EFFICACY AND SAFETY OF DUAL FREQUENCY ULTRASOUND FOR FACIAL LIFTING

KHINE NYEIN CHAN

MASTER OF SCIENCE
IN
DERMATOLOGY

SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE
MAE FAH LUANG UNIVERSITY

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ACKNOWLEDGEMENTS

Firstly, I would like to express my special thanks of gratitude to my parents; U Nyein Chan Aung and Daw Tin Moe Khine. My completion of this research could not have been accomplished without the support of them. Secondly, I would like to thank my respected Advisor Professor Vichit Punyahotra, M. D., Ph. D., coadvisor Paisal Rummaneethorn, M. D., external examiner Associate Professor Wongdyan Pandii, Dr. P. H. and Professor Tanomkhit Pawcsuntam, M. D. School of Anti-aging and Regenerative Medicine, Mae Fah Luang University.

Moreover, I am deeply grateful to my Ti lone, Ko Phyo Pyi, Arnold and Wine for their continuous emotional and mental support. I thankfully acknowledge the financial support from Mae Fah Luang University for my research and glad to be part of the university.

Khine Nyein Chan

Thesis Title	Efficacy and Safety of Dual Frequency Ultrasound for Facial Lifting
Author	Khine Nyein Chan
Degree	Master of Science (Dermatology)
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ABSTRACT

Background: Dual frequency ultrasound is the ultrasound using 2 different frequencies with a quick frequency modulation. This causes micro massage effect which decreases matric metalloproteinases enzyme activity which mainly takes part in collagen degradation. Moreover, this effect also stimulates heat shock protein synthesis, anti-inflammatory effect and skin barrier recovery.

Objective: The objective of this quasi-experimental, clinical study was to evaluate the efficacy and safety of dual frequency ultrasound for facial lifting.

Methods and materials: Subjects 25 to 45 years of age (n=20) with mild to moderate facial skin laxity were recruited. The ultrasounds 1/3 MHz and 3/10 MHz were applied to face for 24 minutes. It is done 2 times per week for 6 weeks. The follow up visits were 4th week, 8th week and 12th week after last treatment session.

Result: Wrinkle score at baseline was lower than 4th week (-8.706) and 8th week (-10.00) statistically significant at the level of 0.05($p<0.05$). The mean of cutometer score (skin elasticity) in each visit statistically significant increased. The sebumeter and tewameter scores are improved with each visit at the level of 0.005. There was no adverse effect in all subjects throughout the study.

Conclusion: Based on these results, wrinkle and skin elasticity are improved with time to time compared to the baseline. The dual frequency ultrasound's face lifting result remains the same for 12th week. Moreover, Sebumeter and Tewameter scores, are decreased over time throughout the treatment which demonstrated that dual frequency ultrasound can also decrease sebum production and make the skin more hydrated.

Keywords: Dual Frequency Ultrasound, Face Lifting, Skin Elasticity, Wrinkle

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CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Due to increased awareness of public and personal hygiene, people's life expectancy become longer. Thus, prevention and treatment of skin aging is becoming popular topic. At the past, only aesthetic surgical lifting procedures were used to correct face and neck laxity. The concept of non-invasive procedures for replacing the surgeon's scalpel has been an interesting topic for more than decade. (Shome et al., 2019; Zandvakili et al., 2016)

Chemical peeling, injectable, and ablative and non-ablative laser therapies are the most used nonsurgical options for facial skin rejuvenation. However, these are not effective options for lifting and tightening of the tissue. Even ablative laser is the promising treatment for skin rejuvenation, it has many adverse effects like very long recovery period, the other better treatment methods emerge (Zandvakili et al., 2016; Oni et al., 2014)

As human gets older and older, skin constantly gets aging process. Human facial aging is inevitable and ongoing process. It includes skin laxity, loss of subcutaneous tissue, photodamage and bony resorption which is associated with chronological and photoaging. Due to aging, skin fibroblasts, many skin appendages and collagen formation decrease both in numbers and functions which lead to skin laxity. Tissues under the skin which maintain the face contour also contributes to skin laxity (Zandvakili et al., 2016; Robins, 2019; Sadick, 2008).

Aging is related with continuous reduced production of heat shock proteins (HSPs) and increased collagen degrading matrix metalloproteinases (MMPs) which cause significant downregulation of collagen and elastin fibers which is the dermal element that provides elasticity and resilience. This is turn leads to reduce in skin laxity which is corrected by facial lifting and tightening procedures (Kang, 2019; Meyer-Rogge et al., 2012).

Ultrasound technology has been employed for both therapeutic and diagnostic purposes for several decade (Wulkan et al., 2016). Ultrasound is recently introduced as a useful tool for cosmetic purposes. It becomes leading method because of having the ability to accurately focus energy and is able to destroy very small number of tissues (Shome et al., 2019; Zandvakili et al., 2016)

Ultrasound is capable of inhibiting the MMPs to restore the skin barrier thus it is used now in antiaging treatment and skincare (Kim et al., 2021). The dual-frequency ultrasound is highly efficient in the modulation of MMPs and HSPs which make it to use for anti-aging treatment and collagen and elastin production through the application of combining with 10MHz ultrasound. The main principle of dual-frequency ultrasound is activating fibroblasts which can increase production of collagen and elastic fibers and protein synthesis by a cavitation and microstreaming. These all make dual frequency ultrasound possible to use for skin tightening and lifting (Lee et al., 2018; Meyer-Rogge et al., 2012).

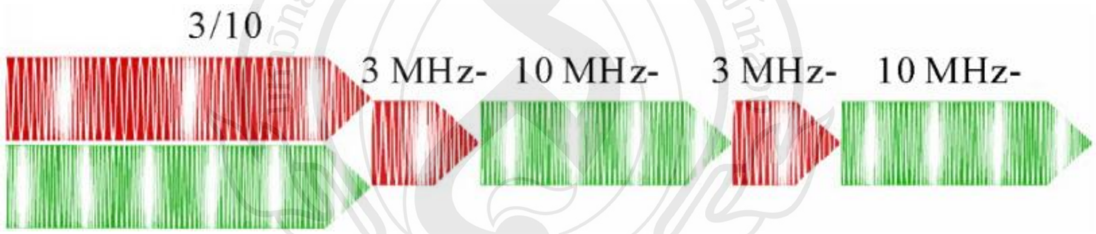

Figure 1.1 Wave formation in dual frequency ultrasound

In this wave, the frequency is oscillating between two different kinds (1 and 3 MHz or 3 and 10 MHz) which causes huge change in pressure gradients of tissue and hence the ultrasound micro-massage effects is produced (Kruglikov & Kruglikova, 2011). This kind of micro-massage effect is assumed to main role of dual-frequency ultrasound treatment suppressing the activity of matric metalloproteinases protein and activating the heat shock protein (Lee et al., 2018).

We assume that dual frequency ultrasound which has micro-massage effect on MMPs and HSP, fibroblast activation and tissue regeneration is the alternative treatment option for face lifting. There is no previous study with the dual-frequency ultrasound machine on the reducing skin and neck laxity. Therefore, to study the efficacy and safety of dual frequency ultrasound for face tightening is the main aim of this study.

1.2 Research Question

Is dual frequency ultrasound considered as a safe and effective method for face lifting and tightening?

1.3 Objective

1.3.1 General Objective

To evaluate the efficacy and safety of dual frequency ultrasound for lifting and tightening of redundant facial skin

1.3.2 Specific Objective

1.3.2.1 Primary Outcome

To compare average mean change of wrinkle, sebum, viscoelasticity and transepidermal water loss measurement from baseline to 4^{th} ,8^{th} and 12^{th} weeks after completion of final treatment by using Visio scan, sebum meter, cutometer and tewameter.

1.3.2.2 Secondary Outcome

1. To assess overall improvement and the patient satisfaction of dual frequency ultrasound for face lifting by using Global Aesthetic Improvement Scale and Participant’s Satisfaction Score.

2. To assess the side effects of dual frequency ultrasound by using research questionnaire.

1.4 Hypothesis

1.4.1 Primary Hypothesis

Dual frequency ultrasound has a good efficacy and satisfactory evaluation in wrinkle, skin elasticity, sebum, and transepidermal water loss for facial skin lifting and tightening.

1.4.2 Secondary Hypothesis

Dual frequency ultrasound can be used safely, measured by assessing of adverse effects (pain, swelling, erythema, burn, numbness and other side effects) after each treatment.

1.5 Conceptual Framework

As we age, the collagen and elastic fibers decrease both in quality and quantity, resulting in many skin alterations and the skin becomes more fragile and thinner. In addition, many skin appendages such as sebaceous and sweat glands, begin to decline in their functions over the years making skin drier, causing fine lines and deep wrinkles. Moreover, extrinsic aging causes the appearance of freckles, sun spots, excessive collagen and elastin loss. The appearance of an uneven skin tone, dryness, roughness and fine lines and wrinkles are also factors of extrinsic aging.

In a healthy skin, intramolecular cross links are weak and reducible covalent aldehyde bonds. In the aged skin, the latter will be replaced through multivalent irreducible cross-links. With the dual frequency ultrasound, additional mechanical forces arise, which can easily shake and break the cross-links between the collagen molecules resulting in stronger tightening effect. In addition, dual frequency ultrasound causes differentiation of the fibrocytes into the fibroblasts, activation of the new collagen production, improvement of the collagen structure and modulation of the collagenase expression through micro massage effect. So dual frequency ultrasound can regenerate new collagen and elastin resulting in relief of facial skin laxity.

