FRACTIONAL CARBON DIOXIDE LASER COMBINED WITH TOPICAL ASTAXANTHIN GEL VERSUS FRACTIONAL CARBON DIOXIDE LASER; A SPLIT FACE COMPARISON IN TREATING ACNE SCARS

BY

MISS CHANYA SUEVERACHAI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (DERMATOLOGY) CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE THAMMASAT UNIVERSITY ACADEMIC YEAR 2018

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ENTITLED

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was approved as partial fulfillment of the requirement for
the degree of Master of Science (Dermatology)

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Thesis Title	FRACTIONAL CARBONDIOXIDE LASER COMBINED WITH TOPICAL ASTAXANTHIN GEL VERSUS FRACTIONAL CARBONDIOXIDE LASER; A SPLIT FACE COMPARISON IN TREATING ACNE SCARS
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ABSTRACT

Background: Acne scars are atrophic scar that is caused by the decreased of the net collagen resulted in a depression of the surface of the skin causing a serious cosmetic appearance problems. Many modalities have been used in treating atrophic acne scar but combined therapies seems to provide the best result in treatment. Astaxanthin is a source of active antioxidant enzymes and is generally accepted to provide cosmetic improvements. However, no studies have yet been carried out to make comparisons between fractional carbon dioxide laser treatment performed with and without combination with topical astaxanthin to treat atrophic acne scars.

Objectives: To assess the effects of applying topical astaxanthin (78.9μm solution) combined with fractional carbon dioxide laser and compare these with the solo use of the fractional carbon dioxide laser for the treatment of atrophic acne scars.

Materials and methods: The study investigated 30 participants, with each receiving treatment on the left or right side of the face, decided randomly. Half of the face was treated with the fractional carbon dioxide laser and topical astaxanthin, while half was treated using only the fractional carbon dioxide laser. The evaluation was carried out using digital photography, Antera® 3D photography,

Goodman and Baron acne scar grading system and patient satisfaction score were recorded at baseline, week 4, week 8 and week 12 following the treatment.

Results: The results for the fractional carbon dioxide laser treatment plus astaxanthin showed a statistically significant fall from 10.18 to 8.75 ($p<0.001$), while for the placebo side there was a statistically significant fall from 10.35 to 9.26 ($p<0.001$). No statistically significant difference was found between the two groups. ($p=0.15$). Furthermore, the use of topical astaxanthin produced no observable side effects.

Conclusions: The application of topical astaxanthin combined with fractional carbon dioxide laser treatment works effectively in reducing atrophic acne scars, but the difference when compared to the fractional carbon dioxide laser treatment alone is not statistically significant. This may be a consequence of the low concentration of topical astaxanthin used, short duration of follow-up, relatively low laser parameter as well as the small sample size may also play a part in these findings.

Keywords: Acne scars, Astaxanthin, Fractional carbon dioxide laser

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Miss Chanya Sueverachai

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Symbols/Abbreviations	Terms
mg	milligrams
mJ	millijoules
mm	millimeters
mm^3	milliliters
ms	milliseconds
nm	nanometers
AU	Arbitrary unit
ALR	Ablative laser resurfacing
AFR	Ablative fractional laser resurfacing
CO2	Carbon Dioxide
FDA	The Food and Drug Administration
FP	Fractional photo thermolysis
FrCO2	Fractional Carbon Dioxide Laser
H&E	Hematoxylin and Eosin
Hz	Hertz
J/cm2	Joule per square centimeter
MTZ	Microthermal Treatment Zone
MMPs	Matrix Metalloproteinases
N	Number
NAFR	Non ablative fractional resurfacing
PIH	Post-inflammatory hyperpigmentation
R	Roughness
TCA	Trichloroacetic acid
3D	Three Dimension
bFGF	Basic fibroblast growth factor
P-Value^w	P-value within group
P-Value^b	P-value between group

CHAPTER 1
INTRODUCTION

Acne vulgaris is commonly found in patient during adolescent years, young adult and in minority cases some can persist through the later adulthood. The main pathogenesis of acnes are increase in sebum production, colonization of *Propionibacterium acnes*, follicular hyper keratinization and peri-follicular inflammation which in every process can damage the skin and resulted in atrophic scars and/or hypertrophic scars.

Two basic types of scar can be found after the acne process is atrophic scar which can occur up to 80 percent of the cases, while the rest develop hypertrophic scars and/or keloid. Atrophic scars can be sub-classified into 3 subtypes; rolling scar, box scar and ice pick scar which each has its own characteristic and each response differently to the treatment.

While many modalities has been introduced as a treatment of choice in treating acne scars such as chemical peels, dermabrasion, laser treatment, dermal grafting, tissue augmentation, needling, there is still no definitive guideline in treating acne scars. However, many study has proposed that combining different methods can help improve the efficacy in treating acne scars and also decreases the downtime and inflammation associated with laser treatment.

Lasers for acne scar can be divided into two major categories, the ablative laser and the non-ablative laser. While the ablative laser has more efficacy in treating the atrophic acne scars, it's disadvantages such as peri-procedure discomfort and longer recovery time has become their biggest limitation. Non-ablative laser, on the contrary, are more tolerable with shorter recovery time but multiple sessions of treatment are required and provide less impressive result than the traditional ablative lasers.

Fractional laser has been introduced in the field of laser to reduce disadvantages of those traditional ablative lasers made while trying to maintain the high efficacy in resurfacing the skin. By the word "fraction," means that only some part of the skin will be affected leaving the unaffected part to help aid in re-epithelialization after the treatment, therefore it decreases the downtime of the post-laser treatment. Fractional carbon dioxide laser has combined the traditional carbon dioxide laser, which is 10,600

nm, with the technology of fractional photo-thermolysis (FP) intervention. This way, patient will experience lesser downtime such as transient erythema, crusting and post-inflammatory pigmentation after each laser session, while still gaining the high efficacy of the traditional CO2 laser. But nevertheless, some patient will still face the inflammation associated laser treatment but in the lesser portion.

Astaxanthin, a xanthophyll carotenoids, is an active antioxidant enzyme that can be found in living cells of *Haematococcus pluvialis*, *Chlorella zofingiensis*, *Chlorococcum*, and *Phaffia rhodozyma.* It's chemical structure contains conjugated bonds of hydroxyl and keto group providing the lipophilic and hydrophilic properties making them able to stay inside and outside of the cell membrane. Astaxanthin has a wide range of biological activities such as antioxidant effects, anti-inflammation, anti-lipid peroxidation activity, anti-diabetic activity, cardiovascular disease prevention, anticancer activity and also acts as immuno-modulators. It has become one of the most potent carotenoid that is use in cosmetic dermatology in various form such as powder, tablets and cream.

In this study, we would like to see the improvement in quality of the acne scar by treating with fractional carbon dioxide laser combined with topical astaxanthin gel, a split face comparison. After the treatment, we will evaluate the efficacy of the treatment by using a standardize acne scar assessment, evaluating by the blinded clinician. Satisfaction and safety score will also be evaluated by both patient and clinician.

Our results will help clinician and patient in providing another option to improve atrophic acne scars by combining the laser treatment and the topical astaxanthin gel and also to have a reduction in laser associated downtime and inflammation.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Acne scar

Acne vulgaris effects 80% of people between the age 11-30 years old, and 5% in adults over 30 year of age. Scarring on visible area such as face and neck can associated with negative psychological impact. Many treatment modality has been introduced to provide the best options for each patient (6).

2.1.1 Pathogenesis

The main pathogenesis of acnes are increase in sebum production, colonization of *Propionibacterium acnes*, follicular hyper-keratinization and perifollicular inflammation which all can damage the skin and resulted in atrophic scars and/or hypertrophic scars. If the inflammation of the associated acne scar result in a net degradation of the collagen fibers during the healing process then the scar become atrophic, on the other hand, if there is a net gain of collagen then the scar become hypertrophic and/or keloid (6).

2.1.2 Classification

As we know, the acne scars are classified into hypertrophic scar or keloid and atrophic scars, the atrophic acne scar occur up to 80% of the cases, while the rest develop hypertrophic scars and/or keloid. Atrophic acne scar itself can be subdivided into 3 types which are ice prick, rolling and boxcar according to its morphology.

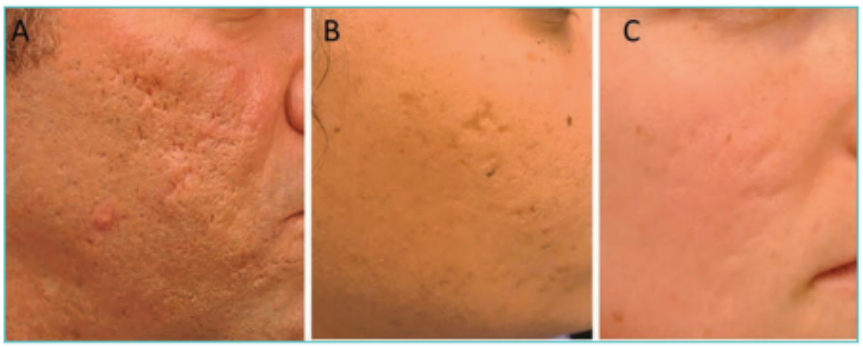


Figure 2.1: The three types of atrophic acne scar: A) icepick, B) boxcar, and C) rolling

The most common subtype is the icepick scar, occur up to 70 percent of all the scar type, is characterized by less than 2mm, V-shaped epithelial tracts with sharp margin that extends downwards to deep dermis or subcutaneous tissue. This character of icepicks make this type of the scar very resistant to the conventional treatment. The second most common subtype is the box scar, occur about 20 percent to 30 percent, is characterized by round to oval depressions with a measure of diameter between 1.5 to 4.0 mm. The least common type is the rolling scar, occur about 15 percent to 25 percent, characterized by superficial shadowing and undulating appearance with the diameter up to 5 mm. Rolling scar has a unique feature of fibrous anchoring of the dermis down to subcutaneous which the treatment must be focus on (6,8). (Figure 2.2)

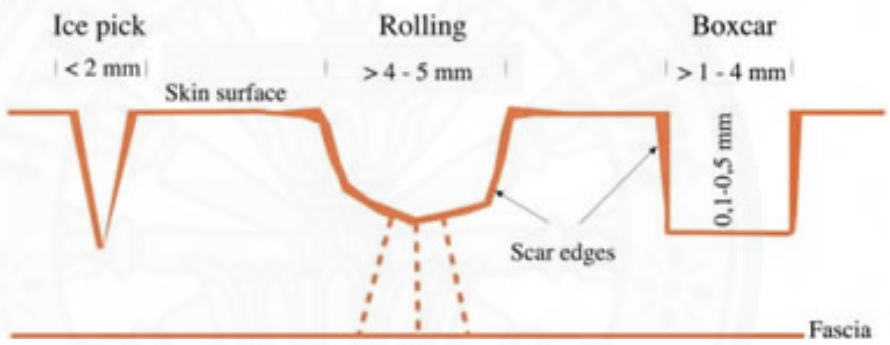


Figure 2.2: Acne scar subtypes (adapted from Fabbrocini et al).

By sub-classifying each type of the atrophic scar also aid the clinician in choosing an adequate treatment for each type of the scar that suits specifically with each patients as each type of scar response differently to each treatment option.

2.1.3 Treatment

Many modalities has been introduced to treat atrophic acne such as chemical peels, dermabrasion, laser treatment, dermal grafting, tissue augmentation, needling, excision and fat transplantation (5,6,7,8,16). But in order to achieve the best result that fits patient individual's need, combination therapy may be required for treatment in the same visit to improved to a degree that could not be obtained by each procedure alone. Each type of the acne scar also response differently to each treatment option, therefore physical examination to evaluate each patient is an important procedure.

When patient present with acne scars clinician have to considered whether the patient has residual erythema or not, if present then usually the first step is

to reduce the redness with vascular lasers such as PDL, IPL or KTP. When patient finish treated with erythema the clinician then address whether the patient has the generalized or individual scars (Figure 2.3). The remaining scars should then be treated according the most suitable method. For the broad areas of scarring, lasers and resurfacing agents remain the mainstay but for the individual scars, injectable fillers or surgical procedure are more appropriate (6). (Figure 2.3)

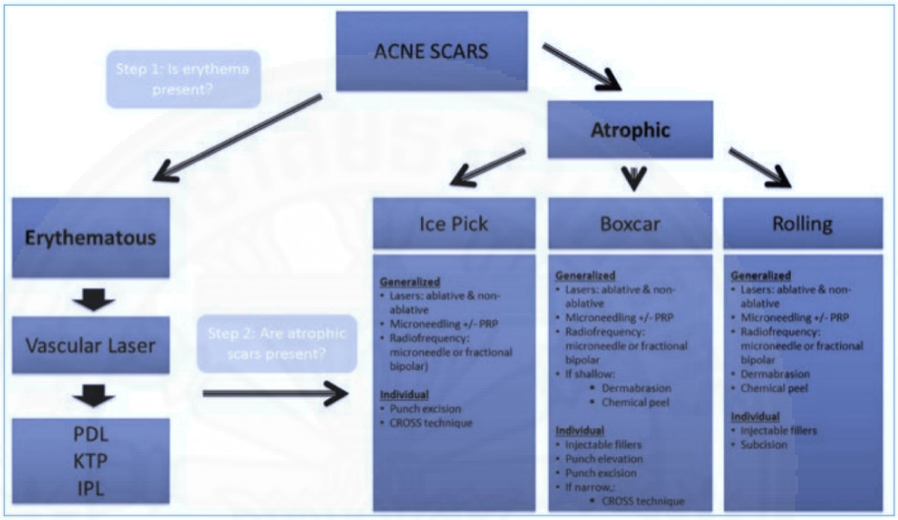


Figure 2.3: Acne scarring treatment algorithm

One of the most popular choice in treating generalized atrophic acne scar is the ablative laser resurfacing because of its ability in treating all type of the scars comparing to other treatment that may lack efficacy in some type of the scar. As shown in Figure 4, each type of scars response to each treatment differently, fractional photothermolysis is the only treatment that is effective in all types of the scar while other treatment modality can provide less effect or no effect at all in one type of the scar. Tissue augmenting agents and subcision can both provide the effective treatment in all types of the scars but in the lesser effect comparing to the fractional photothermolysis and also more suitable in patient with individual scars (6,10). (Table 2.1)

Table 2.1 : Effectiveness of treatments for acne scarring

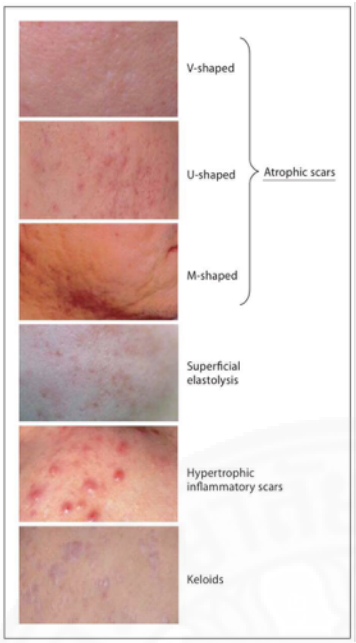
TABLE 2. Procedures to select by lesion type of scars			
TREATMENT	ICE PICK SCARS	ROLLING SCARS	BOXCAR SCARS
Chemical peels TCA CROSS technique	 ++ ++	 – –	 ++ ++
Dermabrasion/microdermabrasion	+	–	+
Laser Ablative and nonablative laser Fractional photothermolysis	 – ++	 ++ ++	 ++ ++
Punch techniques Punch excision Punch elevation Punch replacement grafting	 ++ – ++	 – – –	 + ++ –
Tissue augmenting agents	+	++	+
Needling	–	++	++
Subcision	+	++	+
++ = Effective, + = less effective, – = not effective			

2.1.4 Evaluation

To assess the severity of acne scar and its impact on patient's quality of life, there must be a grading scale that is accepted among the clinician that can be use to evaluate before, during and after the treatment. Grading scale that is commonly use in evaluating the severity of acne scars are such as ECCA grading scale and Goodman and Baron qualitative and quantitative global scarring grading system (9,11,12). For the grading that is use to assess the impact on patent's quality of life are such as patient satisfaction index, SCARS patient-reported outcome tool and facial acne scar quality of life (FASQoL) questionnaire (13).

