transient Potential Vanilloid Receptor 1, VRI, rabbit polyclonal antibody, Biomol International). TRPV1 are found within the basal and suprabasal layers of the epidermis. Immunostaining was effectuated using an amplified immunoperoxidase test (kit Impress, Vector Laboratories) and stained by AEC (3-amino-9-ethylcarbazole). Quantification was effectuated on the totality of the slice using the number of cells expressing the receptor and projecting this count to the total number of keratinocytes within the basal layer of the epidermis.

Clinical materials and methods

The objective of the clinical study was to determine the effect of the Deep Oscillation® on subjects exhibiting dark circles and bags under the eyes. The study comprised dermatological scores from examinations by a dermatologist, ultrasound measurements, and photography.

20 subjects with phototypes ranging from III to V were chosen based on inclusion and exclusion criteria and subjects gave their informed consent to participate in the study. Any subjects with antecedents of allergies, dermatological abnormalities on the face or those having had taken anti-inflammatory medication were excluded from the study. According to the protocol, preliminary analyses were first conducted on the day of inclusion and similar analyses were conducted following 10 treatment sessions with the Deep Oscillation®.

Evaluations, conducted by a dermatologist, rated the subjects' dark circles and bags. Scores ranging from 0 to 4 for the dark circles were to take into account the intensity of the circle's pigmentation and its area under the eye. For bags under the eyes, scores ranging from 0 to 6 were established, based on their volume. Photography of the front and profile of the face was effectuated using a Canon EOS 5OD with a Macro ring MR-14 EX. A complementary analysis was also carried out using SkinStation®, consisting of an ultrasound containing a camera (magnification ×50) to quantify the volume of undereye bags (v/mm^2).

Ten sessions of Deep Oscillation® were conducted twice per week using three frequencies, according to the combined program for anti-inflammation and the alleviation of undereye swelling: 120-180 Hz for three minutes, 10-30 Hz for three minutes, and 85 Hz for two minutes. The subjects had their make-up removed and were placed in a resting position, lying down on an examination bed for five minutes. Each session was conducted with the Deep Oscillation® applied for a few seconds at the level of the neck to facilitate lymphatic drainage before it was applied to the undereye zone in circular movements from the interior towards the exterior of the face.

Statistical analysis

For statistical analysis of these evaluations, an average was calculated for each parameter from the results obtained for each of the 8 skin fragments. For the clinical component of the study, a comparison was made pre- and post-treatment. The statistical analysis was conducted using a reduced deviation Student t-test or the paired test with a $\propto$ risk of 5%.

Results

Ex-vivo model of human skin

The program "Draining and Anti-Inflammatory Effect" was selected to analyze the modification of vessels, edema and for the excretion of pro-inflammatory cytokines, while the "Analgesic Effect" program was chosen to analyze the expression of TRPV1 receptors. Preliminary studies prescribed the optimal time needed preceding evaluation: 24 hours following the end of the 2nd session of Deep Oscillation® for histological and immunohistochemical analyses and 4 hours following the 2nd session for the IL8 assay.

In order to evaluate the draining effect of the machine, the overall percentage of dilated capillaries was calculated to determine any vascular changes. The results concerning the global percentage of dilated capillaries are shown in *figure 1*. Two sessions of the Deep Oscillation® "draining" program permitted a significant reduction of the

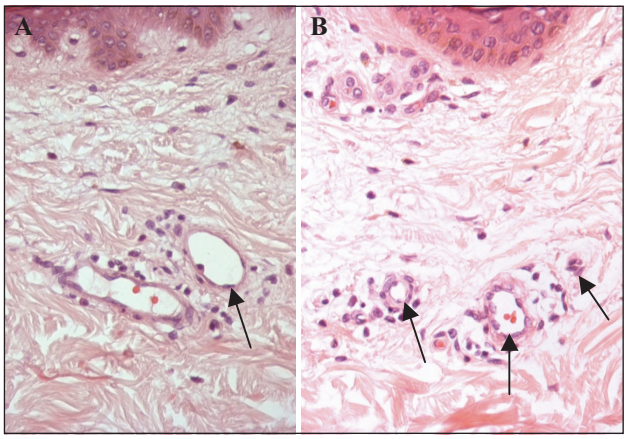

Figure 1. A) Hematoxylin-eosin staining (×400) Untreated skin: Vasodilation of capillaries. **B)** Hematoxylin-eosin staining (×400) Deep Oscillation®-treated skin: reduction of dermal capillaries' dilation.

percentage of dilated capillaries: 75 ± 10.4%, compared to non-treated control skin with 91.7 ± 4.55% dilated capillaries (p = 0.005). The measurement of capillary average areas was also calculated and is expressed in *figure 2A*. Results show that treatment with the Deep Oscillation® induces vasodilatation reduction that is statistically significant compared with control skin: 95.1 ± 26 µm² *versus* 266.5 ± 85.2 µm² (p = 0.001). The results of the analysis of dermal edema are expressed in *figure 2B*. Two sessions using the Deep Oscillation® program "Draining and Anti-Inflammatory Effect" resulted in a significant reduction of dermal edema compared with control skin: a score of 1.66 ± 0.57 *versus* 2.58 ± 0.2 (p = 0.004).