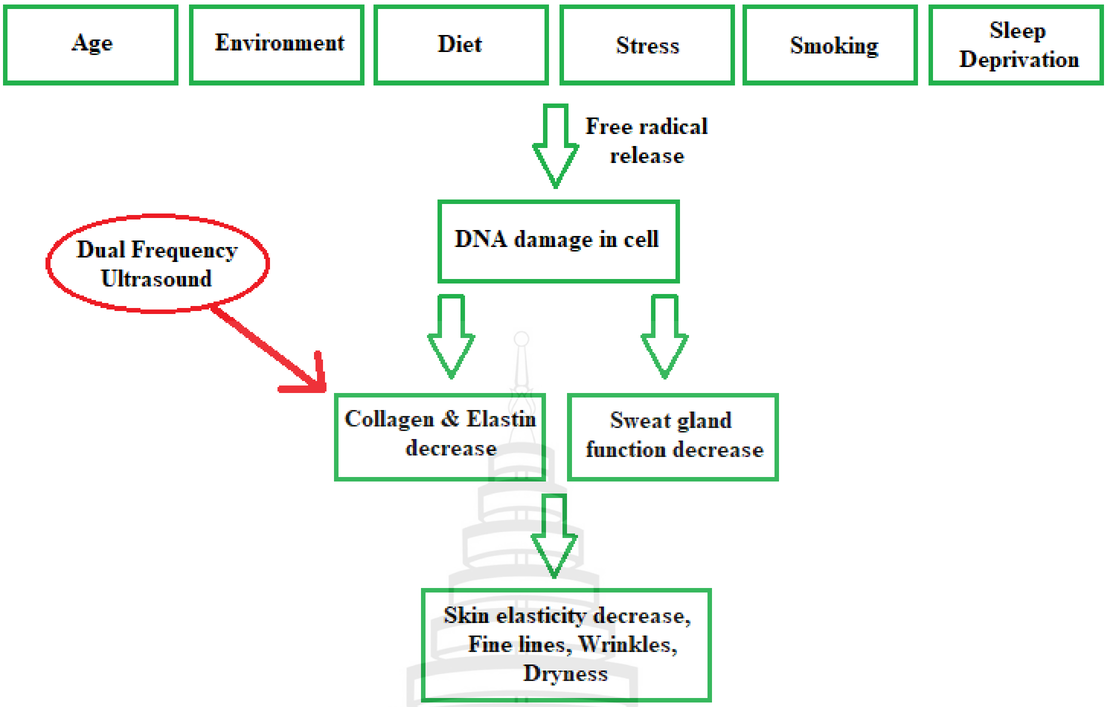

Figure 1.2 Conceptual framework

1.6 The Scope of Research

Twenty participants who are willing to do face lifting procedure, having mild and moderate facial laxity, Fitzpatrick skin type III – V, both male and females, age 25-45 years will be done for face lifting with dual frequency ultrasound. Subjects received a total of 12 treatments for 6 weeks; twice weekly and efficacy measurement will be assessed at 4^{th}, 8^{th}, 12^{th} week after last session at Mae Fah Luang Hospital Bangkok.

The photographic will be done at baseline and follow up visit at 4^{th}, 8^{th}, 12^{th} week after the last treatment session under the same digital camera setting.

The improvements in facial laxity will be independently evaluated by three dermatologists and participants. Sebum, viscoelasticity, transepidermal water loss and wrinkle were assessed by sebum meter, cutometer, tewameter and VISTA® respectively. Side effects were assessed by research questionnaire.

1.7 Operation Definition

1.7.1 Physician Global Aesthetic Improvement Scale (GAIS)

Table 1.1 Physician Global Aesthetic Improvement Scale (GAIS)

Grade	Degree	Description
5	Exceptional improvement	Excellent correction result
4	Very improve	Marked improvement but not completely optimal
3	Improve	Improvement, better compared with the original condition, but not the same with marked improvement
2	No change	The appearance remains the same compared with the original one
1	Worsen	The appearance has become worsen compared with the original one

1.7.2 Participant Satisfaction Score

Table 1.2 Participant Satisfaction Score

Grade	Degree
-1	Unsatisfied
0	Indifferent
1	Somewhat satisfied
2	Moderately satisfied
3	Very satisfied
4	Completely satisfied

1.7.3 Skin Laxity Definition

Table 1.3 Skin Laxity Definition

Grade	Descriptive	Laxity*
0	None	None
1	Mild	Only nasolabial fold
1.5	Mild	Nasolabial with early Melo labial fold
2	Moderate	Nasolabial fold/Melo labial folds, jowls, early Submandibular
2.5	Moderate	Distinct nasolabial fold/Melo labial folds, jowls and submandibular
3	Advance	Distinct nasolabial/Melo labial fold jowls and submandibular, early neck strand
3.5	Advance	Deep nasolabial/Melo labial fold. prominent jowls and submandibular, prominent neck strands
4	Severe	Marked nasolabial/Melo labial folds, jowls and submandibular, neck redundancy and strands

Note * Alexiades–Armenakas criteria for laxity grading.

1.7.4 Fitzpatrick Skin Photo Type

Table 1.4 Fitzpatrick skin Photo Type

Skin Type	Skin Color
I	White; very fair; red or blond hair; blue eye; freckles
II	White; fair; red or blond hair; blue, hazel or green eyes
III	Cream white; fair with any eye or hair color; very common.
IV	Brown; typical Mediterranean Caucasian skin
V	Dark brown; mild-eastern skin types
VI	Black

1.7.5 Efficacy

Efficacy means the ability to produce a desired or intended result after the study.

1.7.6 Device Specification

Dual Frequency Ultrasound Technology; Trade name is LDM® -MED (Importer: Quantum healthcare Thailand, manufactured by WELLCOMET Gmbh company). This device was supported by the company to use in Mae Fah Luang University Hospital.

1.7.6.1 Purpose of use

This device is used to modify matric metalloproteinases and heat shock proteins activities in tissue and thus a therapeutic effect can be achieved for all dermatological and aesthetic indications that are path physiologically linked to abnormal MMP and HSP activities.

1.7.6.2 General features

1. The machine has been certified by The Thai Food and Drug Administration.
2. Body of the machine with applicator weighs 8 kilograms.
3. 10-40 °C in Temperature and 30-75% relative humidity is a suitable environment for machine operation.
4. The dual frequency ultrasound (LDM® -MED) uses 1/3 MHz and 3/10 MHz ultrasound.
5. The machine has two parallel connected outputs for the treatment heads (sonotrodes).
6. Sonotrode1- Frequencies 3MHz/10MHz, 1.2cm^2, Sonotrode2- Frequencies 1MHz/3MHz, size5.0cm^2.
7. It is consisted of main unit with color touch screen.
8. The machine has 22 different programme.11 programs for aesthetics and 11 programs for dermatology.
9. Treatment period spends 24 minutes per session. 12 minutes for each side of face. The device features the following treatment modes

1) Continuous ultrasound with TV=1:1

2) Pulsed ultrasound with TV=1:2 (impulse =5ms, pause =5ms)

3) Pulsed ultrasound with TV=1:5 (impulse =2ms, pause =8ms)

4) Pulsed ultrasound with TV=1:10 (impulse =1ms, pause= 9ms)

5) LDM plus Technology 3/10 MHz with pulse trains for both frequencies between 1 & 20ms.

6) LDM plus Technology 1/3 MHz with pulse trains for both frequencies between 1 & 20ms.

CHAPTER 2

REVIEW OF RELATED LITERATURE

Aging means the progressive decline of most of the body systems and physiologic reserve capacity which is an inevitable biologic process (Kang, 2019). Major feature of aging is redundant facial and neck skin that is due to the reduction of collagens fibers in the dermis and hypodermis (Finzi & Spangler, 2005; Tanaka, 2018).

2.1 Pathophysiology

With increasing age, the skin's antioxidant defenses tend to weaken and so the body cannot protect from oxidative stress resulting in oxidative damage. It causes up regulation of stress related factors and cellular apoptosis which can trigger the aging process [6]. There are 2 pathophysiological mechanisms for skin aging. First one is related to both intrinsic and extrinsic aging. Continuous exposure to UV- or IR-radiation and chronic, intrinsic inflammation causes the overproduction of matrix metalloproteinases. The second pathophysiology of skin aging is based on the continuous decreased heat shock proteins production (HSPs) (Meyer-Rogge et al., 2012)

Aging contributes to the decreased interaction of proteins with water by effecting on the ground substance of the skin; proteoglycans and glycosaminoglycans. In photoaged skin, increased glycosaminoglycans deposition in the abnormal elastotic material prevents the normal water attraction. However, in intrinsic aged skin, there is reduced secretion of hyaluronic acid. Both changes leads to age-related decreases inskin hydration and turgor (Kang, 2019). In addition, the skin and soft tissue support decline with age and so result in lax and sagging of the skin. The gravity force which always acts on the body also enhances this aging process (Gilchrest & Krutmann, 2006)

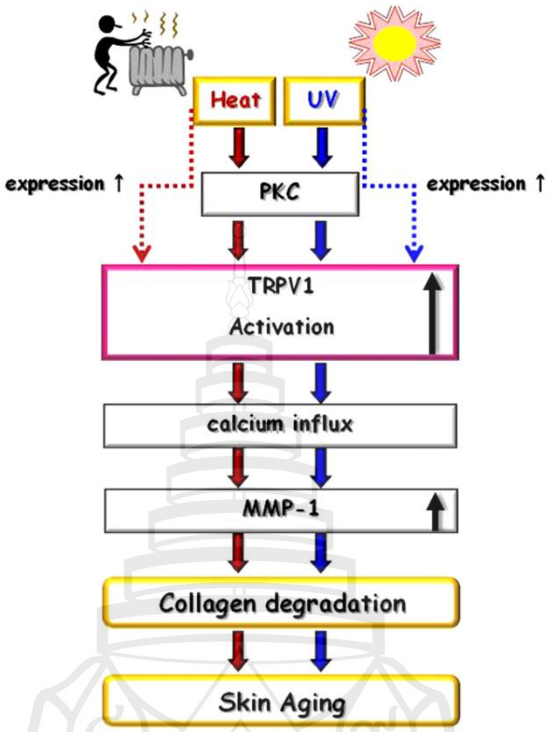

Figure 2.1 Pathophysiology of Skin Aging

2.2 Intrinsic Skin Aging

Intrinsic skin aging which can be also called chronologic skin aging is mainly influenced by genetic and hormonal factors.However, the informations about causes of chronological skin aging are less clear than those of extrinsic aging or photoaging.The intrinsic aging causes continuous reduction of synthetically active fibroblasts whose main function is to produce collagen bundles.In addition,there is also progressive accumulaton of old fibrocytes.This overall causes decreased collagen production and glycosaminoglycans. Moreover, in chronological aging, so many alterations like reduced blood flow,lowered amount lipids and the loss of rete ridges are also found.The result is aging skin which is dry,pale having fine wrinkles,less elasticity and impaired reparative function (Gilchrest & Krutmann, 2006; Kang, 2019; Meyer-Rogg, 2012).