2.1.4.1 ECCA grading scale

ECCA grading scale (echelle d'évaluation Clinique des cicatrices d'acné) is a tool designed to help dermatologist to use in their everyday practice for clinical evaluating of the efficacy of the treatments on acne scar. This grading composed of 6 items corresponding to 6 types of acne scars with each kind of scar is associated with a quantitative score ranging from 0 to 4 and a weighting factor ranging from 15 to 50 (9). (Figure 2.4)



Description	Weighting factor (a)	Semi-quantitative score (b)	Grading (a × b)
V-shaped atrophic scars, diameter of less than 2 mm, and punctiform	15	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
U-shaped atrophic scars, diameter of 2–4 mm, with sheer edges	20	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
M-shaped atrophic scars, diameter of more than 4 mm, superficial and with irregular surface	25	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
Superficial elastolysis	30	0 = absent 1 = mild 2 = moderate 3 = intense	/_____/
Subgrading 1			/_____/
Hypertrophic inflammatory scars, scars of less than 2 years of age	40	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
Keloid scars, hypertrophic scars, of more than 2 years of age	50	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
Subgrading 2			/_____/
Global score (subgradings 1 + 2)			/_____/

Figure 2.4: ECCA grading scale

2.1.4.2 Goodman and Baron quantitative scarring grading system

Goodman and Baron quatitative scarring grading system classified type of scar into 4 types, mild atrophic scars, moderate atrophic scars, severe arophic scars and hypertrophic/keloidal postacne scars. Those of atrophic scars will score according to the number of the lesion the patient has. Mild atrophic scars scores less heavily that moderately atrophic scars and less again than severe atrophic scars. On the other hand, hypertrophic/keloidal post acne scar will score according to the area of the skin involvement (12). (Table 2.2)

Table 2.2: Goodman and Baron Quantitative Scarring Grading System

Grade or Type	Number of lesions 1 (1-10)	Number of lesions 2 (11-20)	Number of lesions 3 (>20)
Milder scarring (1 point each)	1 point	2 points	3 point
Macular erythematous pigmented			
Mildly atrophic dish-like			
Moderate scarring (2 points each)	2 points	4 points	6 points
Moderately atrophic, dish like			
Punched out with shallow bases small cars (<5 mm)			
Shallow but broad atrophic areas			
Severe scarring (3 points each)	3 points	6 points	9 points
Punched out with deep but normal bases, small scars (<5 mm)			
Punched out with deep but abnormal bases, small scars (<5 mm)			
Linear or troughed dermal scarring			
Deep, broad atrophic areas			
Hyperplastic	2 points	4 points	6 points
Papular scars	Area<5 mm	Area 5-20 mm2	Area>20 cm2
Keloidal/Hypertrophic scars	6 points	12 points	18 points

Table 2: (b) Assessment of improvement using Goodman and Baron's quantitative acne scar grading system

Grades	Improvement status
0-5	Minimal reduction in GSGS scores
5-10	Moderate reduction in GSGS scores
10-15	Good reduction in GSGS scores
>15	Very good reduction in GSGS scores

2.1.4.3 Goodman and Baron qualitative scarring grading system

Goodman and Baron qualitative scarring grading system concentrates more on the scar morphologies and disease severity by dividing acne scars into four grade, grade 1, grade2, grade 3 and grade 4 ranging from the least severe to the most severe respectively. If the lesion on patient's face can be sub-classified into two or more grades then the patient will be described according to the highest grade, omitting the milder disease (11). (Table 2.3)

Table 2.3: Goodman and Baron Qualitative Scarring Grading System

TABLE 1. Grades and Examples of Postacne Scarring

Grade	*Level of disease*	*Characteristics*	*Examples of scars*
1	Macular disease	Erythematous, hyper- or hypopigmented flat marks visible to patient or observer irrespective of distance.	Erythematous, hyper- or hypopigmented flat marks
2	Mild disease	Mild atrophy or hypertrophy that may not be obvious at social distances of 50 cm or greater and may be covered adequately by makeup or the normal shadow of shaved beard hair in males or normal body hair if extrafacial.	Mild rolling, small soft papular
3	Moderate disease	Moderate atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in males or body hair if extrafacial, but is still able to be flattened by manual stretching of the skin.	More significant rolling, shallow "box car," mild to moderate hypertrophic or papular scars
4	Severe disease	Severe atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in males or body hair (if extrafacial) and is not able to be flattened by manual stretching of the skin.	Punched out atrophic (deep "box car"), "ice pick", bridges and tunnels, gross atrophy, dystrophic scars significant hypertrophy or keloid

2.1.4.4 SCARS patient reported outcome tool

SCARS patient reported outcome tool is a 5-questions instrument based on what patient look at the skin when they look at themselves in the mirror. This tool starts with the visual analog scales consist of 2 questions for the patient to rate their active acne lesions and atrophic scar lesions this way patient are reminded to separate the lesion between active acne and the atrophic scars. Then, patient rated what they think about atrophic scars based on a 5 point rating scales questionnaire (13). (Table 2.4)

Table 2.4: SCARS patient reported outcome tool

Assessment of Acne Scar Appearance and Severity: Part 1

Please remove all make-up and facial jewelry/piercings, and tie back any loose hair covering your face before answering this questionnaire.

Please begin by answering the questions below. These questions will help you understand the difference between active acne and atrophic acne scars.

a) Active acne includes zits, breakouts, pimples, whiteheads, and blackheads.

When looking at your face in the mirror right now, do you see **zits, breakouts, pimples, whiteheads, or blackheads**?

Yes / No (please circle one)

If yes, please **rate the severity** of the **zits, breakouts, pimples, whiteheads, or blackheads** on your face by putting a vertical line in the place that best describes your acne.

The following question asks you about atrophic acne scars only. Please do not consider active acne (zits, breakouts, pimples, whiteheads, or blackheads), scabs, or flat red or dark marks.

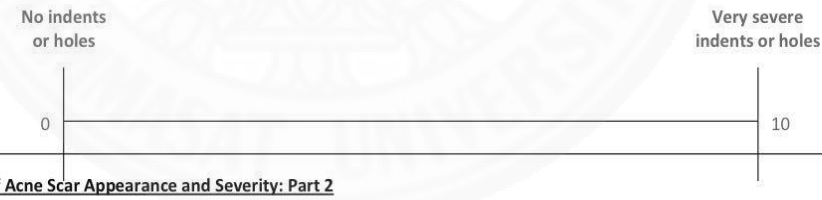
b) Atrophic acne scars are indents or holes in the skin from previous active acne (not from injury, scratching or picking).

When looking at your face in the mirror right now, do you see **indents or holes** in the skin from previous active acne?

Yes / No (please circle one)

If you answered "No" for this question, you have completed this questionnaire. Thank you for your time.

If yes, please **rate the severity** of the **indents or holes** on your face by putting a vertical line in the place that best describes your scars.



Assessment of Acne Scar Appearance and Severity: Part 2

The following questions ask you about atrophic acne scars only. Remember, atrophic acne scars are indents or holes in the skin from previous active acne. Please do not consider active acne (zits, breakouts, pimples, whiteheads, or blackheads), scabs, or flat red or dark marks.

Please read each question, then look in the mirror and think about the indents or holes on your whole face, and then answer the question. Please mark an "X" in the box (☒) that best describes how the indents or holes on your face look RIGHT NOW.

There are no right or wrong answers to these questions. If you want to change your answer, please cross out your original answer and mark an "X" in the correct box.

Please choose only one response.

1. **When looking at your face in the mirror right now, how much of your face looks covered by indents or holes?**

Almost none of my face	A little of my face	Some of my face	A lot of my face	Almost all of my face
☐	☐	☐	☐	☐

2. When looking at your face in the mirror right now, how small or large do the individual indents or holes look?

Very small	Small	Moderate	Large	Very large
☐	☐	☐	☐	☐

3. When looking at your face in the mirror right now, how many indents or holes do you see?

Very few indents or holes	A few indents or holes	Some indents or holes	Quite a lot of indents or holes	Many indents or holes
☐	☐	☐	☐	☐

4. When looking at your face in the mirror right now, how deep do the individual indents or holes look?

Not at all deep	A little deep	Moderately deep	Very deep	Extremely deep
☐	☐	☐	☐	☐

5. When looking at your face in the mirror right now, how visible are the indents or holes to you?

Not at all visible	A little visible	Moderately visible	Very visible	Extremely visible
☐	☐	☐	☐	☐

2.1.4.5 Facial acne scar quality of life (FASQoL)

Facial acne scar quality of life is a 10-questions instrument assessing the impact of scars on emotion, social functioning and work/school with a recall period of the past 7 days based on patient's perspective. This tool is also a 5 points rating scales questionnaire. It has been suggest as a sensitive tool to evaluate the acne scars treatment benefits (13). (Table 2.5)

Table 2.5: Facial acne scar quality of life (FASQoL)

Acne Scar Quality of Life Questionnaire

The following questions ask you about how the atrophic acne scars on your face impact your daily life. Atrophic acne scars are indents or holes in the skin from previous active acne (not from injury, scratching or picking).

When answering these questions, please do not consider any of the following:

- **Zits, breakouts, pimples, whiteheads, or blackheads on the face;**
- **Acne scabs (dry, rough protective crusts that form over zits or pimples before indents or holes develop); or**
- **Flat red or dark marks.**

Please mark an "X" in the box (☒) that best describes the impact that the indents or holes on your face had on you over the PAST 7 DAYS.

There are no right or wrong answers to these questions. If you want to change your answer, please cross out your original answer and mark an "X" in the correct box. Please choose only one response.

1. **Over the past 7 days, have you felt self-conscious when you were with people because of the indents or holes on your face?**

Not at all self-conscious	A little self-conscious	Somewhat self-conscious	Very self-conscious	Extremely self-conscious
☐	☐	☐	☐	☐

2. **Over the past 7 days, have you felt less attractive because of the indents or holes on your face?**

I did not feel less attractive	A little less attractive	Somewhat less attractive	Much less attractive	Extremely less attractive
☐	☐	☐	☐	☐

3. **Over the past 7 days, have you felt annoyed because of the indents or holes on your face?**

Not at all annoyed	A little annoyed	Somewhat annoyed	Very annoyed	Extremely annoyed
☐	☐	☐	☐	☐

4. **Over the past 7 days, have you felt worried that the indents or holes on your face won't go away?**

Not at all worried	A little worried	Somewhat worried	Very worried	Extremely worried
☐	☐	☐	☐	☐

5. **Over the past 7 days, have you felt sad because of the indents or holes on your face?**

Not at all sad	A little sad	Somewhat sad	Very sad	Extremely sad
☐	☐	☐	☐	☐

6. **Over the past 7 days, have you felt upset by negative comments from others because of the indents or holes on your face?**

Not at all upset	A little upset	Somewhat upset	Very upset	Extremely upset
☐	☐	☐	☐	☐

Table 2.5: Facial acne scar quality of life (FASQoL) (Continued)

7. Over the past 7 days, have the indents or holes on your face made you avoid going out with friends or family?

Not at all	A little	Somewhat	Very much	Extremely
☐	☐	☐	☐	☐

8. Over the past 7 days, have you felt bothered by having to hide the indents or holes on your face?

Not at all bothered	A little bothered	Somewhat bothered	Very bothered	Extremely bothered
☐	☐	☐	☐	☐

9. Over the past 7 days, have the indents or holes on your face affected your relationships with others (for example, friends, family, romantic relationships/partner)?

Not at all	A little	Somewhat	Very	Extremely
☐	☐	☐	☐	☐

10. Over the past 7 days, have the indents or holes on your face affected your participation at work or school?

Not at all	A little	Somewhat	Very	Extremely
☐	☐	☐	☐	☐

2.2 Astaxanthin

Astaxanthin, a xanthophyll carotenoids, is an active antioxidant enzyme that can be found in living cells of *Haematococcus pluvialis*, *Chlorella zofingiensis*, *Chlorococcum*, and *Phaffia rhodozyma.* This natural source of antioxidant has various of biological activities such as antioxidant effects, anti-lipid peroxidation activity, anti-inflammation, anti-diabetic activity, anticancer activity and even act as an immunomodulatory. Astaxanthin is also well-known for its cosmetic benefits on the skin such as reduces crow's feet wrinkle, increase skin elasticity and moisture content, inhibition of pigmentation, inhibition of melanin-generation and of light-induced aging and accerelate wound healing have been reported (19,32). (Figure 2.5)

Figure 2.5: Astaxanthin

2.2.1 Antioxidant effects

ROS are involved in all phases of wound healing, including inflammation phase, proliferation phase and remodeling phase. This antioxidant enzyme scavenges the free oxygen radicles and converted them into hydrogen peroxide which then again converted into water, resulted in a breakdown of the reactive oxygen species chain reaction. By terminating the chain reaction will lower the concentrations of ROS which then initiate signaling cascade during the proliferative phase, including homeostasis, formation of granulation tissue, re-epithelialization, and angiogenesis. On the contrary, prolonged generation or large amount of ROS will activate NF-kB/Rel pathway instead which then lead to chronic inflammation and delayed wound healing causing scars.