To evaluate the anti-inflammatory effect of the therapy, an IL8 assay was conducted, its results are expressed in *figure 2C*. Two sessions using the Deep Oscillation® program, "Draining and Anti-Inflammatory Effect" led to a significant reduction of the excretion of IL8 pro-inflammatory cytokines with a level of 234.3 ± 69.3 pg/ml in comparison to the control skin at 285.7 ± 75.7 pg/ml (p = 0.005), equaling a reduction of 22%.

In order to determine the efficacy of the therapy to reduce pain, an immunohistochemical analysis was conducted to evaluate keratinocyte TRPV1 sensory receptors. The expression of keratinocyte TRPV1 levels significantly fell after two treatments of the device under the program "Analgesic Effect" with 4.5 ± 6.2% positive cells per field of vision compared to 11.8 ± 8.5% in control skin (p = 0.01).

Clinical evaluation

20 subjects were included in this study, including two men and 18 women, with an average age of 48.75 ± 7.16 years. A significant average reduction of undereye bags after 10 sessions of treatment was found, with a dermatological score of the right eye at 3.8 pre-treatment to 1.65 post-treatment (26% reduction; $p = 10^{-7}$). The average reduction of undereye bags was higher for the left eye, with scores averaging 1.94, falling from 3.87 at the start of the treatment (50% reduction; $p = 5.10^{-8}$). The before and after photographs of a subject are depicted in *figure 3*. A significant reduction of dark circles following 10 sessions of the Deep Oscillation® was found with an average dermatological score of 1.9 for the right eye in comparison with a score of 3.2 pre-treatment (40% reduction; $p = 4.10^{-9}$). Results showed a similar reduction for the left eye with an average score 1.8 for dark circles after treatment versus 3.17 pre-treatment (43% reduction, $p = 3.10^{-9}$).

Volume analysis consisting of an ultrasound containing a camera (magnification ×50) quantified undereye bags with an average volume of 38.2 v/mm² pre-treatment and 29.1 v/mm² post treatment (24% reduction; p = 0.01).

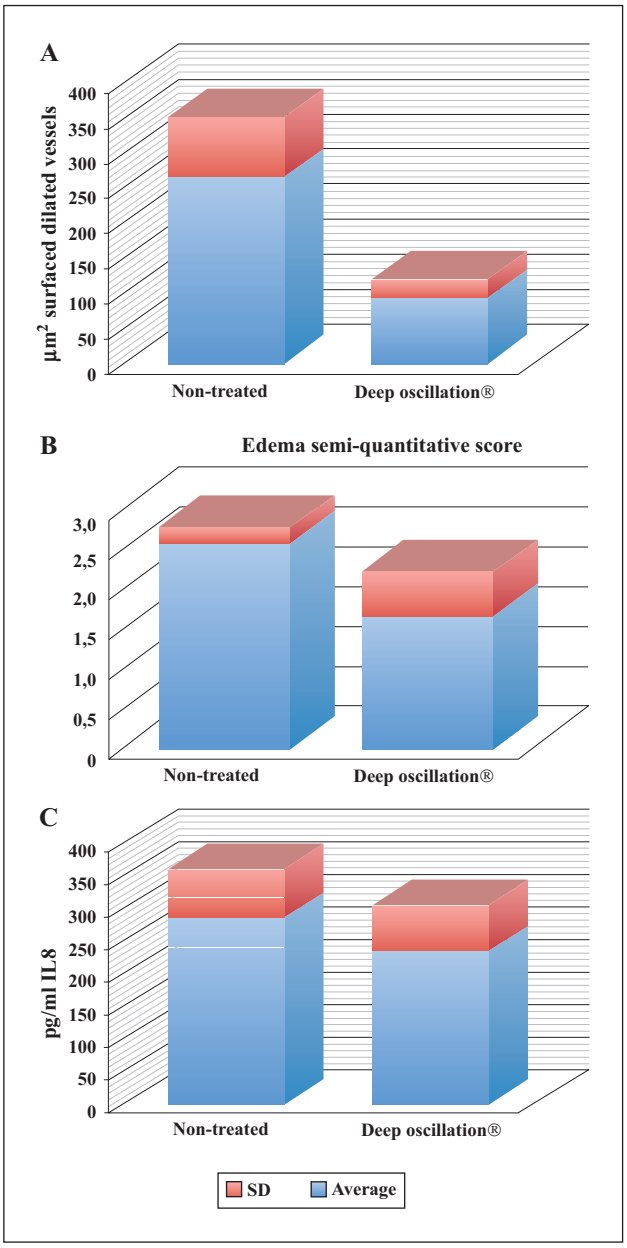

Figure 2. A) Histological quantification of dilated vessels. **B)** Histological evaluation of edema using semi-quantitative scores. **C)** Pro-inflammatory IL8 dosage.