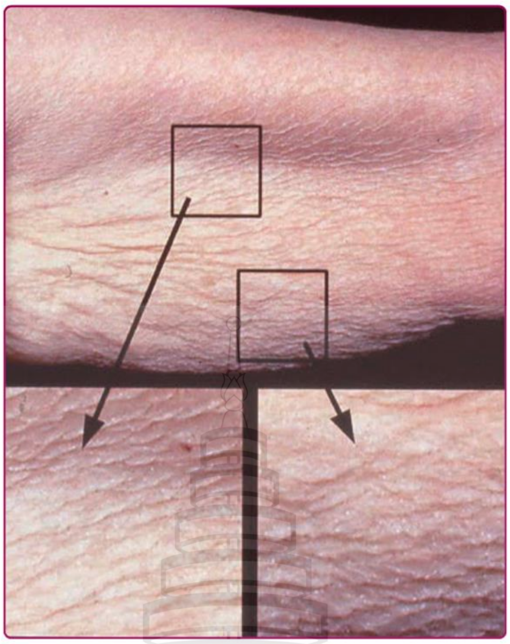

Figure 2.2 Intrinsic aging is characterized by fine wrinkling, increased skin laxity, and sagging

2.3 Extrinsic Skin Aging

Long term exposure to UVA and UVB from sunlight causes photoaging, premature skin aging. Photoaging contributes to changes both in function and structure of all component of the skin, consisting of the epithelium, the dermal connective tissue, the vascular and pigmentary system, and possibly even the subcutaneous fat. The upregulation matrix metalloproteinases (MMPs) and urinary plasminogen activator seen in photodamaged skin which in turn causes down-regulation of collagen synthesis, resulting in collagen loss and abnormal deposition of degenerative elastotic material within the dermis (actinic elastosis) [4, 6, 18]. (Fisher et al., 1997; Kang, 2019; Bolognia, 2012)

Figure 2.3 Photoaging

Table 2.1 Age Associated Changes in the Dermal Components of the Skin

Dermal Component	Structural Changes	Functional changes
Collagen	Increased rigidity Reduction of type I and III collagen Increased fragmentation.	Impaired wound healing Decreased mechanical stress on fibroblasts (→decreased collagen synthesis
Elastin	Reduced number and diameter of elastin fibres Solar elastosis (Photodamage)	Decreased elasticity
Ground substance	Abnormal deposition of Glycosaminoglycans preventing normal interaction with water Reduced hyaluronic acid	Decreased skin hydration

2.4 Non-invasive Skin Tightening Technique

Nowadays, people are very interested in rejuvenation process including, skin tightening procedures (Courage + Khazaka Electronic, Köln—Skin Testing Equipment, n.d.). Among non-invasive and invasive skin-tightening, the first one is more preferred due to rapid return to work, short recovery time, and low risk of complications (Lee et al., 2015). Patients who are not fit for rhytidectomy or who wants to avoid surgery are suitable ones for nonsurgical skin tightening (Sadick, 2008)

2.5 Dual Frequency Ultrasound

Initially, ultrasound was mainly used for various skin diseases' diagnostic purposes and some of the aesthetic problems. Nowadays, ultrasound is widely used for face lifting, body sculpting and skin anti-aging (Kim et al., 2021).

Ultrasound energy has unique characteristics that are appropriate for skin lifting and tightening (Minkis & Alam, 2014). When the technology applies two different frequencies ultrasound waves with a quick frequency modulation, the micro-massage effects is produced. In other words, this micro-massage effect is obtained due to the large pressure gradients. This is the most important effects of dual frequency ultrasound wave which cannot be seen in normal ultrasound waves. Mechanical effects of ultrasound could also be regulated by varying the scale of the massage effect (Lee et al., 2018).

For example, if the frequency changes from 3 MHz to 10 MHz or from 1 MHz to 3 MHz with the same intensity, the pressure amplitudes remain unchanged. But the dimension of every micro-massage unit in the tissue will be declined from 0.75 mm to 0.25 mm (respectively from 0.25 mm to 0.075 mm). The new micro-massage effect plays main role in suppressing the matrix metalloproteinases activity and activating the heat shock protein of the dual frequency ultrasound. This effect of dual-frequency ultrasound of 3/10MHz on matrix metalloproteinases and heat shock proteins in tissue demonstrates strong anti-inflammatory and wound healing effect in tissues (Lee et al., 2018; Tausch & Kruglikov, 2015).

When ultrasound is applied to the skin, the transferred energy makes keratinocytes' spaces widened and thus microcavities formed because of acoustic mechanical stress, resulting in increased epidermis permeability. This sonophoresis cleans the epidermal microenvironment, eventually speeding up the epidermal recovery by reducing inflammatory debris. The disturbed skin barrier can also be recovered by changing the epidermal calcium gradient which can be made by ultrasound.When epidermal gradient changes,lamellar bodies screation enhances the secondary cytokine release.In this way,the barrier recovery becomes quickened (Kim et al., 2021).

Kim et al study reported that the dual-frequency ultrasound can reduce TEWL scores and erythema, suggesting skin barrier renewing and anti-inflammatory action. Meyer-Rogge et multicenter study proved that application of dual-frequency ultrasound markedly enhances ageing skin visual appearance (Meyer-Rogge et al., 2012). Main principle of dual-frequency ultrasound is promoting tissue generation by activating fibroblasts in soft tissue and protein synthesis by cavitating and microstreaming (Lee et al., 2018).

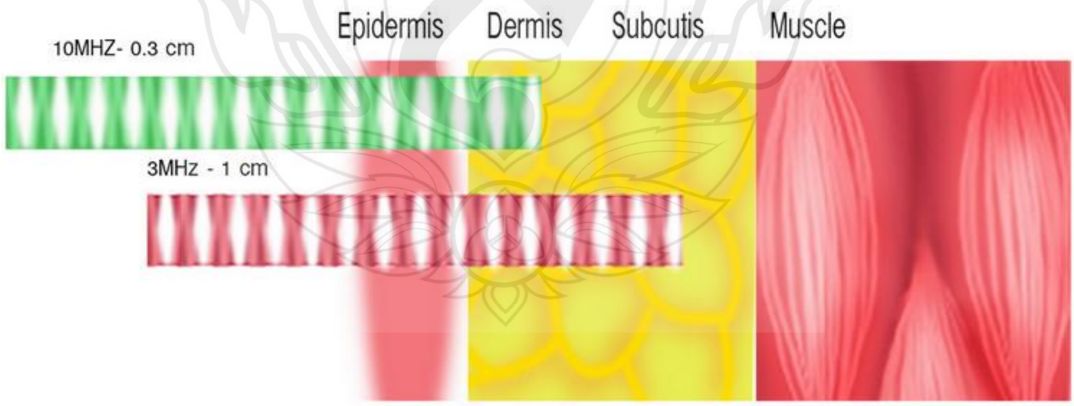

Figure 2.4 Penetration depth of Dual Frequency Ultrasound

2.5.1 Matric Metalloproteases (MMPs)

Collagen-degrading enzymes matric metalloproteases (MMPs) increases with increasing age. Acute UV irradiation transiently upregulates 3 MMPs (MMP-1, MMP-3, and MMP-9) in the skin whereas chronically photodamaged skin causes higher levels of 7 MMPs (MMP-1, MMP-2, MMP-3, MMP-9, MMP-11, MMP-17, and MMP-27), which are primarily derived from dermal fibroblasts. The resulting fragmented collagen cannot produce a sufficient mechanical tension on the fibroblasts to activate collagen formation leading to abnormal collagen homeostasis with increased degradation and decreased production of collagen (Kang, 2019). Ultrasound is able to decrease the matric metalloproteases activity and stimulate TIMPs e.g., TIMP-2 which is an antagonist of MMP under specific conditions (Kruglikov & Krunglikova, 2011). Kim et al. (2021) study stated that matric metalloproteinases' overexpression were also inhibited by dual frequency ultrasound and thus it can be used in correcting the facial laxity by inhibiting the collagen degradation and promoting collagen synthesis.

2.5.2 Heat Shock Protein

Many chemical and physical stimuli including heat can initiate the heat shock protein synthesis. Among many functions of HSP protein, prevention and refolding of misfolded proteins, destruction of unstable proteins and signal transduction are important ones. Inadequate HSPs production is one of the reasons for some abnormalities and apoptosis (Kruglikov & Krunglikova, 2011; Sontag & Kruglikov, 2009). Stimulation of HSPs secretion by chemical stimulus is popular for anti-aging pharmaceuticals, because it can not only prolong the lifespan but also delays aging process. Heat shock protein synthesis is stimulated by a frequency of 10 MHz which is both intensive and long lasting. Using of dual frequency ultrasound with 10MHz is a new emerging way for delaying skin aging (Sontag & Kruglikov, 2009).

2.5.3 Patient Selection

The most appropriate subjects for nonsurgical face liftings are ones having only mild and moderate tissue laxity (Minkis & Alam, 2014). The procedure is dependent not only on patient's wound healing response, like age, dermatoheliosis but also personal habits like smoking, which all adversely effects on the collagen formation. Moreover, subjects with BMI<30 can give better outcome (Wulkan et al., 2016). This kind of lifting and tightening devices should not be used in patients with previous cosmetic procedures like fillers, and those who cannot be satisfied with a moderate result (Kim et al., 2021).

2.5.4 Safety

Ultrasound can be safely used in mild skin tightening and lifting procedure. Ultrasound cannot attack melanin and thus it is good for all skin types. In Tausch and Kruglikov, 2015 study has proved that in injection lipolysis procedure, dual frequency ultrasound can be used to reduce the pain that is caused by injection lipolysis and only welts and striations occurred (Wulkan et al., 2016). Kim et al. (2021) study reported that, throughout the dual frequency ultrasound treatment, no adverse effects were observed. Kruglikov study about venous ulcer treatment found that using dual-frequency ultrasound of 3 and 10 MHz was completely free from adverse reactions (Kruglikov & Kruglikova, 2011)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

Quasi-Experimental, Clinical Study

3.2 Study Population

3.2.1 Sample

Twenty subjects, age 25-45years with Fitzpatrick skin ranging from III and V and mild to moderate skin laxity.