Astaxanthin has unique molecular structure which gives a powerful antioxidant effects to scavenge the ROS, decrease the oxidative damage caused by the free radicals that may react with proteins, lipid and DNA inside and outside of the cell. In one study, the biological markers of oxidative stress marker, iNos, showed a significantly lowered expression in mice skin that were injured and then recieve topical astaxanthin gel in the real time PCR analysis. The reduction of iNos marker represent the reduction in the ROS which is involved in every step of wound healing (19,24,32).

2.2.2 Anti-lipid peroxidation activity

Due to its unique molecular structure of having one end of hydroxyl and another end of keto group enabling them to have both lipophilic and hydrophilic properties. Astaxanthin are able to stay within the cell and outside of the cell by fitting with the lipophilic-hydrophilic-lipophilic property of the cell membrane, while other

antioxidant agent such as vitamin C, and beta carotene can only be positioned within the lipid bilayer. This characteristic makes astaxanthin 500 times more powerful than vitamin E, 800 times more powerful than coenzyme Q10 and up to 6,000 times more powerful than vitamin C to protect the cell membrane structure by inhibiting lipid peroxidation from both inside and outside of the cell (19). (Figure 2.6)

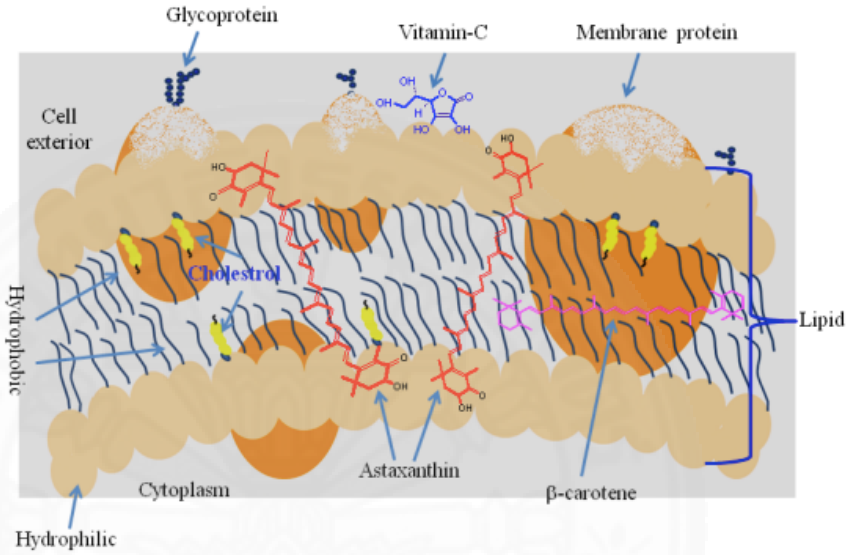


Figure 2.6: Superior position of astaxanthin in the cell membrane

2.2.3 Anti-inflammation

Astaxanthin has shown to reduced inflammatory cells in the guinea pig that were induced asthma with aerosol ovalbumin. Astaxanthin potentially reduce inflammatory cells such as eosinophil, neutrophil and macrophage by roughly 1.5 folds, moreover, it enhances the activity of cAMP and cGMP levels by 1.4 and 2.04 fold respectively in the lung tissues of guinea pig after feeding with astaxanthin. Another study shows astaxanthin efficacy in reducing bacterial load and gastric inflammation in Helicobacter pylori-infected mice. A study in human given oral dose of astaxanthin also shows the same result, by the lower level of C- reactive protein in the blood level which is well-established marker of inflammatory status (19,24,32).

2.2.4 Effects on cutaneous wound healing

One of the study on cutaneous wound healing in mouse shown that, topical astaxanthin has the potential effects in accelerate the cutaneous wound healing via upregulation of basic fibroblast growth factor (bFGF), collagen 1A1 and

pro-collagen which are wound healing biological markers (22,24).

Basic fibroblast growth factor itself has effect in granulation tissue formation, re-epithelialization, matrix formation and remodeling. It regulates the synthesis and deposition of various extracellular matrix and also increase keratinocyte motility during re-epithelialization thus promote and stimulate the migration of fibroblast. A study shows an upregulation in bFGF can be seen since day 1 after topically applied astaxanthin in the injured skin.

Collagen 1A1 has an important role in cutaneous wound healing especially during the proliferation phase and remodeling phase. Col1A1 turns to be collagen type 3 by the help of fibroblast. A study in mice has shown that there is upregulation of col1A1 in the real time PCR at day 6 after applied topical astaxanthin on the injured skin (17).

Pro-collagen, like collagen1A1, has seen upregulated in the real time PCR in vocal fold of mice that were injured and then dosed with astaxanthin. Pro-collagen is believed to have a role in wound healing as it can turn to collagen by the help of procollagen N proteinase and procollagen C proteinase (22,24).

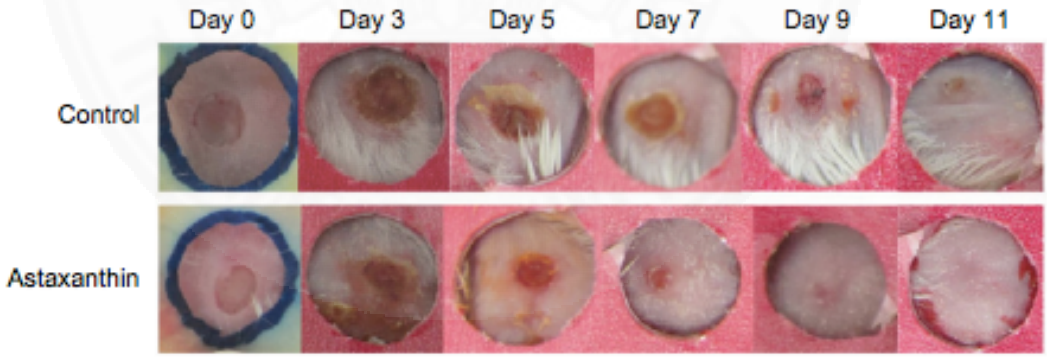


Figure 2.7: Images of wound contraction in astaxanthin and control groups from day 0 and day 11.

In histopathology of the wound, a cross section shows almost complete re-epithelialization in topical astaxanthin group by day 3. By day 7, it shows a complete re-epithelialization, mild degree of edema, well arranged blood vessels, collagen bundle, few mononuclears and a well elongated epidermis with keratinization is observed in mice treated with astaxanthin. On the contrary, the control group shows poor re-

epithelialization, highly edema, few capillaries and scattered fibroblasts and granulation tissues (24). (Figure 2.7 and 2.8)

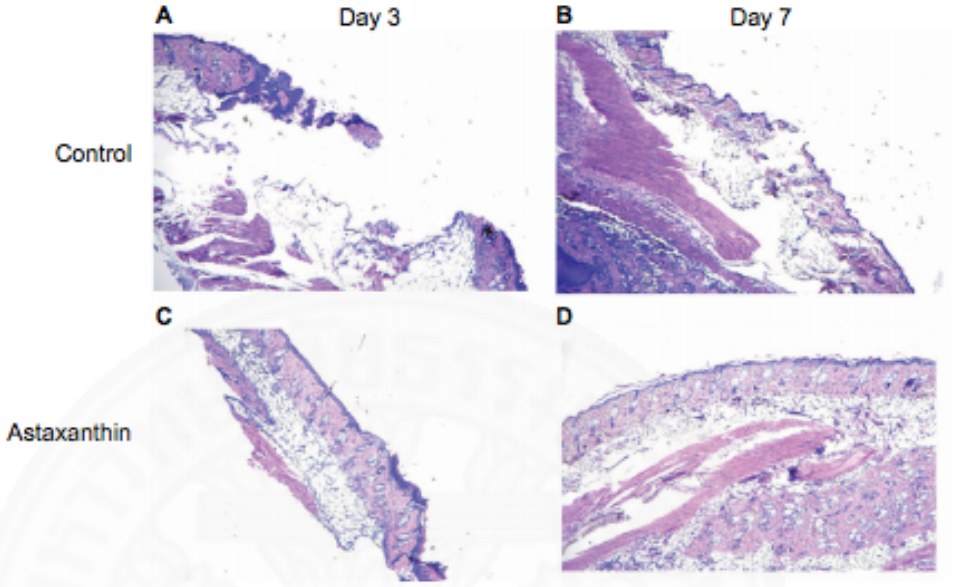


Figure 2.8: Skin wound sections from the astaxanthin-treated group and the control group at day 3 and 7 stained with hematoxylin and eosin. Notes: (A,B) Control; (C,D) astaxanthin-treated group; (A,C) and (B,D) represent day 3 and day 7 respectively.

2.2.5 Safety of astaxanthin

No report on any side effect from topical astaxanthin in wounded mice or topical form of astaxanthin in human subjects (21,22,24). Astaxanthin is reported safe with no serious side effects in the research so far (19). Excessive consumption of oral astaxanthin may lead to pigmentation of the skin in yellow to red coloration (19).

2.3 Fractional carbondioxide laser

2.3.1 Fractional CO2 laser and its application

Laser resurfacing remains the most effective treatment option for acne scar. It can be classified into two major categories, the ablative laser and the non-ablative laser. While the ablative laser has more efficacy in treating the atrophic acne scars, it's disadvantages such as peri-procedure discomfort and longer recovery time has become their biggest limitation. Non-ablative laser, on the contrary, are more tolerable

with shorter recovery time but multiple sessions of treatment are required and provide less impressive result than the traditional ablative lasers (6).

Each ablative and non-ablative laser can be sub-catergorized into traditional laser which has higher incidence of post-treatment adverse effects such as burning, erythema and edema while fractionated laser which seem to have the potential to overcome these adverse event. (Table 2.6)

Table 2.6: Laser for acne scarring

TABLE 1. Lasers for acne scarring

LASER CATEGORY	TRADITIONAL	FRACTIONATED
Ablative	10,6000nm CO_2 2,940nm ER: YAG	Fractional 10,6000nm CO_2 Fractional 2940nm ER: YAG Fractional 2,790nm YSGG
Non-ablative	1,064nm Nd:YAG 1,320nm Nd:YAG 1,450nm diode 755nm picosecond pulse duration laser 585nm PDL 595nm PDL 532nm KTP	Fractional 1,550nm Er-doped Fractional 1,540nm Er:glass

PDL: pulsed dye laser; KTP: potassium titanyl phosphate

By the word “fraction” means that the laser creates microscopic zones of injury to the dermis with skip areas in between. Only some part of the skin will be affected leaving the unaffected part to help aid in re-epithelialization after the treatment. Therefore, the fractional laser decreases the downtime of the post-laser treatment, as the skin has the ability to heal faster comparing to the traditional ablative lasers (35). (Figure 2.9)

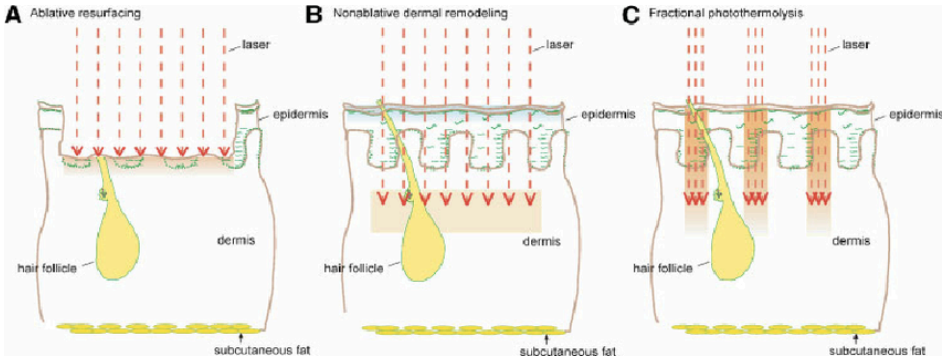


Figure 2.9: Comparison of ablative skin resurfacing, non-ablative.

Fractional carbon dioxide laser has combined the traditional carbon dioxide laser, which is 10,600 nm, with the technology of fractional photo-thermolysis (FP) intervention. This way, patient will experience lesser down time such as transient erythema, crusting and post-inflammatory pigmentation after each laser session, while still gaining the high efficacy of the traditional CO2 laser. But nevertheless, some patient will still face the inflammation associated laser treatment but in the lesser portion (35).

2.3.2 Safety of fractional CO2 laser

The safety and adverse event of fractional CO2 laser has been reported in many study despite its high safety profile comparing to the traditional ablative laser. Some adverse event may be unavoidable, patient will still experinced erythema, edema and PIH but can be resolved within one month after treatment. One report of infection but it is due to the use of high setting over the area of the neck which is thinner (6,25,27).

CHAPTER 3
OBJECTIVES

3.1 Primary objectives:

To evaluate the effect of topical astaxanthin in combined with fractional carbondioxide laser versus fractional carbondioxide laser alone in treating atrophic acne scars

3.2 Secondary objectives:

To evaluate the downtime and inflammation associated with laser treatment in the topical astaxanthin combined with fractional carbondioxide laser group.

To evaluate the safety and efficacy of topical astaxanthin in cutaneous healing.

3.3 Hypothesis

Fractional CO2 laser in combined with topical astaxanthin will improve the acne scar quality and decreases in downtime and inflammation associated with laser treatment.

CHAPTER 4
SIGNIFICANCE OF THE RESEARCH

Acne scars is considered as one of the disease that has a high prevalence among the adolescents which is up to 80%. It can involve in all body areas where there are high concentration of pilosebaceous glands but the most common affected areas are face, neck and chest which are the area that is easy to see. As these lesion can result in permanent scarring on the exposed area, it has a major impact in quality of life as it can cause cosmetic discomfort, low self-esteem and depression and may persist through out their life.

Treating acne scars does not only treat the physical problems but also treat the social, psychological and emotional problems. The physical problems, we focus on the outer appearances of the patient which is the skin, the improvement in the acne scar which need multimodal approach of treatment to get the maximized result. As we treated the long standing disease, we also treat the patient's social, psychological and emotional problems by helping them feel better about themselves. The skin is considered the first thing people see as the outer appearances therefore it has a big impact in living a life.

Better skin will make patient feel beautiful, more confident about themselves. On the opposite, if left untreated it can cause a huge impact in the quality of life, depression, impaired social functioning and even suicidal ideation.

Development of this research will give both patient and clinician another additional option in treating atrophic acne scar. Also to ensure that the patient will get a significant improvement in the quality of atrophic acne scar and at the same time using a shorter recovery time in the after laser treatment.

CHAPTER 5
RESEARCH METHODOLOGY

5.1 Study Sample

5.1.1 Target population

Patients presenting to Out Patients Department (OPD) of Dermatology, Thammasat University Hospital who had been diagnosed with atrophic acne scars fulfilling the inclusion criteria were included in the study.