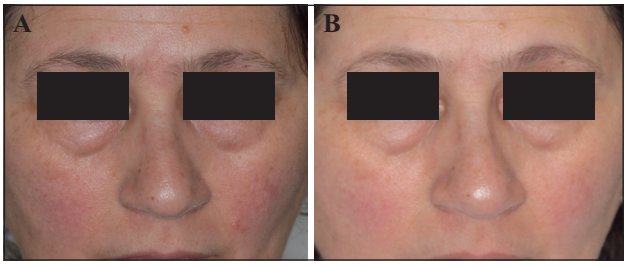

Figure 3. A) Clinical evaluation at D0. **B)** Clinical evaluation at D37.

Discussion

The Deep Oscillation® works by creating precise oscillations ranging from 5-200 Hz in treated tissue, using electrostatic attraction and friction. Seidl describes the therapy effect as intense resonance oscillation of the entire tissue segment undergoing treatment [10]. The oscillations are unique because they act deeply on all tissue components to a depth of 8 cm (through skin, connective tissue, subcutaneous fat, muscles, blood and lymph vessels) [1]. Because of the non-invasive, non-traumatic, gentle nature of this therapy, very early application is possible e.g. following injury and post operatively. The therapy treats a number of human disorders based on the biological effects of this low intensity (non-thermal) electro-mechanical action.

The vasculary efficacy of the Deep Oscillation® was shown by using an *ex-vivo* model of longterm skin culture mimicking the *in-vivo* environment, avoiding both biopsies and animal testing [7-9]. Significant anti-inflammatory effects, including reduced vasodilation, œdema and pro-inflammatory IL8 excretion were found in this study. The second component of the study, a clinical evaluation, confirmed the *ex-vivo* results and demonstrated the draining effect of the Deep Oscillation® with reduced bags and lightening of dark circles under the eyes.

To better understand the mechanism of the Deep Oscillation®, the device acts mainly on intercellular circulation of the interstitial connective tissue, leading to a normalization of circulation fluidity. In fact, Deep Oscillation® therapy has been effective in the physical treatment of lymphedema [2, 11]. Repeating intense resonant vibrations in rapid succession does not only lead to an improvement in the rate of blood flow at the microcirculation level but the mechanical stimuli also have a dispersing effect on edema, even if indurated. Interstitial liquid, as well as content materials, become transportable this way and distribution is promoted on the level of the interstitium. As a result of an increased absorption area, lymphatic drainage might be enhanced. In addition, repeating mechanical stimuli might act on interstitial collagen fibre structures and, through tractive forces on anchoring filaments, on the endothelial structures of initial lymph vessels (capillaries and precollectors).

It is possible to compare the Deep Oscillation® mechanism with that obtained using electrical-field-induced molecular vibrations or with electromagnetic therapy. Indeed, it is theorized that application of an appropriate electrical current, either directly through wires or indirectly through induction by a magnetic field, may affect tissues in several ways. Blood, like all tissues, contains electrically charged ions. When a magnetic field with a series of alternating North and South poles is placed over a blood vessel, the influence of the field will cause positive and negative ions (for example, Na+ and Cl-) to bounce back and forth between the sides of the vessel, creating flow currents in the moving blood like those in a river [13, 14].

Therefore, the quick repetitions of the electro-mechanical action (vibration) of the Deep Oscillation® lead to the modification of membrane polarity and consequently to rhythmic deformations of blood and lymphatic wall capillaries. Moreover, alternating electrical fields induce redistribution of cell membrane proteins, which could initiate signal transduction responsible for the reorganization of cellular structures such as vascular walls [15].

A pain-reduction effect was also observed, with fewer TRPV1 sensory receptors in the epidermis. The observed pain reduction and anti-inflammatory effects of Deep Oscillation® could be associated with the described distribution of interstitial liquid and enhancement of lymphatic drainage, which might result in a reduction of other potentially noxious substances (traumatic acidosis, prostaglandin etc.). If there are other possibilities, such as direct cell modulating effects by electrical fields, as proven for cell information therapy [12], further investigation is required. Other studies using static and pulsating electromagnetic field therapy have proven effective in the relief of pain. It is unlikely that the effect is related to a reduction in nerve conductivity; the field required to produce a 10% reduction in nerve conductivity is roughly 24 Tesla [16]. Another study in humans showed that magnetic treatment actually induced hyperalgesia in a tooth pain model [17].

In conclusion, in this clinical study, a reduction of bags and dark circles in 20 subjects was obtained with use of the Deep Oscillation®. A study on a greater number of subjects testing a non-treated side of the face to a treated side could augment proof of this treatment's efficacy. Nevertheless, this preliminary study shows a good correlation between *ex-vivo* and clinical results. ■

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