3.2.2 Sample Size Determination

The similar article: high-intensity focused ultrasound for the treatment of wrinkles and skin laxity in seven different facial area is used for sample size population (Park et al., 2015)

Physicians' evaluation score of dual frequency ultrasound for the preauricular group showed 1.74 ± 0.60 at baseline to 1.26 ± 0.50 at 6 months compared to the overall score; 2.37 ± 0.68 at baseline to 1.47 ± 0.51 at 6 months.

Different change in periauricular group =0.48

Different change in overall sore =0.9

From the formula, $\alpha = 0.05$ (two-tailed) $Z_{0.025} = 1.96$, $Z_{0.10} = 1.28$

$n_1 = 20 \quad n_2 = 20$

$\sigma_1 = 0.50 \quad \sigma_2 = 0.51$

$S^2_p = [(n1 - 1) S^2_1 + (n2 - 1) S^2_2] / n_1 + n_2 – 2$

S^2_p (pooled variance) = 0.25505

$$n = (Z_{\alpha/2} + Z_{\beta})^2 \sigma^2 / (\mu_1 - \mu_2)^2$$

$$= (1.96 + 1.28)^2\, 0.25505 / 0.1764 = 15.178 \text{ or } 16 \text{ subjects}$$

Note

n = sample size

σ = variance

Estimated dropout rate is 20%. Thus 20 subjects (n=20) are recrited.

3.2.3 Inclusion Criteria

3.2.3.1 Participants age 25-45 years with mild to moderate skin laxity and Fitzpatrick skin type between III to V

3.2.3.2 Body mass index (BMI) less than 30 and historically healthy.

3.2.3.3 Subjects who accept the treatment of facial lifting with dual frequency ultrasound

3.2.3.4 Subjects who willingly to give informed consent and come every follow up visits.

3.2.4 Exclusion Criteria

3.2.4.1 Pregnant and breastfeeding women

3.2.4.2 Current cigarette smoking, heavy drinking and drug abuse.

3.2.4.3 Subjects with underlying medical illnesses and active skin diseases that could affect the wound healing process.

3.2.4.4 BMI > 30

3.2.4.5 Subjects who have cardiac pacemakers, metal and electronic implants

3.2.4.6 A hemorrhagic condition or regular use an anticoagulation therapy

3.2.4.7 Subjects with long term corticosteroid and immunosuppressive therapy

3.2.4.8 Subjects receiving vitamin A treatment or other face lifting cosmetic procedures within 6 months before study

3.2.5 Discontinuation Criteria

3.2.5.1 Participants want to discontinue the program due to any reasons

3.2.5.2 Participants can also be withdrawn if any exclusion criteria become apparent

3.2.5.3 Participants get serious adverse complication

3.2.5.4 Subjects who get pregnant during trial

3.2.5.5 Failure in a follow-up visit

3.3 Study Location

Mae Fah Luang University Hospital, Bangkok.

3.4 Research Instrument

3.4.1 Patient record form

3.4.2 Description of the trial objectives and purpose

3.4.3 Description of the trial design

3.4.4 Informed consent form

3.4.5 Clinical evaluation record form

3.4.6 Ultrasound conducting gel

3.4.7 Dual frequency ultrasound machine

3.4.8 Questionnaires and satisfaction assessment form

3.4.9 Adverse effect record form

3.5 Variables of the Study

Independent variable: Dual frequency ultrasound

Dependent variable: Physician Global Aesthetic Improvement Scale
Skin Viscoelasticity by Cutometer
Wrinkle by Visioscan

TEWL by Tewameter

Sebum by Sebum Meter

Adverse effect

Participant Satisfaction Score

3.6 Study Procedures

Participants received a course of 12 treatment sessions scheduled twice weekly for 6 consecutive weeks.

3.6.1 Preparation of Research Subjects

3.6.1.1 Participants were selected by fulfilled criteria.

3.6.1.2 The researcher explained a purpose and step of procedures during the study including the benefit and possible adverse reaction after treatment.

3.6.1.3 Participants signed an informed consent form for research participation.

3.6.1.4 The information of the subject was recorded.

3.6.2 Treatment Process

3.6.2.1 All subjects will be measured with the Cutometer, Tewameter, Visioscan and Sebum meter before treatment procedure.

3.6.2.2 Standardized photographs will be taken prior to the treatment (baseline) and at each follow-up visit 4^{th}, 8^{th} and 12^{th} weeks using digital camera. Photographic documentation using identical camera setting the same light and the same position both before and after treatment. Photographic facial positions: Left 45°, Center 0°, Right 45°

3.6.2.3 All metal jewelry in area treated should be removed.

3.6.2.4 The area to be treated is cleaned thoroughly with alcohol so that no makeup, cream, or any dirt is left. This is to prevent anything from interfering with the delivery of the ultrasound.

3.6.2.5 Apply the ultrasound gel on the face.

3.6.2.6 Apply the treatment head 3/10 MHz, size 5.0 cm^2 with intensity 1.5 W/cm on the left side of the face. After 6 minutes, the treatment size is changed to the right side of the face.

3.6.2.7 Then apply the treatment head 1/3 MHz, size 10.0 cm^2 with intensity 1.0 W/cm^2 on the left side of the face. After 6 minutes, the treatment size is changed to the right side of the face.

3.6.2.8 The researcher evaluated the subjects' post-treatment complications such as pain, swelling, erythema, burn, numbness and other side effects after each treatment.

3.6.2.9 The researcher advised post treatment care to all subjects.

Table 3.1 Treatment Procedure

Frequency MHz	**Mode**	**Intensity W/qcm**	**Time (min)**	
3/10	10ms/10ms	3MHz →1.5W	3	
		10MHZ →1.5W		L
3/10	1ms/1ms	3MHz →1.5W	3	
		10MHZ →1.5W		
3/10	10ms/10ms	3MHz →1.5W	3	
		10MHZ →1.5W		R
3/10	1ms/1ms	3MHz →1.5W	3	
		10MHZ →1.5W		
1/3	10ms/10ms	1MHz →1.0W	3	
		3MHz →1.0W		L
1/3	1ms/1ms	1MHz →1.0W	3	
		3MHz →1.0W		
1/3	10ms/10ms	1MHz →1.0W	3	
		3MHz →1.0W		R
1/3	1ms/1ms	1MHz 1.0W	3	
		3MHz 1.0W		

3.6.3 Follow up Visit (4^{th}, 8^{th} and 12^{th} week after final treatment)

3.6.3.1 Each follow-up visit, the researcher took a photograph of all participants under identical camera setting as before treatment. Photographic facial positions: Left 45°, Center 0°, Right 45°

3.6.3.2 All subjects will be measured skin elasticity, sebum, wrinkles and transepidermal water loss by cutometer, sebum meter, visioscan and tewameter.

3.6.3.3 Any adverse reaction occurred to the participants at follow up visit will be evaluated by research questionnaire.

3.7 Outcome Measurement and Data Collection

3.7.1 Subjective Evaluation

3.7.1.1 The improvement evaluated by comparing digital photographs at baseline and 4^{th}, 8^{th} and 12^{th} week after final treatment by Global Aesthetic Improvement Scale (GAIS)

1 = worse
2 = no change
3 = improve
4 = very improve
5 = Exceptional improve

3.7.1.2 Satisfaction will be evaluated by participant's satisfaction score by using grading scale at baseline and 4^{th}, 8^{th} and 12^{th} week after last treatment

-1 = unsatisfied
0 = indifferent
1 = somewhat satisfied
2 = moderately satisfied
3 = very satisfied
4 = completely satisfied

3.7.2 Objective Evaluation

3.7.2.1 Wrinkles will be evaluated by using Visio scan at baseline and follow up visit 4th, 8th and 12th week after last treatment.

3.7.2.2 Skin elasticity will be evaluated by cutometer at baseline and follow up visit 4th, 8th and 12th week after last treatment.

3.7.2.3 Sebum will be measured by sebum meter at baseline and follow up visit 4th, 8th and 12th week after last treatment.

3.7.2.4 Transepidermal water loss (TEWL) will be measured by tewameter at baseline and follow up visit 4th, 8th and 12th week after last treatment.

3.7.2.5 Adverse effect will be evaluated at 4th, 8th and 12th week after last treatment by research questionnaire.

3.8 Research Materials

3.8.1 Cutometer MPA 580

Figure 3.1 Cutometer MPA 580

This device measures upper skin elasticity by using suction method. It is useful for many different kinds of cosmetic formulation mainly antiaging or firmness enhancing products.(*Courage + Khazaka Electronic, Köln - Skin Testing Equipment*, n.d.)

3.8.2 Tewameter

Tewameter is to detect Transepidermal Water loss (TEWL) by evaluating the barrier function of the skin. It measures the density gradient of water evaporation directly by two pairs of sensors (temperature and relative humidity) inside the hallow cylinder. It can notice very slight skin water barrier damage even at an early phase (*Courage + Khazaka Electronic, Köln - Skin Testing Equipment*, n.d.).

3.8.3 VISIA complexion Analysis System

Figure 3.2 VISIA® Complexion Analysis System

VISIA® Complexion Analysis System (Canfield, Fairfield, NJ) can visualize skin pigmentation, dark spots, UV spots, photodamage area, inflammation, pores, wrinkles, skin textures, and porphyrins area.

3.8.4 Sebum Meter® SM 815

The sebum meter is used in dermatological basic research. It is the direct measurement of sebum secretion on skin, hair and scalp.

3.8.5 Dual Frequency Ultrasound

For this study, we will use Wellcomet LDM®MED dual frequency ultrasound machine.

Figure 3.3 LDM® MED

3.9 Data Analysis

3.9.1 Participants' eligibility in this research will be determined by applying inclusion and exclusion criteria and their personal information are highly confidential in data analysis.

3.9.2 Participants' medical record data and outcomes from this study trial conducted in Mae Fah Luang University Dermatology Clinic, we will analyze by using SPSS 18 software and Microsoft Excel 2010

3.9.3 General demographic data of participants will be recorded by using descriptive statistical analysis to supply descriptive information such as means, medians, modes, ranges and standard deviations.

3.9.4 Physician Global Aesthetic Improvement Scale and Participant Satisfaction Scores' outcomes are ordinal (non-parametric) time factor (each follow) with repeated measurement will be tested the difference by Friedman test.