5.1.2 Sample size

A total of 34 patients are recruited for this study.

$$n = \frac{(z_{1-\alpha} + z_{1-\beta})^2 \sigma^2}{(\epsilon - \delta)^2}$$

$$\epsilon = \mu - \mu_0$$

Figure 5.1: Sample size calculation

Reference value (μ0) = 45.84

Mean (π) = 62.2

Standard deviation (σ) = 30

Margin (ծ) = 0

α = 0.01-0.05

β = 0.1-0.2

Sample size (n) = 27 subjects

Drop out rate 20% = 34 subjects

5.1.3 Inclusion Criteria

1.3.1 Patient age between 20 to 40 years old.

1.3.2 Moderate to severe acne scar according to Goodman and Baron's acne scar grading system.

1.3.3 Patient with atrophic scars.

5.1.4 Exclusion Criteria

1.4.1 Patients' refusal to participate the study

1.4.2 Patient with atrophic scars with active acne lesion

1.4.3 Patient with active inflammation, infection or ulcer

1.4.4 Patient with oral isotretinoin used in 6 months prior

1.4.5 Patient with history of ablative laser resurfacing treatment such as FrCO2, FrErYag 4 months prior

1.4.6 Patient with history of predisposition to keloids/hypertrophic scars

1.4.7 Patient with underlying disease of collagen vascular disease, bleeding diathesis or diabetes mellitus

1.4.8 Pregnant or planning pregnancy woman

1.4.9 Lactating women

1.4.10 Patient on immunosuppressive drug, chemotherapy or radiation

1.4.11 Patient suspected allergy to astaxanthin

1.4.12 Unreliable and poor compliance patient

5.1.5 Discontinuation Criteria

1.5.1 Patients' refusal to participate the study

1.5.2 Patient suffering serious adverse effect of fractional CO2 laser

1.5.3 Patient suffering serious adverse effect of astaxanthin

1.5.4 Unreliable and poor compliance patients

5.2 Research Design

Interventional: Therapeutic, prospective, evaluator blind, split-face control trial will be conduct in December 2018 until March 2019.

A total of 3 sessions of treatment will be given to each patient with a 4 weeks

± 1 weeks interval for each treatment. The last follow up session for evaluation will be at 4 ± 1 weeks after the last treatment session given to the patient, making a total course duration approximately of 12 ± 1weeks in total. For the first session, patient will be followed up at day 3 after treatment for evaluation and treatment for any adverse event after the therapy. Making a total visit of 5 times.

5.3 Materials and Methods

5.3.1 Laser

5.3.1.1 FrCo2 laser

- Eco2 (High speed fractional CO2), Lutronic®, USA.
- Parameter:
- Beam size 120 μm, static mode.
- Peak power 30 W
- Pulse energy 50 mJ
- Density 50 spots/cm2 (3.2% Coverage)
- 1 passes, 10% overlapping

Figure5.2: Eco2 (High speed fractional CO2), Lutronic®, USA.

5.3.2 Drugs

Astaxanthin composed of 78.9 μM of astaxanthin extracted from *H. pluvialis* supplied by Cosmaprof company.

Placebo composed of cream base colored as same as astaxanthin supplied by Cosmaprof company.

5.3.3 Study place

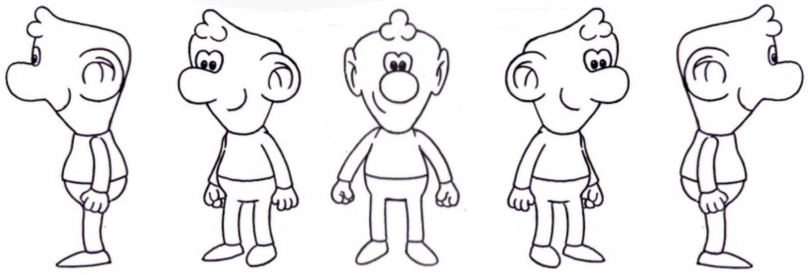
The study will be conducted at Thammasat University hospital.

5.3.4 Preparation of research subjects

Subjects will be selected to enroll according to the inclusion and exclusion criteria. The inform consent and nature of the study will be given out to the subject. The subjects will sign an inform consent form to participate in the study. Data include patients' personal history (age, gender, body mass index, skin type, smoking habits, drinking habits, underlying disease, family history, age of scar onset, duration of scar, history of prior treatment and family history will be collected at the first day of the treatment session. Physical examination will be done by a single clinician evaluating type of acne scar each subject has according to the Goodman and Baron acne scar grading system at the baseline.

5.3.5 Treatments

Prior each treatment session, patients were asked to remove all the make-up and cream using Cetaphil Gentle Skin Cleanser (from Galderma, Switzerland). Digital photograph will be taken directly at the distance of 50 cm. at both side of the faces which will be captured at 5 angles which are full frontal (0°), profile from the left (45° and 90°) and profile from the right (-45° and -90°). Lastly, the Antera®3D will be used to measure skin texture and scar volume on both cheeks before each treatment session. (Figure 5.3)



90° -45° 0° 45° 90°

Figure5.3: Digital photograph taken at 50 cm.

After the photo session, patient were then be applied with 10.56% lidocaine topical numbing cream from Koru pharma®, Korea, under occlusion for 45 minutes on the treatment area to achieve a satisfactory anesthetic effect. After 45 minutes of anesthetic cream, patient will then cleaned with normal saline solution painted on the treatment area and wait for the skin to dry out about 15 minutes. Protective eyewear will be given to both the patient and physician.

One row of FrCO2 laser will be applied on each cheek of the subjects before beginning the full treatment. Then, FrCO2 will be applied to whole face using Eco2 (High speed fractional CO2), Lutronic®, USA. with a setting of 120 μm Beam size, static mode, peak power of 30 W, pulse energy of 50 and density of 50 spots/cm2, 1 pass with 10% overlapping. The endpoint is mild erythema.

Subject will be asked to determine their level of pain ranging from a scale of 0 to 10 which is no pain to most painful, respectively. Moreover the subjects will be asked to tick the box according to the symptoms the subjects feel immediately after the laser such as sting, erythema, edema, burn, post-inflammatory hypo-hyperpigmentation and desquamation to evaluate the safety of the laser treatment. Noted that every single session will be the same physician who's doing the FrCO2.

For patient who experienced PIH less than 80% of the face surface area from previous treatment, the next laser session will not be postponed but in the next laser visit, physician will adjust the parameter by decreasing the setting by 50% of the previous setting. On the contrary, for patient who experienced PIH more than 80% of the face surface area, patients will be withdraw from the study.

After laser resurfacing, thirty four patient will be divided into 2 groups. Clinician's assistant will give out cream A to be applied on the right side of the face while on the contralateral side of the face will be receiving cream B. First group of seventeen patients will receive cream A which is astaxanthin to apply on the right side of the face while receiving cream B which is cream base to apply on the left side of the face. On the contrary, the second group will received cream base as cream A to apply on the right side of the face and receive astaxanthin as cream B to apply on the left side of the face. This way, only clinician's assistant knows whether cream A or cream B is the actual astaxanthin cream while the other is the placebo for each patient.

The patient's face will be equally divided in half, by an imaginary line starting from middle of the hairline of the forehead, passing mid glabella, mid tip of the nose, middle of philtrum, upper lip, lower lip and stops at the middle of the chin. Patient will be advised to continue the cream in the same order as given topically twice daily which is in the morning and before bed during the study. (Figure 5.4)

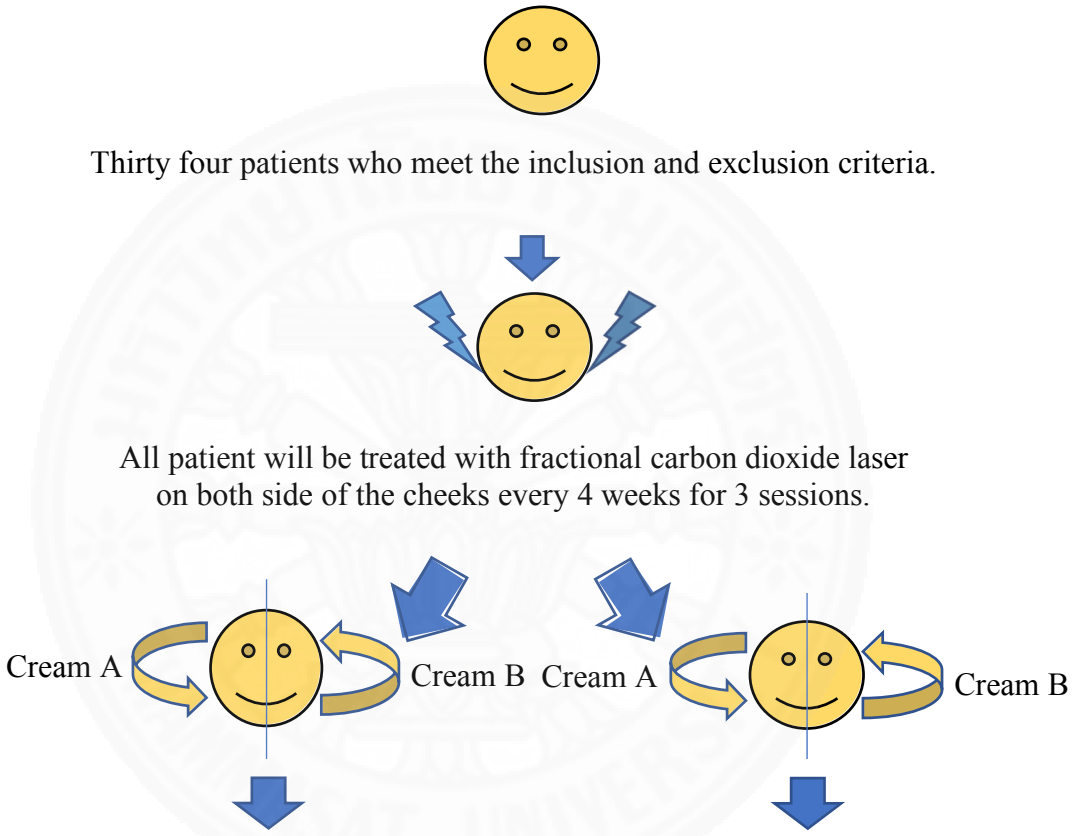
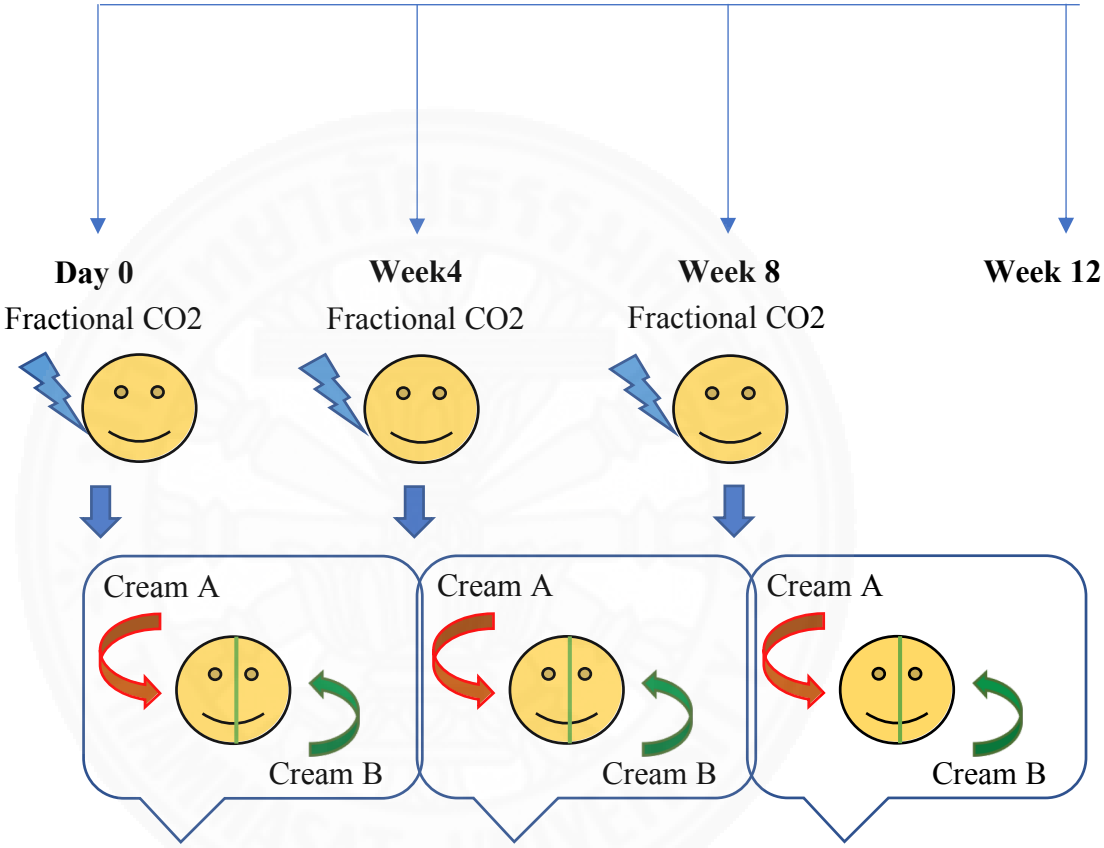


Figure 5.4: Treatment intervention

During the first day after the FrCo2 treatment, patient will be asked to avoid contact with water. Patient will be asked to use sunscreen of at least SPF30+ and PA+++ every day during the study but avoiding during the first day after the laser treatment. Make-up were asked to avoid during the first 3 days after laser resurfacing. (Figure 5.5)



Cream A and cream B apply topically on the right and left side respectively.

Figure 5.5: Summary of intervention

5.3.6 Postoperative care

After treatment, patient may experience burning sensation for 1-2 hours, ice pack with sterile dry gauze will be provided to relief the symptom. Patient will be ask to continued cream A and cream B in the same order topically in the morning and night time during the study. After laser treatment, patient will be ask to avoid contact with water for 24 hours. Other treatment and topical product, friction and heat will be asked to avoid during the study time. Patient will be ask to use mild facial cleanser during the study. Broad spectrum sunscreen with at least SPF30+ and PA+++ will be allow to

use a day after the laser treatment and will be asked to continue using during the study period.