3.9.5 Compare the mean of the value from Wrinkles, Sebum, Viscoelasticity, Transepidermal Water Loss Scores before treatment with each follow up visits by using repeated measure ANOVA for statistical significance.

3.9.6 Adverse events after treatment of dual frequency ultrasound will be analyzed by descriptive statistical analysis.

3.9.7 Statistical significance was defined as a p value of less than 0.05.

3.10 Ethical Considerations

This research has been performed with Good Clinical Practice (GCP), which considered as ethical and scientific standards for the design, conduct, recording and reporting of clinical research involving the participation of human subjects and must be approved by the human research committee of Mae Fah Luang University. The participants will gain assurance that the rights, safety, and well-being of research subjects are respected according to the ethical considerations.

The research should be justified on basis of a favorable risk and benefit assessment during the study protocol. Safety, rights and the well-being of participants is the most important thing to keep in mind, and it takes priority over academic and social benefits. All participants will be informed the standard information on how clinical trial should be conducted before the process of informed consent. All information related to the research will be kept confidential. In addition, the results of the research will not include personal data as well. The researcher will inform possible side effect and feeling during the treatment in order to relieve nervousness of the participants.

For benefits of this study, the participants will receive a new modality of non-invasive treatment for face lifting and tightening with dual frequency ultrasound technology. This is an opportunity to learn how dual frequency ultrasound effects on collagen and elastic synthesis and fibroblast activity leading to the development of medical knowledge that is waiting for researchers to conduct further studies in the future.

CHAPTER 4

RESULTS

4.1 General Characteristic of the Sample

The general demographics data for 20 subjects were analyzed by using descriptive statistical method.

Table 4.1 Participants' demographic characteristic

Demographic	n=20
Male	3
Female	17
Age Groups (years)	
Mean ± SD	35.75±2.35
Range	25-45
Occupation (n)	
Employee	10
Student	8
Officer	2
Underlying disease (n)	
YES	0
NO	20
Personal Medication (n)	
YES	0
NO	20
History of allergy (n)	
YES	0
NO	20

17 of total participants are women and the rest, 3 are men. The mean age is 35.75±2.35. The subjects include 10 employees, 8 students and 2 officers. All the subjects have no relevant medical, allergy and drug history.

4.2 Wrinkle Score Results Evaluated by VISIA

Table 4.2 Statistical analysis of Wrinkle Scores at baseline, follow-up visits 4th, 8th, and 12th week after last treatment session

Wrinkle Score	Mean	SD
Baseline	57.53	23.20
4th Week after Treatment	66.24	16.40
8th Week after Treatment	67.53	18.54
12th Week after Treatment	69.24	18.60
P-value	**0.01***	

Note P-value determine by Repeated measure ANOVA

* Statistically significant at the 0.05 level

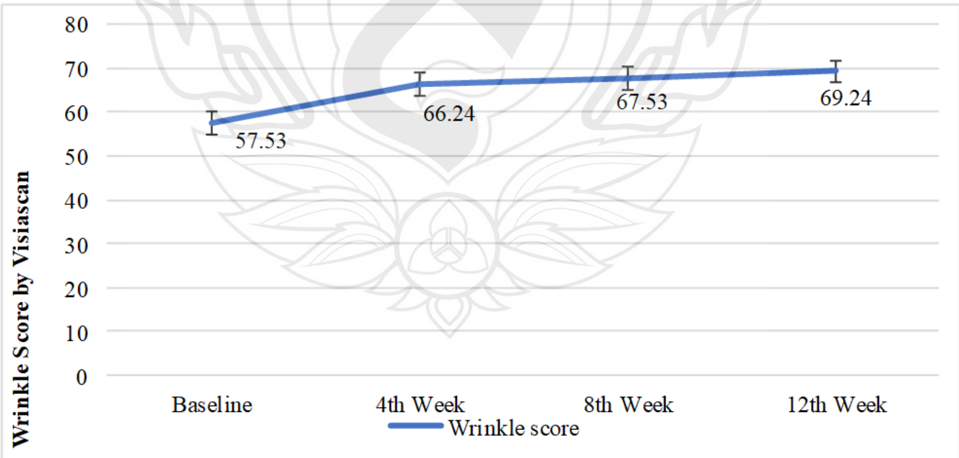

Figure 4.1 Line graph showing Wrinkle scores at baseline, follow-up visits 4th, 8th, and 12th week after last treatment session

According to the statistical analysis results, mean of Wrinkle score at baseline was 57.34±23.20, 4th week 66.24±16.40, 8th week 67.53±18.54, and 12th week 69.24±18.60. The mean of Wrinkle score in each visit statistically significant increased at the level of 0.05 ($p<0.001$).

Table 4.3 Multiple comparison of Wrinkle Scores at baseline, follow-up visits 4th, 8th and 12th week after last treatment sessions

Time	Pair Comparison	Mean difference	P-value
Baseline	4th Week	-8.706	0.024*
	8th Week	-10.00	0.012*
	12th Week	-11.71	0.099
4th Week	Baseline	8.71	0.024*
	8th Week	-1.30	1.000
	12th Week	-3.00	1.000
8th Week	Baseline	10.00	0.012*
	4th Week	1.29	1.000
	12th Week	-1.71	1.000
12th Week	Baseline	11.71	0.099
	4th Week	3.00	1.000
	8th Week	1.71	1.000

Note Multiple comparison determines by the Bonferroni method

* The mean difference is significant at the 0.05 level

According to multiple comparison result from table 4.3, Wrinkle score at baseline was lower than 4th week (-8.706) and 8th week (-10.00) statistically significant at the level of 0.05 ($p<0.05$).

4.3 Cutometer Score (Skin Elasticity Measurement)

Table 4.4 Statistical analysis of Cutometer Scores at baseline, follow-up visits 4th, 8th, and 12th week after last treatment session

Cutometer Score	Mean	SD
Baseline	0.4266	0.1380
4th Week after Treatment	0.5278	0.1651
8th Week after Treatment	0.5397	0.1783
12th Week after Treatment	0.5685	0.1875
P-value	**0.001***	

Note P-value determine by Repeated measure ANOVA

* Statistically significant at the 0.05 level

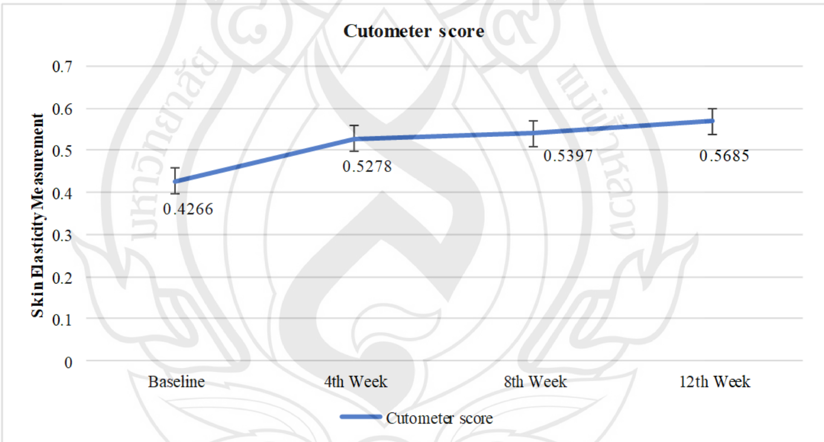

Figure 4.2 Line graph showing Cutometer scores at baseline, follow-up visits 4th, 8th, and 12th week after last treatment session

According to the statistical analysis results from table 4.4 and figure 4.2, mean values of cutometer score on baseline was 0.4266±0.1380, 4th week 0.5278±0.1651, 8th week 0.5397±0.1783, and 12th week 0.5685±0.1875. The mean of cutometer scores in each visit statistically significant increased at the level of 0.05 ($p<0.001$).

Table 4.5 Multiple comparison of Cutometer scores at baseline, follow-up visits 4th, 8th and 12th week after last treatment sessions

Time	Pair Comparison	Mean difference	P-value
Baseline	4th Week	-0.101	0.014*
	8th Week	-0.113	0.010*
	12th Week	-0.142	0.004*
4th Week	Baseline	0.101	0.014*
	8th Week	-0.012	1.000
	12th Week	-0.041	0.083
8th Week	Baseline	0.113	0.010*
	4th Week	0.012	1.000
	12th Week	-0.029	0.016*
12th Week	Baseline	0.142	0.004*
	4th Week	0.041	0.083
	8th Week	0.029	0.016*

Note Multiple comparison determines by the Bonferroni method

* The mean difference is significant at the 0.05 level

According to the multiple comparison result from table 4.5, it is found that Cutometer at baseline was lower than follow up 4th week (-0.101), 8th week (-0.113) and 12th week (-0.142) statistically significant at the level of 0.05 ($p<0.05$).Moreover, the cutometer score between follow up 8th week and 12th week is significantly different ($p=0.016$).

4.4 Tewameter Score (Skin Barrier Function Measurement)

Table 4.6 Statistical analysis of Tewameter score at baseline, follow-up visits 4th, 8th, and 12th week after last treatment session

Tewameter Score	Mean	SD
Baseline	17.59	2.74
4th Week after Treatment	15.95	2.03
8th Week after Treatment	15.41	2.10
12th Week after Treatment	15.04	2.03
P-value	**0.001***	

Note P-value determine by Repeated measure ANOVA

* Statistically significant at the 0.05 level

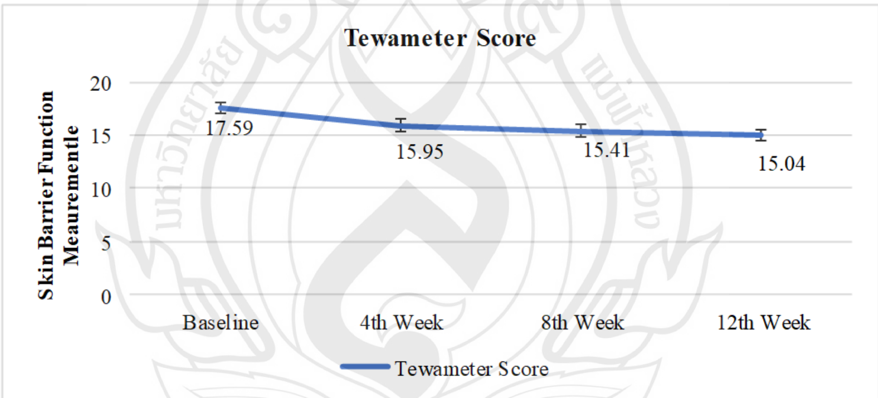

Figure 4.3 Linear graph showing Tewameter scores at baseline, follow-up visits 4th, 8th, and 12th week after last treatment session

According to the statistical analysis results from table 4.6 and figure 4.3, mean of Tewameter scores on baseline was 17.59±2.74, 4th week 15.95±2.03.38, 8th week 15.41±2.10, and 12th week 15.04±2.03. The mean of tewameter scores in each visit statistically significant reduced at the level of 0.05 ($p<0.001$).

Table 4.7 Multiple comparison of Tewameter score at baseline, follow-up visits 4^{th}, 8^{th} and 12^{th} week after last treatment sessions

Time	Pair Comparison	Mean difference	P-value
Baseline	4^{th} Week	1.635	0.004*
	8^{th} Week	2.182	0.000*
	12^{th} Week	2.547	0.001*
4^{th} Week	Baseline	-1.635	0.004*
	8^{th} Week	0.547	0.188
	12^{th} Week	0.912	0.153
8^{th} Week	Baseline	-2.182	0.000*
	4^{th} Week	-0.547	0.188
	12^{th} Week	0.365	0.549
12^{th} Week	Baseline	-2.547	0.001*
	4^{th} Week	-0.912	0.153
	8^{th} Week	-0.365	0.549

Note Multiple comparison determines by the Bonferroni method

* The mean difference is significant at the 0.05 level

According to the multiple comparison result from table 4.7, Tewameter score at baseline was higher than 4^{th} week (1.635), 8^{th} week (2.182) and 12^{th} week (2.547) statistically significant at the level of 0.05 ($p<0.05$).

4.5 Sebumeter Score (Sebum Measurement)

Table 4.8 Statistical analysis of Sebumeter score at baseline, follow-up visits 4th, 8th, and 12th week after last treatment session

Sebumeter Score	Mean	SD
Baseline	123.00	41.79
4th Week after Last Treatment	101.90	18.47
8th Week after Last Treatment	95.10	20.72
12th Week after Last Treatment	88.65	16.91
P-value	**0.001***	

Note P-value determine by Repeated measure ANOVA

* Statistically significant at the 0.05 level

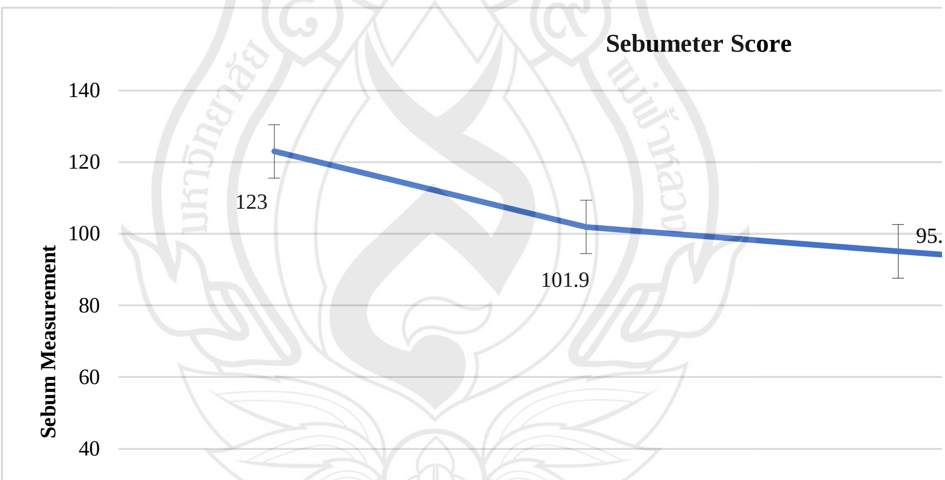

Figure 4.4 Linear graph showing sebumeter scores at baseline, follow-up visits 4th, 8th, and 12th week after last treatment session

According to the statistical analysis results from table 4.8 and figure 4.4, mean of Sebumeter at baseline was 123.00±41.79, 4th week 101.90±18.47, 8th week 95.10±20.72, and 12th week 88.65±16.91. The mean of Sebumeter in each visit statistically significant decreased at the level of 0.05 (p<0.001).

Table 4.9 Multiple comparison of Sebumeter score at baseline, follow-up visits 4th, 8th and 12th week after last treatment sessions

Time	Pair Comparison	Mean difference	P-value
Baseline	4^{th} Week	21.10	0.031*
	8^{th} Week	27.90	0.016*
	12^{th} Week	34.35	0.002*
4^{th} Week	Baseline	-21.10	0.031*
	8^{th} Week	6.80	0.587
	12^{th} Week	13.25	0.007*
8^{th} Week	Baseline	-27.90	0.016*
	4^{th} Week	-6.80	0.587
	12^{th} Week	6.45	0.048*
12^{th} Week	Baseline	-34.35	0.002*
	4^{th} Week	-13.25	0.007*
	8^{th} Week	-6.45	0.048*

Note Multiple comparison determines by the Bonferroni method

* The mean difference is significant at the 0.05 level

According to the multiple comparison result from table 4.9, it is found that Sebumeter score at 8^{th} week is decreased than baseline(-27.90) statistically significant at the level of 0.05 ($p<0.05$).Moreover, Sebumeter score at 12^{th} week lower than baseline (-34.35), 4^{th} (-13.25) and 8^{th} week (-6.45) statistically significant at the level of 0.05 ($p<0.05$).

4.6 Global Aesthetics Improvement Score (GAIS)

Global Aesthetics Improvement Score (GAIS) is graded as

1 = worse

2 = no change

3 = improve

4 = very improve

5 = exceptional improve

Table 4.10 Statistical analysis of GAIS on follow-up 4^{th}, 8^{th}, and 16^{th} week

	GAIS, n (%)				
	1	**2**	**3**	**4**	**5**
4^{th} Week	-	2(10.0)	12(60.0)	6(30.0)	-
8^{th} Week	-	-	14 (70.0)	6 (30.0)	-
12^{th} Week	-	-	6 (30.0)	14 (70.0)	-

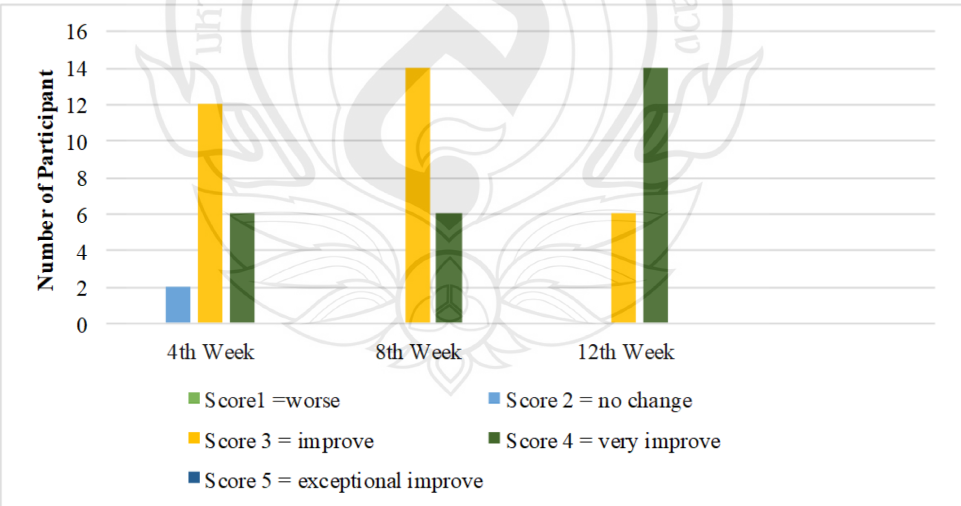

Figure 4.5 Bar graph showing GAIS on follow-up 4^{th}, 8^{th}, and 12^{th} week

Table 4.11 Statistical analysis of GAIS score

GAIS Score	Mean	SD
4th Week	3.20	0.62
8th Week	3.30	0.47
12th Week	3.70	0.47
P-value	**0.001***	

Note P-value determine by Friedman test.

* Statistically significant at the 0.05 level

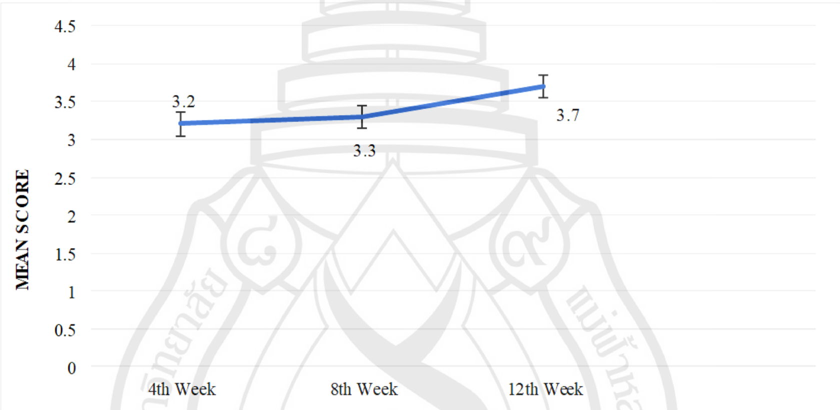

Figure 4.6 Linear graph showing GAIS score at follow-up visits 4^{th}, 8^{th}, and 12^{th} week after last treatment session

According to table 4.10 and figure 4.5, out of 20 participants, 12 participants were rated as improved, 6 participants very improved whereas 2 participants are rated as no change by dermatologist. At follow up visit 8^{th} week, 14 participants are rated as improve and 6 participants very improve. By 12^{th} week, very improve was seen in 14 participants and improve was seen in 6 participants. Compare to 4^{th} week, more improvement was found in 12^{th} week. According to statistical analysis, the results of GAIS score was increased and it was statistically significant ($p<0.001$).

Table 4.12 Multiple comparison of GAIS between follow-up 4th, 8th and 12th week

Time	Pair comparison	P-value
4th Week	8th Week	0.157
4th Week	12th Week	0.002*
8th Week	12th Week	0.005*

Note Significance values have been adjusted by the Bonferroni correction for multiple tests.