5.3.7 Outcome measurement

Each patients will have 3 sessions of FrCo2 laser starting at day 0, 4 weeks and 8 weeks after the first session. Additional follow up sessions other than FrCo2 session will be at day3 and 12 weeks after the first session, making a total of 5 visits for each patient according to the summary table 7 shown below. Noted that the treatment session and follow-up session are the same day for day 0 , 4 weeks and 8 weeks after the first session. For day 3 and 12 weeks after the baseline, patient will have only photograph, 3D photography taken and other evaluation assessment done but no treatment session. (Table 5.1)

Table 5.1: Summary of outcome measurement

Time (After baseline)	Baseline (Before Tx)	Day 0 (After Tx)	Day 3	Week 4	Week 8	Week 12
Take photograph	☺		☺	☺	☺	☺
Antera® 3D photograph	☺		☺	☺	☺	☺
Goodman & Baron scar grading	☺			☺	☺	☺
Patient evaluation of improvement				☺	☺	☺
Patient universal pain assessment		☺	☺	☺	☺	☺
Adverse event assessment		☺	☺	☺	☺	☺

5.3.7.1 Evaluation of efficacy

(1) A. Digital Photograph:

Patient will be captured at a distance of 50 cm. on both side of the faces in every visit. Patient will be capture at five different angles which are full

frontal (0°), profile from the left (45° and 90°) and from the right side (-45° and -90°). The camera setting and lighting in the room will be

(2) B. Antera 3D Photography:

Antera®3D will be used to measure the skin texture and scar volume at the baseline, 4 weeks, 8 weeks and 12 weeks after the first treatment by the same physician. (Figure 5.6)

Figure 5.6: Antera® 3D camera for skin analysis

(3) C. Goodman and Baron's acne scar grading system:

Goodman and Barons's acne scar grading system is a global scar grading system that allows invertigators, educators and proceduralists to compare their cases more accurately and to have a more objective discussion of the efficacy of operative interventions or therapies. It consists of 2 parts which are qualitative part and quantitative part.

Goodman and Baron Quatitative Scarring Grading System classified type of scar into 4 types, mild atrophic scars, moderate atrophic scars, severe arophic scars and hypertrophic/keloidal postacne scars. Those of atrophic scars will score according to the number of the lesion the patient has. Mild atrophic scars scores less heavily that moderately atrophic scars and less again than severe atrophic scars. On the other hand, hypertrophic/keloidal post acne scar will score according to the area of the skin involvement. (Table 5.2)

Mild atrophic scar scores a maximum of 6 points. Three of theses points may be earned for over 20 lesions of either macular scars (hypopigmented, hyperpigmented or erythematous) or mildy atrophic scars. Number of the scars between 11-20 will score 2 points, likewise the number of scar less than or equal to 10 will score only 1 point.

Moderate scaring score more heavily with a maximum score of 18 points. Six of these points are able to be attained by over 20 scars of either moderate atrophic small scars, small punched out scars with shallow bases (shallow box scar), or broad areas of moderate atrophy. Number of the scars between 11-20 will score lesser with 4 points for each classification and the number of scar less than or equal to 10 will score only 2 point for each classification.

For the severe form of atrophic acne scar may score a maximum of 36 points. Nine of theses points are able to be attained by over 20 scars of 1.) small, deep punch-out scars of 5 mm or less (deep box scars), with normal base, 2.) small, deep punch-out scars of 5 mm or less with abnormal base, 3.) linear or troughed dermal scarring and 4.) deep broad atrophic scar. Number of the scars between 11-20 will score lesser with 6 points for each classification and the number of scar less than or equal to 10 will score only 3 point for each classification.

For hyperplastic scarring, subdevided into papular scars and keloidal scars. Papular scars will scored according to the atrophic scar, with number of lesions more than 20 scorring 6 points, 11- 20 lesions scorring 4 points and less than or equal to 10 lesions scorring 2 points. On the opposite, keloidal or hypertrophic scars will score base on the skin surface area involvement. For lesions with area more than 20 cm2 will score 18 points, lesion with area between 5-20 cm2 will score 12 points and lesion with area less than 5 cm2 will scor6 points. These are all summarizes according to the table 2 down below.

For the assessment of improvement using Goodman and Baron’s quantitative acne scar grading system, if the total score decrease less than 5 it is refer to a minimal redction, if the total score decrease between 5 to 10, it is considered moderate reduction, if the score decrease between 10 to 15, it is considered good reduction and if the total score

Table 5.2: Goodman and Baron Quantitative Scar Grading System

Grade or Type	Number of lesions 1 (1-10)	Number of lesions 2 (11-20)	Number of lesions 3 (>20)
Milder scarring (1 point each) Macular erythematous pigmented Mildly atrophic dish-like	1 point	2 points	3 point
Moderate scarring (2 points each) Moderately atrophic, dish like Punched out with shallow bases small cars (<5 mm) Shallow but broad atrophic areas	2 points	4 points	6 points
Severe scarring (3 points each) Punched out with deep but normal bases, small scars (<5 mm) Punched out with deep but abnormal bases, small scars (<5 mm) Linear or troughed dermal scarring Deep, broad atrophic areas	3 points	6 points	9 points
Hyperplastic Papular scars Keloidal/Hypertrophic scars	2 points Area<5 mm 6 points	4 points Area 5-20 mm2 12 points	6 points Area>20 cm2 18 points

Table 2: (b) Assessment of improvement using Goodman and Baron's quantitative acne scar grading system

Grades	Improvement status
0-5	Minimal reduction in GSGS scores
5-10	Moderate reduction in GSGS scores
10-15	Good reduction in GSGS scores
>15	Very good reduction in GSGS scores

Goodman and Baron Qualitative Scarring Grading System concentrates more on the scar morphologies and disease severity by dividing acne scars into four grade, grade 1, grade 2, grade 3 and grade 4 ranging from the least severe to the most severe respectively. If the lesion on patient's face can be sub-classified into two or more grades then the patient will be described according to the highest grade, omitting the milder disease. (Table 5.3)

For grade one disease, patient only have macular disease of erythematous, hypo- or hyperpigmented marks irrespective of the distance example of erythematous, hyper- or hypopigmented flat marks.

Grade two disease, mild disease, is defined by mild atrophy or hypertrophy that may not be obvious at social distances of 50 cm or greater and can be covered adequately with make up or normal shadow of shaved beard in male patient for examples such as mild rolling scars and small, soft papular scar.

For grade three disease, moderate disease, patient has moderate atrophic or hypertrophic scarring that is obvious at social distance of 50 cm. or greater and cannot be easily cover up with make up or the normal shadow of shaved beard hair in males. But the scars are still able to be flattened by manual stretching of the skin for example, more significant rolling shallow box car scar, mild to moderate hypertrophic or popular scars.

For the most severe disease, the fourth grade, is defined by severe atrophic or hypertrophic scarring that is can be seen at distance of 50 cm. or greater. The scars cannot be easily covered with make up or normal shadow of shaved beard in males and scar cannot be flattened by manual stretching of the skin. Examples of the lesion for this categories are punched out atrophic or deep box car, ice pick scar, bridges and tunnels, gross atrophy, dystrophic scars and significant hypertrophy or keloid scar.

Table 5.3: Goodman and Baron Qualitative Scar Grading System

TABLE 1. Grades and Examples of Postacne Scarring

Grade	*Level of disease*	*Characteristics*	*Examples of scars*
1	Macular disease	Erythematous, hyper- or hypopigmented flat marks visible to patient or observer irrespective of distance.	Erythematous, hyper- or hypopigmented flat marks
2	Mild disease	Mild atrophy or hypertrophy that may not be obvious at social distances of 50 cm or greater and may be covered adequately by makeup or the normal shadow of shaved beard hair in males or normal body hair if extrafacial.	Mild rolling, small soft papular
3	Moderate disease	Moderate atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in males or body hair if extrafacial, but is still able to be flattened by manual stretching of the skin.	More significant rolling, shallow ''box car,'' mild to moderate hypertrophic or papular scars
4	Severe disease	Severe atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in males or body hair (if extrafacial) and is not able to be flattened by manual stretching of the skin.	Punched out atrophic (deep ''box car''), ''ice pick'', bridges and tunnels, gross atrophy, dystrophic scars significant hypertrophy or keloid

5.3.7.2 Evaluation of satisfaction

Patient will be asked to fill out the satisfaction evaluation form after each treatment session using SCARS patient reported outcome tool and Facial acne scar quality of life (FASQoL).

(1) A. SCARS patient reported outcome tool:

SCARS patient reported outcome tool is a 5-questions instrument based on what patient look at the skin when they look at themselves in the mirror. This tool starts with the visual analog scales consist of 2 questions for the patient to rate their active acne lesions and atrophic scar lesions this way patient are reminded to separate the lesion between active acne and the atrophic scars. Then, patient rated what

they think about atrophic scars based on a 5 point rating scales questionnaire. Noted that the questionnaire will be translated into a simple Thai language in order for a better standing for the patient. (Table 5.4)

Table 5.4: SCARS patient reported outcome tool

Assessment of Acne Scar Appearance and Severity: Part 1

Please remove all make-up and facial jewelry/piercings, and tie back any loose hair covering your face before answering this questionnaire.

Please begin by answering the questions below. These questions will help you understand the difference between active acne and atrophic acne scars.

a) Active acne includes zits, breakouts, pimples, whiteheads, and blackheads.

When looking at your face in the mirror right now, do you see **zits, breakouts, pimples, whiteheads, or blackheads**?

Yes / No (please circle one)

If yes, please **rate the severity** of the **zits, breakouts, pimples, whiteheads, or blackheads** on your face by putting a vertical line in the place that best describes your acne.

The following question asks you about atrophic acne scars only. Please do not consider active acne (zits, breakouts, pimples, whiteheads, or blackheads), scabs, or flat red or dark marks.

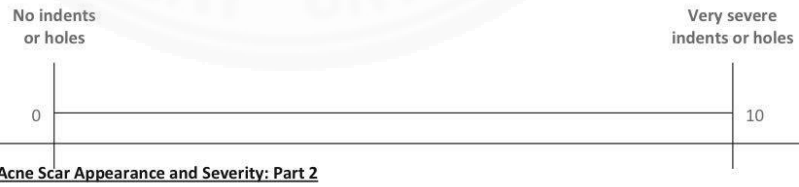
b) Atrophic acne scars are indents or holes in the skin from previous active acne (not from injury, scratching or picking).

When looking at your face in the mirror right now, do you see **indents or holes** in the skin from previous active acne?

Yes / No (please circle one)

If you answered "No" for this question, you have completed this questionnaire. Thank you for your time.

If yes, please **rate the severity** of the **indents or holes** on your face by putting a vertical line in the place that best describes your scars.



Assessment of Acne Scar Appearance and Severity: Part 2

The following questions ask you about atrophic acne scars only. Remember, atrophic acne scars are indents or holes in the skin from previous active acne. Please do not consider active acne (zits, breakouts, pimples, whiteheads, or blackheads), scabs, or flat red or dark marks.

Please read each question, then look in the mirror and think about the indents or holes on your whole face, and then answer the question. Please mark an "X" in the box (☒) that best describes how the indents or holes on your face look RIGHT NOW.

There are no right or wrong answers to these questions. If you want to change your answer, please cross out your original answer and mark an "X" in the correct box.

Please choose only one response.

1. **When looking at your face in the mirror right now, how much of your face looks covered by indents or holes?**

Table 5.4: SCARS patient reported outcome tool (Continued)

Almost none of my face	A little of my face	Some of my face	A lot of my face	Almost all of my face
☐	☐	☐	☐	☐

2. When looking at your face in the mirror right now, how small or large do the individual indents or holes look?

Very small	Small	Moderate	Large	Very large
☐	☐	☐	☐	☐

3. When looking at your face in the mirror right now, how many indents or holes do you see?

Very few indents or holes	A few indents or holes	Some indents or holes	Quite a lot of indents or holes	Many indents or holes
☐	☐	☐	☐	☐

4. When looking at your face in the mirror right now, how deep do the individual indents or holes look?

Not at all deep	A little deep	Moderately deep	Very deep	Extremely deep
☐	☐	☐	☐	☐

5. When looking at your face in the mirror right now, how visible are the indents or holes to you?

Not at all visible	A little visible	Moderately visible	Very visible	Extremely visible
☐	☐	☐	☐	☐

(2) B. Facial acne scar quality of life (FASQoL):

Facial acne scar quality of life is a 10-questions instrument assessing the impact of scars emotion, social functioning and work/school with a recall period of the past 7 days based on patient's perspective. This tool is also a 5 points rating scales questionnaire. It has been suggest as a sensitive tool to evaluate the acne scars treatment benefits. This questionnaire will also be adapted into Thai language for patient's better understanding. (Table 5.5)

Table 5.5: Facial acne scar quality of life (FASQoL)

Acne Scar Quality of Life Questionnaire

The following questions ask you about how the atrophic acne scars on your face impact your daily life. Atrophic acne scars are indents or holes in the skin from previous active acne (not from injury, scratching or picking).

When answering these questions, please do not consider any of the following:

- **Zits, breakouts, pimples, whiteheads, or blackheads on the face;**
- **Acne scabs (dry, rough protective crusts that form over zits or pimples before indents or holes develop); or**
- **Flat red or dark marks.**

Please mark an "X" in the box (☒) that best describes the impact that the indents or holes on your face had on you over the PAST 7 DAYS.

There are no right or wrong answers to these questions. If you want to change your answer, please cross out your original answer and mark an "X" in the correct box. Please choose only one response.

1. **Over the past 7 days, have you felt self-conscious when you were with people because of the indents or holes on your face?**

Not at all self-conscious	A little self-conscious	Somewhat self-conscious	Very self-conscious	Extremely self-conscious
☐	☐	☐	☐	☐

2. **Over the past 7 days, have you felt less attractive because of the indents or holes on your face?**

I did not feel less attractive	A little less attractive	Somewhat less attractive	Much less attractive	Extremely less attractive
☐	☐	☐	☐	☐

3. **Over the past 7 days, have you felt annoyed because of the indents or holes on your face?**

Not at all annoyed	A little annoyed	Somewhat annoyed	Very annoyed	Extremely annoyed
☐	☐	☐	☐	☐

4. **Over the past 7 days, have you felt worried that the indents or holes on your face won't go away?**

Not at all worried	A little worried	Somewhat worried	Very worried	Extremely worried
☐	☐	☐	☐	☐

5. **Over the past 7 days, have you felt sad because of the indents or holes on your face?**

Not at all sad	A little sad	Somewhat sad	Very sad	Extremely sad
☐	☐	☐	☐	☐

6. **Over the past 7 days, have you felt upset by negative comments from others because of the indents or holes on your face?**

Not at all upset	A little upset	Somewhat upset	Very upset	Extremely upset
☐	☐	☐	☐	☐

Table 5.5: Facial acne scar quality of life (FASQoL) (Continued)

7. Over the past 7 days, have the indents or holes on your face made you avoid going out with friends or family?

Not at all	A little	Somewhat	Very much	Extremely
☐	☐	☐	☐	☐

8. Over the past 7 days, have you felt bothered by having to hide the indents or holes on your face?

Not at all bothered	A little bothered	Somewhat bothered	Very bothered	Extremely bothered
☐	☐	☐	☐	☐

9. Over the past 7 days, have the indents or holes on your face affected your relationships with others (for example, friends, family, romantic relationships/partner)?

Not at all	A little	Somewhat	Very	Extremely
☐	☐	☐	☐	☐

10. Over the past 7 days, have the indents or holes on your face affected your participation at work or school?

Not at all	A little	Somewhat	Very	Extremely
☐	☐	☐	☐	☐

(3) C. Patient overall satisfaction score:

Satisfaction questionnaires were completed by the participants to assess the improvement they perceived in their scars, wrinkles, post-inflammatory hyperpigmentation, pores and overall skin texture. A scale of 0%-100% was used, with 0-20 representing minimal improvement, 20-40 moderate improvement, 40-60 good improvement, 60-80 very good improvement, and 80-100 excellent improvement. Patients ticked the box according to their perceptions, and the score was reported four weeks, eight weeks and twelve weeks after the treatment. (Table 5.6)

Table 5.6: Patient overall satisfaction score

Overall Satisfaction Score

No. _____

Please mark the appropriate box that indicate your degree of satisfaction after the treatment by evaluating each side of the face separately.

1. Degree of improvement in **acne scars** of the treated area.

Side	Minimal (0-20%)	Moderate (20-40%)	Good (40-60%)	Very good (60-80%)	Excellent (>80%)
Rt					
Lt					

2. Degree of improvement in **wrinkles** of the treated area.

Side	Minimal (0-20%)	Moderate (20-40%)	Good (40-60%)	Very good (60-80%)	Excellent (>80%)
Rt					
Lt					

3. Degree of improvement in **dark spots** of the treated area.

Side	Minimal (0-20%)	Moderate (20-40%)	Good (40-60%)	Very good (60-80%)	Excellent (>80%)
Rt					
Lt					

4. Degree of improvement in **pores** of the treated area.

Side	Minimal (0-20%)	Moderate (20-40%)	Good (40-60%)	Very good (60-80%)	Excellent (>80%)
Rt					
Lt					

5. Degree of **overall** improvement of the treated area.

Side	Minimal (0-20%)	Moderate (20-40%)	Good (40-60%)	Very good (60-80%)	Excellent (>80%)
Rt					
Lt					

5.3.7.3 Evaluation of safety

(1) A. Universal pain assessment tool:

Patient will be asked to evaluate the pain score after each laser session and also day3 after the first laser session using universal pain assessment tool. The score will range from 0 which is no pain to 10 which is worst pain ever. The score from the previous visit which the patient rated will be given to the patient to compare with their prior result. (Figure 5.7)

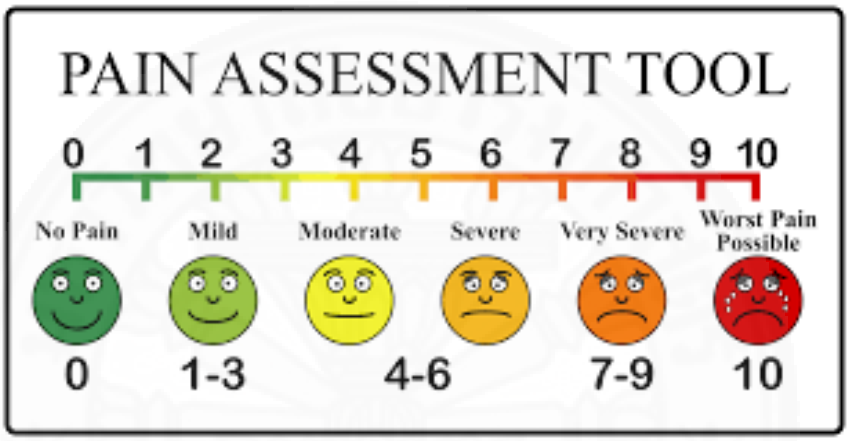


Figure 5.7: Pain assessment tool

(2) B. Adverse event assessment:

Patients will be evaluated by the physician on the adverse event associated the laser treatment including erythema, edema, petechiae, dyschromia, oozing, crusting and PIH at day0, day3, 1 weeks, 4 weeks, 8 weeks and 12 weeks after the first treatment session according to the table below. (Table 5.7)

Table 5.7: Patient adverse event data

Erythema	None	0	Edema	None	0
	Trace	1		Trace	1
	Mild	2		Mild	2
	Moderate	3		Moderate	3
	Severe	4		Severe	4
Dyschromia	None	0	Petechiae	Yes	No
	Trace	1	Oozing	Yes	No
	Mild	2	Crusting	Yes	No
	Moderate	3	Rash	Yes	No
	Severe	4	PIH	Yes	No

(3) C. Treatment for adverse events:

If any side effects occur during the study, the patient will be treated according to their symptoms such as dyschromia will be treated with 4% hydroquinone, acne eruption will be treating with topical antibiotics, other adverse event are according to the table list down below. For the patient who experienced PIH, the next treatment session will be postponed until complete recovery. Twelves weeks after first laser session, if there are still any adverse side effects left then there will be extended follow-ups every month until full recovery. (Table 5.8)

Table 5.8: Treatment for adverse events

Clinical presentation	Treatment
PIH	4% Hydroquinone
Acne flare up	Topical antibiotics
Brusing	Cold compression
Erythema	IPL/PDL/0.03% Tacrolimus
Milia	Electroconfiguration
Bacterial Infection	Oral antibiotics
Herpetic infection	Oral acyclovir

5.4 Data analysis

Paired *t*-test was used to compare acne scar index score, Goodman and Baron's acne scar grading score, SCARS assessment, FASQoL assessment and self-assessment satisfaction score by the patient before and after the treatment. Wilcoxon signed ranks test was used to compare the adverse events between the fractional carbon dioxide plus astaxanthin treated side and fractional carbon dioxide plus placebo side. A *P*-value of <0.05 was considered to be significant.

CHAPTER 6
RESULTS

At first, thirty seven subjects were enrolled into the study. Four of the subjects were excluded because they did not meet the inclusion criteria . During the treatment protocol, three of the subjects did not complete the protocol due to poor compliance and were excluded from the study. Finally, the total of thirty subjects completed the 12 week protocol treatment (Figure 6.1). Demographic data of the subjects were shown in table 15. (Table 6.1)

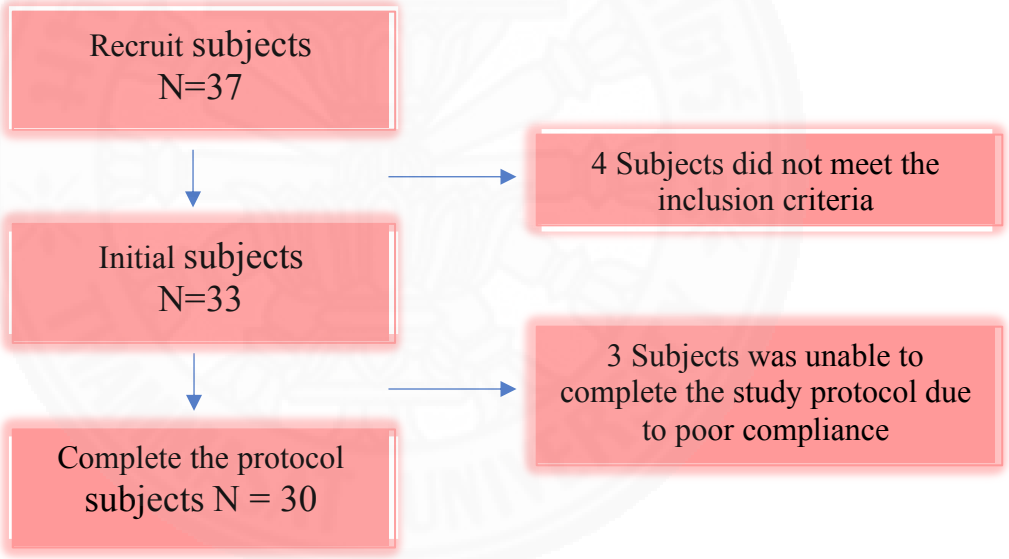


Figure 6.1: Number of enrolled patients

Table 6.1: Summarized demographic data of all participant

Demographic data		Number (n=30)	Percentage (%)
Sex (M:F)	Male	19	63.3%
	Female	11	36.7%
Age group (In years)	21-25	9	30%
	26-30	13	43.3%
	31-35	5	16.67%
	36-40	3	10%
Fitzpatrick's skin type	V	7	23.3%
	IV	16	53.3%
	III	7	23.3%
Duration of disease (Years)	1-5	6	20%
	5-10	20	66.67%
	10-15	0	0%
	15-20	4	13.33%
History of previous treatment	Yes	15	50%
	No	15	50%
Goodman and Baron qualitative acne scar grading system	4	16	53.33%
	3	14	46.67%
Goodman and Baron quantitative acne scar grading system (Maximum = 60)	20 - 40	17	56.67%
	40 – 60	13	43.33%

The study had 30 participants of whom 11 were female and 19 were male. Male to female ratio was 1:7. Their ages were in the range of 20-40 years with the mean age of 28 years. All patient were of III to V Fitzpatrick skin type. (Table 6.1) The average duration of disease was 27.93 years. At the baseline, 14 and 16 of the patient were classified into Goodman and Baron Qualitative acne scar grade 3 and grade 4 correspondingly with a Goodman and Baron Quantitative acne scar score ranging from 27 to 56. (Figure 6.1, Table 6.1)

The exclusion criteria removed any patients who had other dermatologic conditions or might be allergic to astaxanthin. Also excluded were those who had recently used isotretrinoin, those with active inflammation, infections or ulcers, those who had undergone ablative laser resurfacing treatments in the previous 4 months, those with predisposition to keloids or hypertrophic scars, those with collagen vascular disease, diabetes mellitus, or bleeding diathesis. Pregnant or lactating females were also excluded.

At the baseline we evaluate the efficacy with photographs using digital camera, skin texture and scar volume both using Antera® 3D photography and Goodman and Baron acne scar grading system. For the skin texture, we evaluate the roughness of the skin in arbitrary unit using Antera® 3D photography and for the scar volume, we evaluate the volume of depression in mm^3 unit using Antera® 3D photography. At the baseline, no statistically significant difference was found in the skin texture between the two groups ($FrCO_2$ + topical astaxanthin = 10.18 ± 2.82, FrCo2 + Placebo =10.35 ± 3.52, P-value = 0.672). Also, no statistically significant difference was found in the scar volume at the baseline between the two groups ($FrCO_2$ + topical astaxanthin = 7.55 ± 4.03 mm^3, FrCo2 + Placebo = 8.03 ± 4.30 mm^3, P-value = 0.427). Similarly, the Goodman and Baron acne scar grading system also show that there is no statistically significant difference between the two groups at the baseline. (Goodman and Baron qualitative : FrCO2 + Astaxanthin = 3.53 ± 0.51, FrCO2 + Placebo = 3.53 ± 0.51, P-value = 1) (Goodman and Baron quantitative: FrCO2 + Astaxanthin = 31.87 ± 8.80, FrCO2 + Placebo = 31.47 ± 9.5, P-value = 0.442) (Table 6.2)

Table 6.2: Mean baseline of skin texture, scar volume and Goodman and Baron acne scar grading system in both FrCO2 + Astaxanthin and FrCO2 + Placebo group.

Baseline score	FrCO2 + Astaxanthin	FrCO2 + Placebo	P-value
Skin texture (AU)	10.18 ± 2.82	10.35 ± 3.52	0.672
Scar volume (mm^3)	7.55 ± 4.03	8.03 ± 4.30	0.427
Goodman and Baron qualitative	3.53 ± 0.51	3.53 ± 0.51	1
Goodman and Baron quantitative	31.87 ± 8.80	31.47 ± 9.5	0.442

(AU = Arbitrary unit)

Mean improvement of skin texture was used to evaluate at baseline, 4th, 8th and 12th weeks which were statistically significant at 4th, 8th and 12th weeks in both groups. The mean skin texture in fractional carbon dioxide plus astaxanthin group was 10.18 ± 2.82 at baseline, then it reduced to 9.27 ± 2.12 at the 4th week, 8.94 ± 2.14 at 8th week and continue decreasing to 8.75 ± 2.23 at 12th week. The mean change of improvement from baseline of skin texture in fractional carbon dioxide plus astaxanthin group was 0.91 ± 1.25 (p<0.001), 1.24 ± 1.61 (p<0.001) and 1.44 ± 1.39 (p<0.001) at 4th, 8th and 12th week, respectively statistically significant change in every follow up week. (p<0.001*)

While in fractional carbon dioxide plus placebo group, the mean skin texture was 10.35 ± 3.21 at baseline, then it reduced to 8.72 ± 2.49 at the 4th week, 9.19 ± 2.55 at 8th week and decrease to 9.26 ± 2.94 at 12th week. The mean change of improvement from baseline of skin texture in fractional carbon dioxide plus placebo group was 1.63 ±1.52 (p<0.001), 1.16 ± 1.79 (p=0.001) and 1.08 ± 1.46 (p<0.001) at 4th, 8th and 12th week, respectively with also statistically significant change in every follow up week. (p<0.001*)

When comparing the mean reduction of skin texture between the two group, there is a statistically significant difference between the two group only at week 4 (P=0.018) with a higher drop down in FrCo2 combined with placebo group when comparing to the FrCo2 combined with astaxanthin group (-1.63 ±1.52 and -0.91± 1.25 respectively,

P=0.018). However, there was no statistically significant difference in the skin texture improvement between the two groups at week 8 and week 12. (P=0.781 and P=0.150).

Moreover, in comparison between the skin texture at the end of the treatment period (12th week), it was demonstrated that both regimens still could reduce the skin texture significantly at p-value <0.001 in both FrCO2 plus astaxanthin and FrCO2 plus placebo group. By week 12, the acne scar index values had fallen for both groups, and while mean difference showed a greater decline in the $FrCO_2$ plus astaxanthin group, the difference was not significant (p=0.15). (Figure 6.2, Table 6.3).