* Statistically significant at the 0.05 level

According to the multiple comparison result from table 4.5, It found that GAIS at 16th week higher than 4th and 8th week and at 8th week higher than 4th week statistically significant at the level of 0.05 ($p<0.05$).

4.7 Patient Satisfaction Score

Patient satisfaction score is graded as

-1 = unsatisfied

0 = indifferent

1 = somewhat satisfied

2 = moderately satisfied

3 = very satisfied

4 = completely satisfied

Table 4.13 Statistical analysis of patient satisfaction score on follow-up 4th, 8th, and 12th week

	Patients' Satisfaction, n (%)					
	-1	**0**	**1**	**2**	**3**	**4**
4th Week	-	-	12 (60.0)	8 (40.0)	-	-
8th Week	-	-	-	2 (10.0)	18 (90.0)	-
12th Week	-	-	-	1 (5.0)	19 (95.0)	-

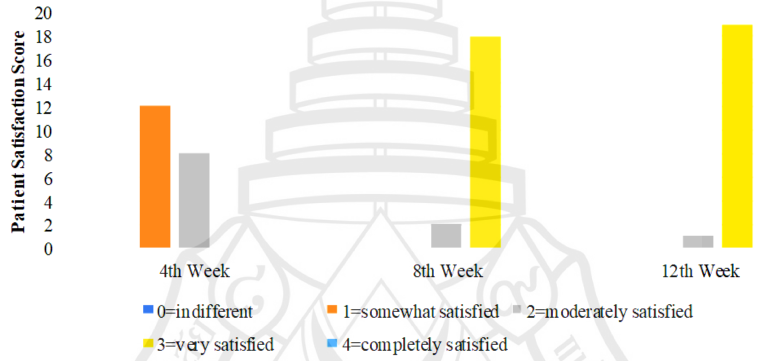

Figure 4.7 Bar Graph showing patient satisfaction score on follow-up 4th, 8th, and 12th week

Table 4.14 Statistical analysis of Patient Satisfaction score

Patient Satisfaction Score	**Mean**	**SD**
4th Week	1.40	0.50
8th Week	2.90	0.31
12th Week	2.95	0.22
P-value	**0.001***	

Note P-value determine by Friedman test.

* Statistically significant at the 0.05 level

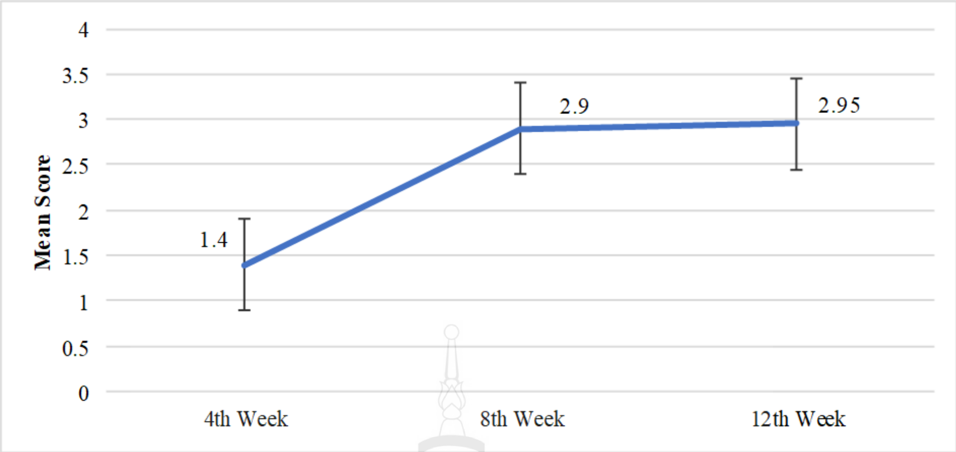

Figure 4.8 Linear graph showing Patient Satisfaction score at follow-up visits 4th, 8th, and 12th week after last treatment session

According to table 4.13 and figure 4.6, out of 20 participants, 12 participants rated somewhat satisfied and 8 participants rated moderately satisfied. At 8th week, 2 participants gave moderately satisfied whereas 18 participants gave very satisfied. By follow up visit 12th week, 19 participants are completely satisfied with treatment. Compare to 4th week, participants were more satisfied with treatment in 12th week. According to statistical analysis, the results of patient satisfaction score were increased and it was statistically significant ($p<0.001$).

Table 4.15 Multiple comparison of patient satisfaction score between follow-up 4th, 8th and 12th week

Time	Pair comparison	P-value
4th Week	8th Week	0.001*
4th Week	12th Week	0.001*
8th Week	12th Week	0.317

Note Significance values have been adjusted by the Bonferroni correction for multiple tests.

* Statistically significant at the 0.05 level

According to the multiple comparison result from table 4.15, It is found that patient satisfaction score at 12^{th} week higher than 4^{th} and 8^{th} week and 8^{th} week higher than 4^{th} week significant at the level of 0.05 ($p<0.05$).

Figure 4.9 Patient Photographs (Front View/ Right Lateral View) Between Before Treatment and 12^{th} week After Treatment

4.8 Adverse Effects

Throughout the research process, there was no adverse effect in all subjects.

CHAPTER 5

DIESUSSION, CONCLUSION, SUGGESTION

5.1 Discussion

In the past, there was only one option for face and skin laxity correction which was aesthetic surgical lifting procedure. Despite being the gold standard treatment for face lifting, many patients prefer less invasive procedure. Ultrasound was first used for diagnostic procedure. Starting from 2009, ultrasound was accepted as treatment for brow lift, followed by chin lift by United States Food and Drug Administration (FDA) (Wulkan et al., 2016)

Dual frequency ultrasound is the one which use two different frequencies oscillating between, creates very rapid pressure change (ultrasound micro massage effects) which can suppress the matrix metalloproteinases and activate heat shock protein. This is in turn aim to reduce the redundant skin (Kang, 2019; Lee et al., 2018).

This was the study of Dual frequency ultrasound for face lifting. In this study, 20 participants were included; 17 for females and 3 for males. All the participants don't have any relevant medical, drug and allergy history. To prove dual frequency ultrasound as safe and effective method for redundant skin, wrinkle scores, Cutometers, Tewameter and Sebumeter are measured. For subjective evaluation, Global Aesthetic Improvement Scale (GAIS) and Patients Satisfaction Scores are used.

The wrinkle scores by VISIA at baseline 57.53±23.20, was raised to 69.24±18.60 at the end of 12th week which was statistically significant at the level of $p<0.001$. When comparing the mean change of each visit, statistical different was seen between baseline and 4th week, 8th week. These results indicated that the effect of wrinkle reduction was seen until 12th week post treatment.

Skin elasticity was also measured with cutometer. Mean scores were increased with each treatment and it was statistically significant ($p<0.001$). These mean scores were 0.4266 0.1380 at baseline, 0.5278 0.1651 at 4th week, 0.5397 0.1783 at 8th week, and 0.5685 0.1875 at 12th week respectively. This stated that the ultrasound can make redundant skin firmer and more elastic.

According to Tewameter and Sebumeter scores, the scores are decreased statistically significant at the level of $P<0.001$. Moreover, for the Sebumeter scores, statistically different was also seen between 8th week and 12th week.

Surprisingly, the dermatologists rated very improve for 14 patients and improve for 6 patients at the end of the 12th week. Compared to the 4th week follow up, GAIS scores are much higher in 8th and 12th week. By seeing this, dual frequency ultrasound effect on face lifting was best seen at the 12th week follow up. In addition, nearly 20 participants except only one were very satisfied with the lifting result. Throughout the studies, there were no serious side effects.

Therefore, according to the results of this study, dual frequency ultrasound can be used as an effective and safe treatment procedure for face lifting and also well tolerated.

5.2 Conclusion

Regarding to this study, wrinkle and skin elasticity are improved with time to time compared to the baseline. The dual frequency ultrasound's face lifting result remains the same for 12th week. Moreover, Sebumeter and Tewameter scores, are decreased over time throughout the treatment which demonstrated that dual frequency ultrasound can also decrease sebum production and make the skin more hydrated. Any harmful effects were not seen. In conclusion, dual frequency ultrasound can be used safely for face lifting and considered as an effective treatment- options for redundant skin.

5.3 Limitation

This study cannot know exactly how long the dual frequency ultrasound face lifting effect still persist. It is better to study the effect of dual frequency ultrasound in larger population. Some sedentary lifestyle changes including smoking, diet, sleep habit which can affect on the result cannot be controlled well.

5.4 Recommendation

This study may be useful as database for further research about dual frequency ultrasound in facial rejuvenation, facial moisturizing and acne treatment.

Furthermore, dual frequency ultrasound may have benefits in other dermatologic and skin condition. Therefore, this may be used as a referent paper for further research.

More researches should be conducted in the future to know other effect of dual frequency ultrasound not only in aesthetic fields but also in therapeutic treatment.

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APPENDICES

APPENDIX A

ETHICAL APPROVAL DOCUMENT

บันทึกข้อความ

From Research Administration Division, Mae Fah Luang University Research and Innovation Institute, Phone No. 7170 (Chantra)

MFU 7742(1)/1221 April 18th, 2022

Subject The consideration result of the research protocol and associated documents

To Khine Nyein Chan, M.D.

Followed by your research protocol submission, ประสิทธิภาพและความปลอดภัยของอัลตราซาวนด์ความถี่คู่ในการยกกระชับใบหน้า (Efficacy and safety of dual frequency ultrasound for facial lifting), EC 22034-20.

The Mae Fah Luang University Ethics Committee on Human research has considered the protocol which the consideration result is

Approval

Please read and try to understand the post-approval regulations then sign with the date specified on page 2 and page 4 of the Certificate of Approval (COA) as attached. Then send back the signed COA through the email rec.human@mfu.ac.th together with giving the address for sending the COA and associated documents. For more information, please contact us through the email rec.human@mfu.ac.th or the phone No. 0-5391-6551 or 0-5391-7170-1.

For your consideration and further implementations.