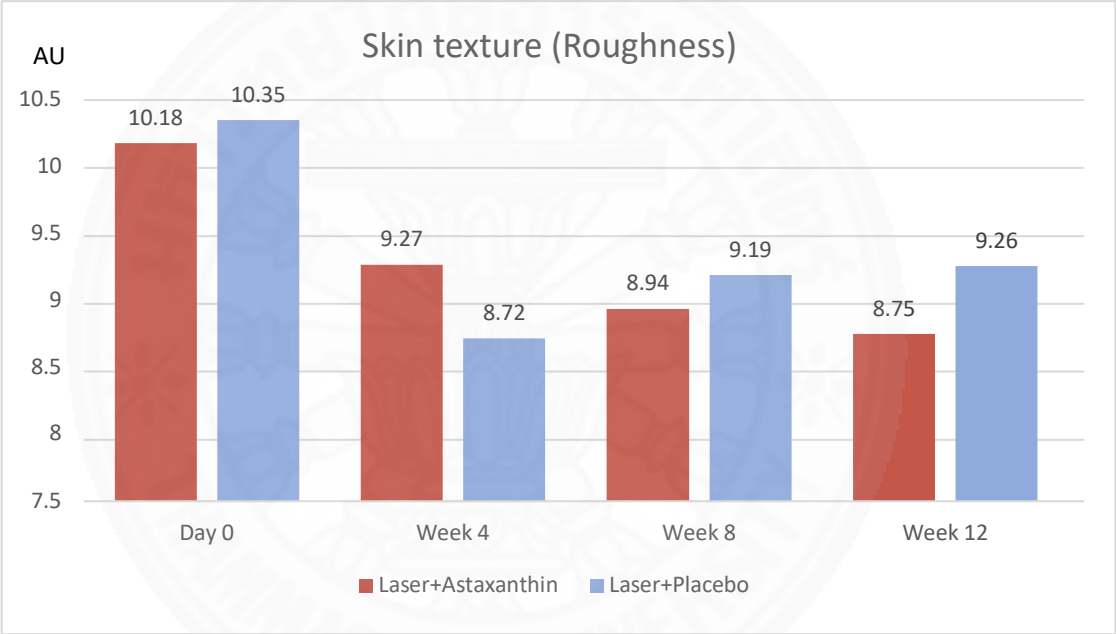


Figure 6.2: Skin texture (Roughness measure in Arbitrary Unit) in fractional carbon dioxide plus astaxanthin and fractional carbon dioxide plus placebo group at baseline, week 4, week 8 and week 12 (AU = Arbitrary Unit)

Table 6.3: Inter and intra group comparison of skin texture in both group.

Skin texture (Roughness) (AU)	Laser + Astaxanthin	P-Value^{w}	Laser + Placebo	P-value^{w}	P-Value^{b}
Day 0	10.18 ± 2.82		10.35 ± 3.21		0.672
Week 4	9.27 ± 2.12		8.72 ± 2.49		0.065
Week 8	8.94 ± 2.14		9.19 ± 2.55		0.418
Week 12	8.75 ± 2.23		9.26 ± 2.94		0.126
Change at wk 4	-0.91 ± 1.25	<0.001*	-1.63 ± 1.52	<0.001*	0.018*
Change at wk 8	-1.24 ± 1.61	<0.001*	-1.16 ± 1.79	0.001*	0.781
Change at wk 12	-1.44 ± 1.39	<0.001*	-1.08 ± 1.46	<0.001*	0.150

(P-Value^{w}: P-value within group, P-Value^{b}: P-value between group)

(AU = Arbitrary unit)

Mean improvement of scar volume was used to evaluate at baseline, 4th, 8th and 12th weeks which were statistically significant at 4th, 8th and 12th weeks in both groups. The mean scar volume in fractional carbon dioxide plus astaxanthin group was 7.55 ± 4.03mm^3 at baseline, then it reduced to 6.23 ± 3.04 mm^3 at the 4th week, 5.78 ± 3.11 mm^3 at 8th week and continue decreasing to 5.43 ± 3.24 mm^3 at 12th week. The mean change of improvement from baseline of scar volume in fractional carbon dioxide plus astaxanthin group was 1.31 ± 1.76 mm^3 (p<0.001*), 1.76 ± 2.31 mm^3 (p<0.001*) and 2.12 ± 2.07 mm^3 (p<0.001*) at 4th, 8th and 12th week, respectively statistically significant change in every follow up week. (p<0.001*)

While in fractional carbon dioxide plus placebo group, the scar volume was 8.03 ± 4.30 mm^3 at baseline, then it reduced to 5.79 ± 3.27 mm^3 at the 4th week, 6.35 ± 3.37 mm^3 at 8th week and decrease to 6.48 ± 3.97 mm^3 at 12th week. The mean change of improvement from baseline of scar volume in fractional carbon dioxide plus placebo group was 2.17 ± 2.09 mm^3 (p<0.001*), 1.63 ± 2.32 mm^3 (p=0.001*) and 1.50 ± 2.01mm^3 (p<0.001*) at 4th, 8th and 12th week, respectively with also statistically significant change in every follow up week. (p<0.001*) When comparing the mean reduction of scar volume between the two groups, there is a statistically significant difference between the two group only at week 4 (p=0.026) with a higher drop drown in FrCO2 combined with placebo group when comparing to the FrCO2 combined with astaxanthin group (-2.17 ± 2.09 mm^3 and -1.31 ± 1.76 mm^3 respectively, p=0.026)

However, when compare the scar volume change starting on week 8 onwards, the fractional carbon dioxide laser plus astaxanthin shows a greater drop down at 1.76 ± 2.31 mm^3(P<0.001*) by week 8 and 2.12 ± 2.07 mm^3 (P<0.001*) by week 12 while in the fractional carbon dioxide laser plus placebo group was at 1.63 ± 2.32 mm^3 (P=0.001*) by week 8 and 1.5 ± 2.01 mm^3 (P<0.001*) by week 12. Though mean difference showed a greater decline in the fractional carbon dioxide plus astaxanthin group, the difference between the two groups was not significant (Week 8; p = 0.714, Week 12; p = 0.057). (Figure 6.3, Table 6.4).

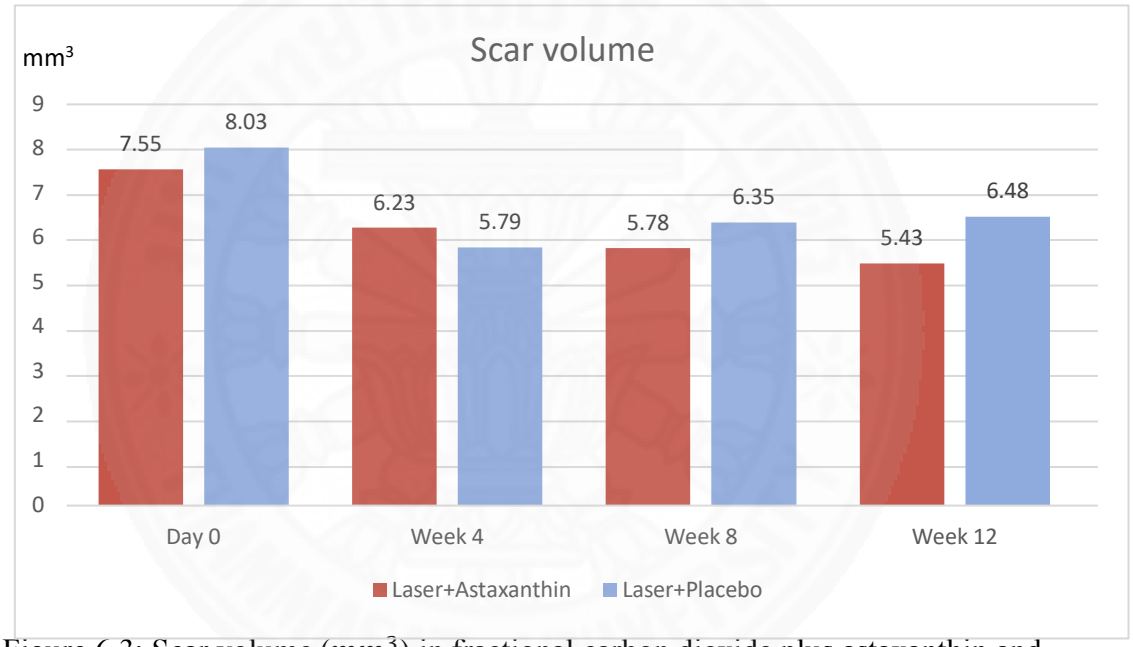


Figure 6.3: Scar volume (mm^3) in fractional carbon dioxide plus astaxanthin and fractional carbon dioxide plus placebo group at baseline, week 4, week 8 and week 12

Table 6.4: Inter and intra group comparison of scar volume in both group.

Scar volume (mm^3)	Laser + Astaxanthin	P-Value^w	Laser + Placebo	P-value^w	P-Value^b
Day 0	7.55 ± 4.03		8.03 ± 4.30		0.427
Week 4	6.23 ± 3.04		5.79 ± 3.27		0.199
Week 8	5.78 ± 3.11		6.35 ± 3.37		0.141
Week 12	5.43 ± 3.24		6.48 ± 3.97		0.019*
Change at wk 4	-1.31 ± 1.76	<0.001*	-2.17 ± 2.09	<0.001*	0.026*
Change at wk 8	-1.76 ± 2.31	<0.001*	-1.63 ± 2.32	0.001*	0.714
Change at wk 12	-2.12 ± 2.07	<0.001*	-1.50 ± 2.01	<0.001*	0.057

(P-Value^w: P-value within group, P-Value^b: P-value between group)

For the Goodman and Baron qualitative acne scar grading system were used to compare the overall morphologies of the acne scar of the whole face ranging from grade 0 to grade 4 at, baseline, week 4, week 8 and week 12 after the first treatment, in which this study only include patient with moderate to severe scar who falls into grade 3 and grade 4 of the Goodman and Baron qualitative acne scar score. Patient who were graded macular and mild disease which were rated grade 1 and grade 2 were excluded from this study.

At baseline, Goodman and Baron qualitative acne scar score of the whole face was at 3.53 ± 0.51 and continue to remain in this score during the follow session of week 4 and week 8. The change begin to drop down at week 12 from 3.53 ± 0.51 to 3.43 ± 0.57 making a mean difference change of 0.1 ± 0.31 but no statistical significant change. (P=0.083) (Table 6.5)

Table 6.5: Goodman and Baron qualitative acne scar score

Goodman and Baron Qualitative acne scar (Max=4)	Score	P-Value^{w}
Day 0	3.53 ± 0.51	
Week 4	3.53 ± 0.51	
Week 8	3.53 ± 0.51	
Week 12	3.43 ± 0.57	
Change at week 4	0 ± 0	1
Change at week 8	0 ± 0	1
Change at week 12	-0.1 ± 0.31	0.083

(P-Value^{w} = P=Value within group)

For the Goodman and Baron quantitative acne scar grading system were used to calculate numbers of acne scar to compare the result at baseline, week 4, week 8 and week 12 after the first treatment by evaluating in whole face and also in splitted face manor.

The Goodman and Baron quantitative acne score of the both side at the baseline were rated at 40.07 ± 8.55 and then it reduced to 36.4 ± 8.59 at the 4th week, 32.27 ± 8.04 at 8th week and decrease to 25.13 ± 7.33 at 12th week. The mean change of improvement from baseline of Goodman and Baron Quantitative acne scar score of the total both side was 3.67 ± 3.54 ($p<0.001$), 7.8 ± 4.72 ($p=0.001$) and 14.93 ± 6.53 ($p<0.001$) at 4th, 8th and 12th week respectively with a statistical significant in every follow up

session. (Table 6.6)

When evaluated separated side, the baseline of the astaxanthin group were rated 31.87 ± 8.8 then it reduced to 28.77 ± 8.05 at the 4th week, 25.9 ± 6.79 at 8th week and decrease to 20.63 ± 5.1 at 12th week. The mean change of improvement from baseline of Goodman and Baron Quantitative acne scar score in fractional carbon dioxide plus astaxanthin group was 3.1 ± 3.69 ($p<0.001$), 5.97 ± 4.04 ($p=0.001$) and 11.23 ± 6.43 ($p<0.001$) at 4th, 8th and 12th week respectively with a statistical significant in every follow up session. (Table 6.6)

While in fractional carbon dioxide plus placebo group, at the baseline of Goodman and Baron quantitative acne scar show a score of 31.47 ± 9.5, then reduced to 28.3 ± 8.71 at the 4th week, 25.6 ± 7.2 at 8th week and continue decreasing to 19.77 ± 5.74 at 12th week. The mean change of improvement from baseline of Goodman and Baron Quantitative acne scar score in fractional carbon dioxide plus placebo group was 3.17 ± 3.65 ($p<0.001$), 5.87 ± 4.17 ($p=0.001$) and 11.70 ± 6.43 ($p<0.001$) at 4th, 8th and 12th week respectively with also show a statistical significant change. (Table 6.6)

When comparing the mean reduction of Goodman and Baron Quantitative acne scar score between the two group, there is no statistically significant difference between the two group at week 4 ($P=0.326$) or week 8 ($P=0.586$) or week 12 ($P=0.214$). (Table 6.6). The digital images and antera3D® images providing the color image of acne scar comparison between both side at day 0, week 4, week 8 and week 12 of the same patient is display in figure 6.5-6.8 (Figure 6.5-6.8)

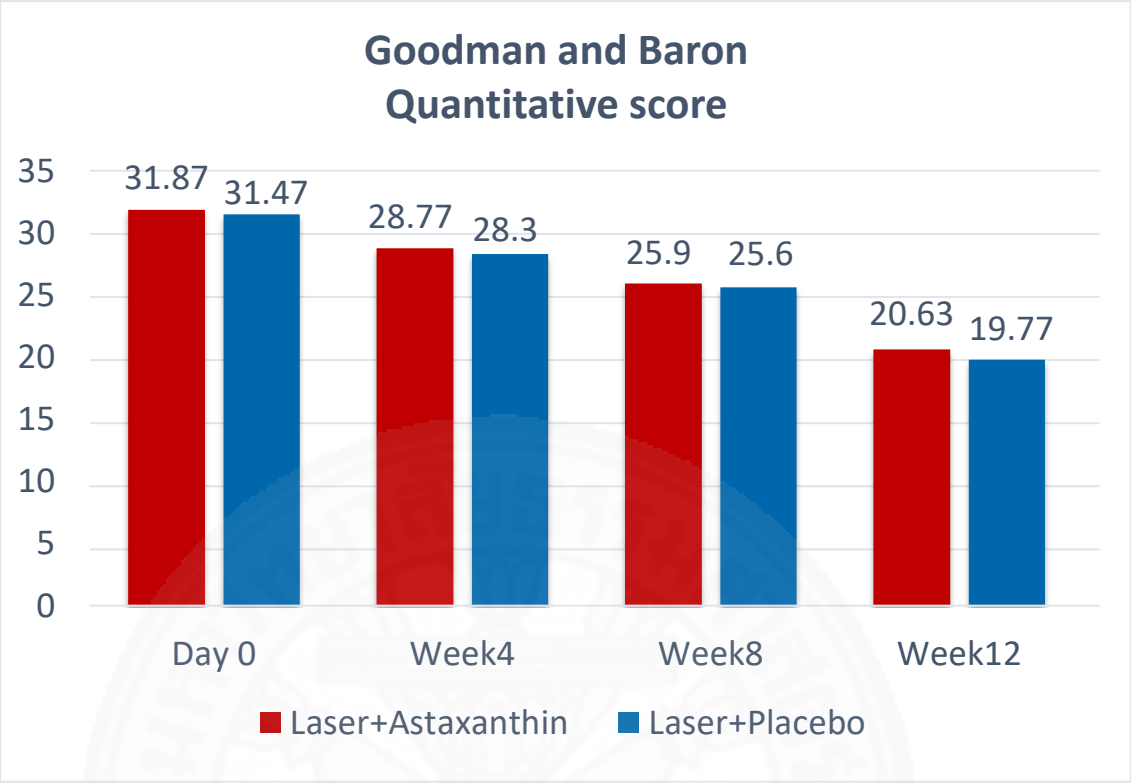


Figure 6.4: Goodman and Baron quantitative acne scar score

Table 6.6: Goodman and Baron quantitative acne scar score.