Best Regards,

(Karnkul Bumrungchad)

Head of Research Administration Division, Committee and Vice Secretary

The Mae Fah Luang University Ethic Committee on Human Research

APPENDIX B

INFORMATION SHEET

1. TITLE

EFFICACY AND SAFETY OF DUAL FREQUECNCY ULTRASOUND FOR FACIAL LIFTING

2. OBJECTIVE

2.1 General Objective

To evaluate the efficacy and safety of dual frequency ultrasound for lifting and tightening of lax facial skin.

2.2 Specific Objective

2.2.1 Primary Outcome

To compare average mean change of wrinkle, sebum, viscoelasticity and transepidermal water loss measurement from baseline to 4^{th} ,8^{th} and 12^{th} weeks after completion of final treatment by using Visioscan, sebum meter, Cutometer and Tewameter.

2.2.2 Secondary Outcome

To assess overall improvement and the patient satisfaction of dual frequency ultrasound for face lifting by using Global Aesthetic Improvement Scale and Participant's Satisfaction Score.

3. BACKGROUND

As human gets older and older, skin constantly gets aging process. Human facial aging is inevitable and ongoing process. It includes skin laxity, loss of subcutaneous tissue, photodamage and bony resorption which is associated with chronological and photoaging. Due to aging, skin fibroblasts, many skin appendages and collagen formation decrease both in numbers and functions which lead to skin laxity (Robins, 2019) (Sadick, 2008).

Ultrasound is capable of inhibiting the MMPs to restore the skin barrier thus it is used now in antiaging treatment and skincare (Kim et al., 2021). The dual-frequency ultrasound is highly efficient in the modulation of MMPs and HSPs which make it to use for anti-aging treatment and collagen and elastin production through the application of combining with 10MHz ultrasound. The main principle of dual-frequency ultrasound is activating fibroblasts which can promote tissue regeneration (collagen, elastin) and protein synthesis by a cavitation and microstreaming. These all make dual frequency ultrasound possible to use for skin tightening and lifting. The new type of micro-massage effect is assumed to be key mechanism of dual-frequency ultrasound treatment suppress the activity of MMPs and activate the HSP (Lee et al., 2018).

4. STUDY LOCATION

Mae Fah Luang University Hospital, Bangkok

5. PROCEDURE

Participants received a course of 12 treatment sessions scheduled twice weekly for 6 consecutive weeks.

5.1 Preparation of Research Subjects

a. Participants were selected by fulfilled criteria.

b. The researcher explained a purpose and step of procedures during the study including the benefit and possible adverse reaction after treatment.

c. Participants signed an informed consent form for research participation.

d. The information of the subject was recorded.

5.2 Treatment Process

a. All subjects will be measured with the Cutometer, Tewameter, Visioscan and Sebum meter before treatment procedure.

b. Standardized photographs will be taken prior to the treatment (baseline) and at each follow-up visit 4^{th} ,8^{th} and 12^{th} weeks using digital camera. Photographic documentation using identical camera setting the same light and the same position both before and after treatment. Photographic facial positions: Left 45°, Center 0°, Right 45°

c. All metal jewelry in area treated should be removed.

d. The area to be treated is cleaned thoroughly with alcohol so that no makeup, cream, or any dirt is left. This is to prevent anything from interfering with the delivery of the ultrasound.

e. Apply the ultrasound gel on the face.

f. Apply the treatment head 3/10 MHz, size 5.0 cm^2 with intensity 1.5 W/cm on the left side of the face. After 6 minutes, the treatment size is changed to the right side of the face.

g. Then apply the treatment head 1/3 MHz, size 10.0 cm^2 with intensity 1.0 W/cm^2 on the left side of the face. After 6 minutes, the treatment size is changed to the right side of the face.

h. The researcher evaluated the subjects' post-treatment complications such as pain, swelling, erythema, burn, numbness and other side effects after each treatment.

i. The researcher advised post treatment care to all subjects.

5.3 Follow up Visit (4^{th}, 8^{th} and 12^{th} week after final treatment)

Each follow-up visit, the researcher took a photograph of each subject by using identical camera setting the same light and the same position as before treatment. Photographic facial positions: Left 45°, Center 0°, Right 45°All subjects will be measured skin elasticity, sebum, wrinkles and transepidermal water loss by cutometer, sebum meter, visioscan and tewameter. All participants will be evaluated participant's satisfaction by using Participant's satisfaction Score. Overall improvement will be evaluated by Physician Global Aesthetic Improvement Scale (GAIS).Any adverse reaction occurred to the participants at follow up visit will be evaluated by research questionnaire.

APPENDIX C

INFORMED CONSENT FORM

ชื่อโครงการวิจัย ……Efficacy and Safety of Dual Frequency Ultrasound For Facial Lifting
ประสิทธิภาพและความปลอดภัยของ อัลตราซาวนด์ความถี่คู่ ในการยกกระชับใบหน้า

ข้าพเจ้า นาย/นาง/นางสาว... ที่อยู่..
…………………………………………ได้อ่านรายละเอียดจากเอกสารชี้แจงข้อมูลแก่อาสาสมัคร ผู้เข้าร่วมในโครงการวิจัยวิจัย ฉบับวันที่............................

ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย และสำเนาเอกสารแสดงเจตนายินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนามและลงวันที่ ทั้งนี้ก่อนที่จะลงนาม ข้าพเจ้าได้รับการอธิบายโดยละเอียดจากผู้วิจัยถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้น ประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น

ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัย โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้ารับทราบจากผู้วิจัยว่า หากเกิดอันตรายใด ๆ จากการวิจัย ข้าพเจ้าจะได้รับการรักษาพยาบาล ตามที่ระบุในเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย

ข้าพเจ้ามีสิทธิที่จะถอนตัวออกจากโครงการวิจัยเมื่อใดก็ได้ การถอนตัวนี้ไม่มีผลต่อการรักษาพยาบาลและสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ การรายงานหรือสรุปผลการวิจัยจะไม่ระบุชื่อนามสกุลของข้าพเจ้า การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่าง ๆ ที่เกี่ยวข้อง จะกระทำด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความสมัครใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

.... ลงนามผู้เข้าร่วมในโครงการวิจัย

(..) ชื่อ-สกุล ผู้เข้าร่วมในโครงการวิจัย (ตัวบรรจง)

วันที.......เดือน..............พ.ศ.................

ข้าพเจ้าได้อธิบายโดยละเอียดถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้นประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น ให้ผู้เข้าร่วมในโครงการวิจัยได้ทราบและมีความเข้าใจดีแล้ว พร้อมทั้งลงนามในเอกสารแสดงเจตนายินยอมด้วยความสมัครใจ

.... ลงนามผู้วิจัยชื่อ-สกุล ผู้วิจัย (ตัวบรรจง)

.... ลงนามพยาน

(..) ชื่อ-สกุล พยาน (ตัวบรรจง)

วันที่ ....เดือน................พ.ศ................

APPENDIX D

RESEARCH PROFILE (CONFIDENTIAL)

General Information (Only official)

1. Date
2. Sex □ Male. □ Female
3. Pregnancy □ Yes □ No
4. Occupation -
5. Underlying disease:
6. Personal medication and supplement:
   a.Chemo-radiotherapy
   b.Active inflammatory skin disease
   c.History of malignant of premalignant lesions in the treatment area
7. History of food or drug allergy:
8. Current treatment :
9. History of following treatment before the study

   □ Yes (within 6 months) ...Identify
   -Radiofrequency
   -Botox
   -Microfocused Ultrasound
   -HIFU
   -Ablative and/or Non-ablative Laser Treatment
   -Microdermabrasion
   □ No

APPENDIX E

PHYSICIAN RECORD FORM

RESEARCH RECORD: (RESEARCHERS' PART)
EFFICACY AND SAFETY OF DUAL FREQUENCY ULTRASOUND FOR FACIAL LIFTING

ID.........................

Tasks at the first visit

Date ..../......................../...............

Please use □ (□) symbol

Check list for the first visit Process		
Process	**Yes**	**No**
Physical examination for fitness of the volunteer		
The volunteer has completed research profile		
All of the profile data is fit to selection criteria		
Assure that the volunteer can understand information in appendix and how to care his or her face after treatment		
The volunteer has written the informed consent form		
The researcher asked the volunteer for any curiosity		
The researcher answered the volunteer's questions		
Photography of the face were performed		
Cutometer, Tewameter, Visioscan and sebum meter was performed		

CASE RECORD FORM, CRF

ID

1. Cutometer MPA 580

Cutometer	Right	Left	Mean
Baseline			
Week 4th after final session			
Week 8th after final session			
Week 12th after final session			

2. Tewameter

Tewameter	Right	Left	Mean
Baseline			
Week 4th after final session			
Week 8th after final session			
Week 12th after final session			

3. Sebum Meter

Sebum Meter	Right	Left	Mean
Baseline			
Week 4th after final session			
Week 8th after final session			
Week 12th after final session			

4. Global Aesthetic Improvement Scale by 3 Dermatologists

Baseline	Week 4 after final session	Week 8 after final session	Week 12 after final session
GAIS score			

1 = worse

2 = no change

3 = improve

4 = very improve

5 = Exceptional improve

APPENDIX F

ADVERSE RECORD FORM

รหัสอาสาสมัคร ....

1. อาการข้างเคียงที่ไม่พึงประสงค์

โปรดใส่เครื่องหมายถูก (✓) ลงในช่องว่างที่มีอาการไม่พึงประสงค์หลังการรักษาในแต่ละครั้ง

อาการ	**ใช่**	**ไม่ใช่**	**ระยะเวลา**	**หมายเหตุ**
เจ็บหรือปวด				
บวม				
แดง				
ไหม้				
ชา				
อื่น ๆ				

2. คะแนนความพึงพอใจ

การประเมินความพึงพอใจ โดยอาสาสมัคร (โปรดทำเครื่องหมายวงกลม ◯)

-1	0	1	2	3	4

-1 หมายถึง ไม่พึงพอใจ

0 หมายถึง ไม่แตกต่างจากเดิม

1 หมายถึง พึงพอใจระดับน้อย

2 หมายถึง พึงพอใจระดับปานกลาง

3 หมายถึง พึงพอใจระดับมาก

4 หมายถึง พึงพอใจที่สุด

3. หากเลือกได้ ท่านยินดีที่จะรับการรักษาแบบเดียวกันนี้ในอนาคตอีกไหม

☐ รับ ☐ ไม่รับ