Goodman and Baron Quantitative score (Max=60)	Both side	P-Value^{w}	Laser + Astaxanthin	P-Value^{w}	Laser + Placebo	P-Value^{w}	P-Value^{b}
Day 0	40.07 ± 8.55		31.87 ± 8.8		31.47 ± 9.5		0.442
Week 4	36.4 ± 8.59		28.77 ± 8.05		28.3 ± 8.71		0.427
Week 8	32.27 ± 8.04		25.9 ± 6.79		25.6 ± 7.2		0.406
Week 12	25.13 ± 7.33		20.63 ± 5.1		19.77 ± 5.74		0.079
Change at week 4	-3.67 ± 3.54	<0.001*	-3.1 ± 3.69	<0.001*	-3.17 ± 3.65	<0.001*	0.326
Change at week 8	-7.8 ± 4.72	<0.001*	-5.97 ± 4.04	<0.001*	-5.87 ± 4.17	<0.001*	0.586
Change at week 12	-14.93 ± 6.53	<0.001*	-11.23 ± 6.22	<0.001*	-11.7 ± 6.43	<0.001*	0.214

(P-Value^{w}: P-value within group, P-Value^{b}: P-value between group)

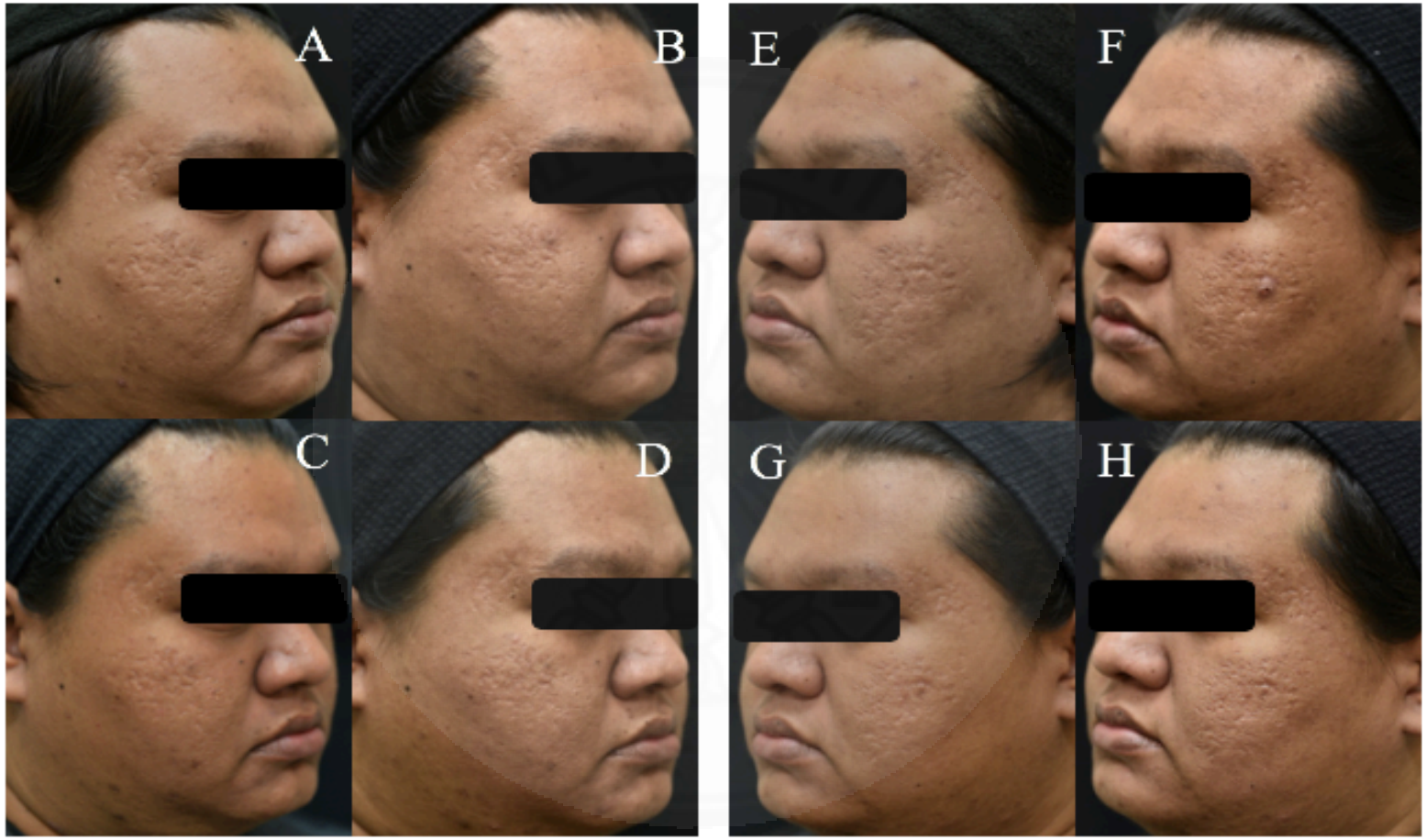


Figure 6.5: Digital images at the baseline (A) at week 4 (B), week 8 (C) and week 12 (D) of FrCO2 + topical astaxanthin on the right cheek and baseline (E) at week 4 (F), week 8 (G) and week 12 (H) of FrCo2 + placebo on the left cheek

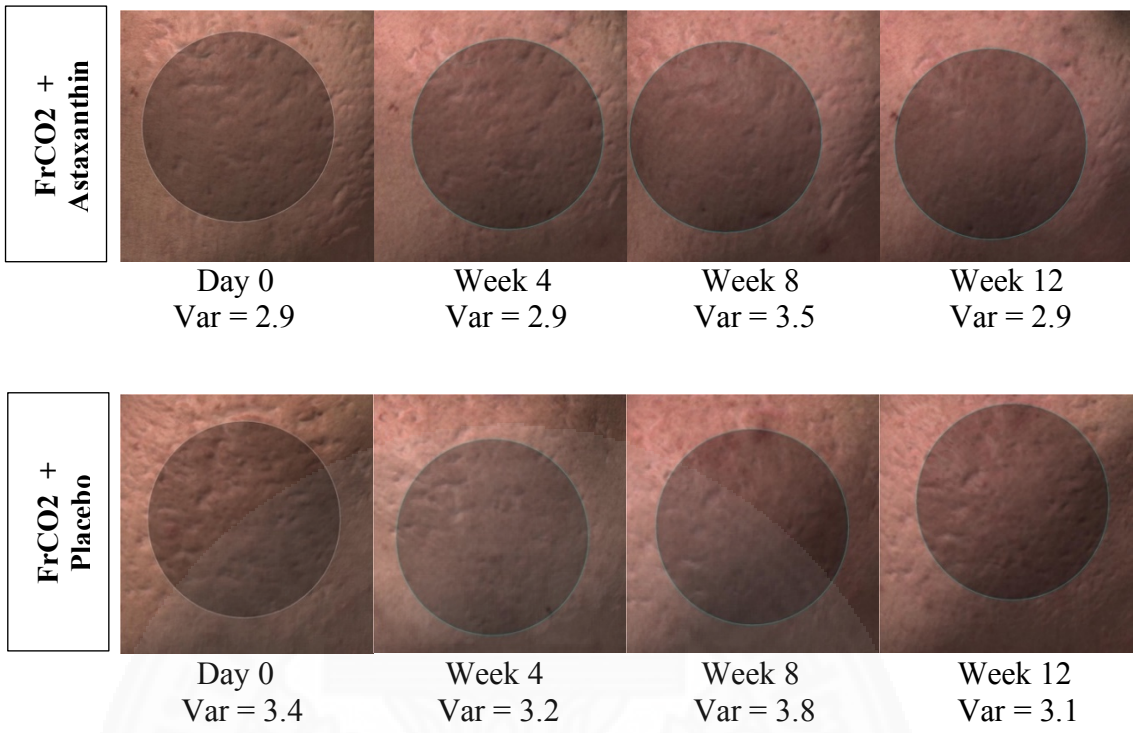


Figure 6.6: Antera3D® images providing the color image of acne scar comparison between day 0, week 4, week 8 and week 12 (Var = Variation)

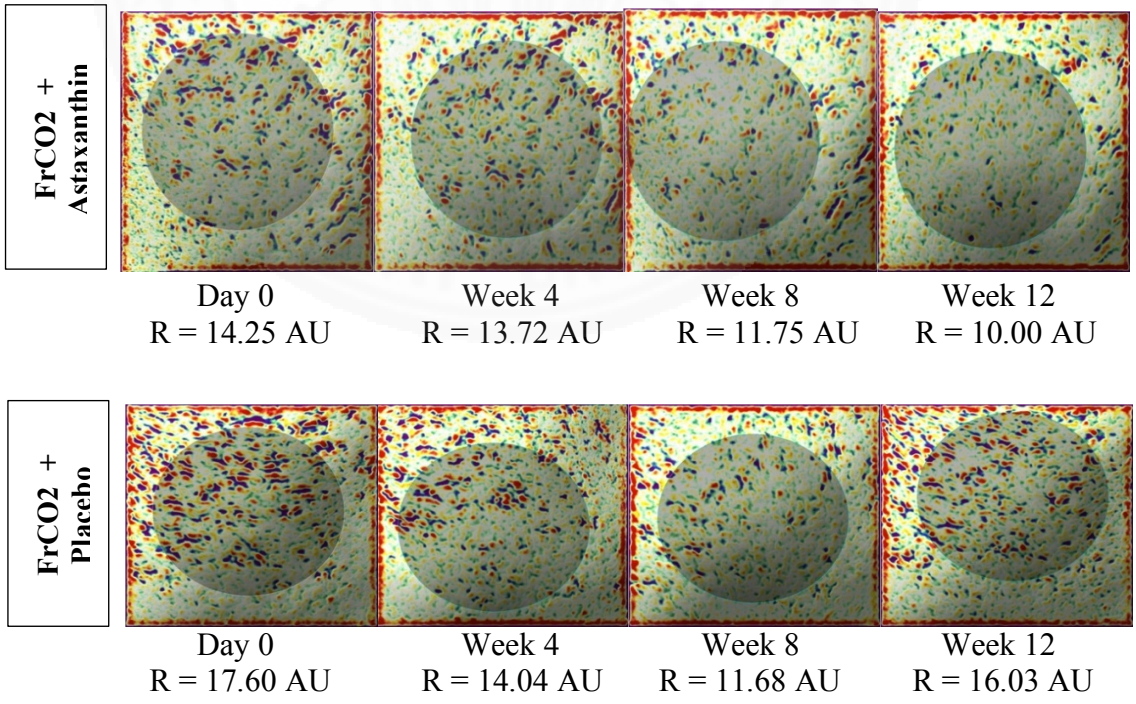


Figure 6.7: Antera3D® images evaluating skin texture comparing the roughness between day 0, week 4, week 8 and week 12 (R =Roughness). (AU=Arbitrary Unit)

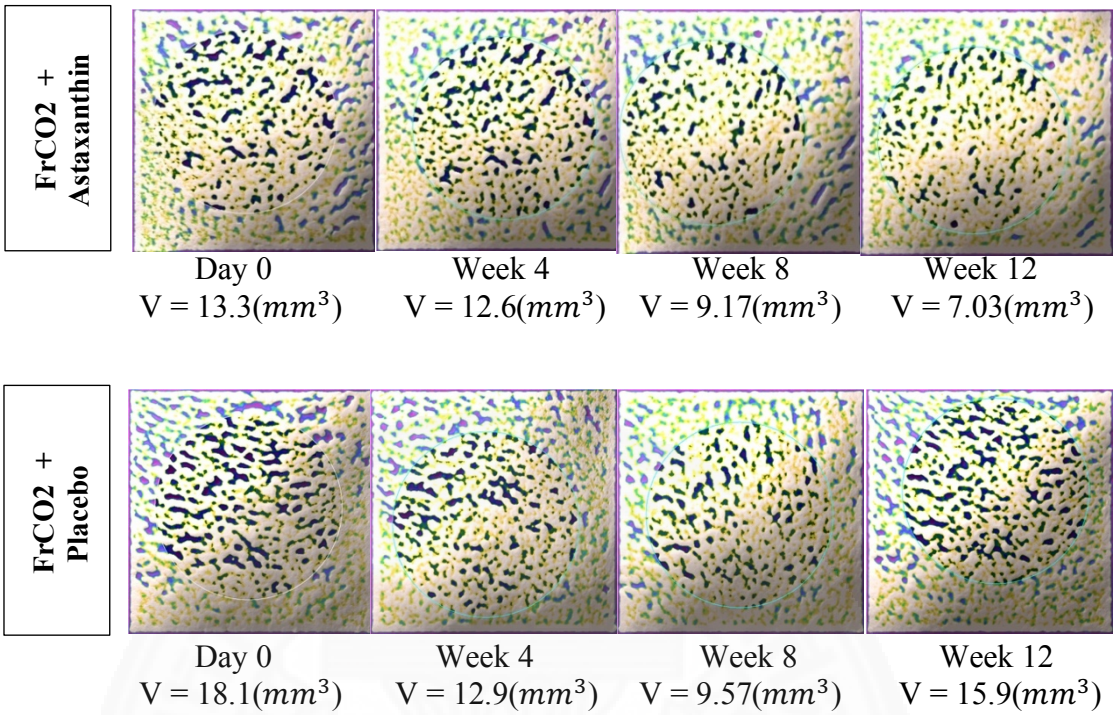


Figure 6.8: Antera3D® images evaluating scar volume comparing between day 0, week 4, week 8 and week 12 (mm^3)

For the evaluation of satisfaction, SCARS patient reported outcome tool, facial acne scar quality of life (FASQoL) and patient overall satisfaction score were used to evaluate the improvement in percentage comparing between the prior the treatment and at week 4, week 8 and week 12. The lower of the percentage meaning the lesser the scars effect their live, on the contrary, the higher of the percentage means the higher scar effect their live.

For SCARS, patient reported a percentage of 52.27 ± 15.76 at week 4 and drop down to 49.33 ± 12.65 at week 8 and down to 43.6 ± 14 at week 12 in the FrCO2 plus astaxanthin group with a statistically significant improvement can be seen at week 12 (P=0.005). Likewise in the FrCO2 plus placebo group also show a percentage of 52.27 ± 15.76 at week 4, a drop down to 45.20 ± 12.31 at week 8 and down to 41.27 ± 12.33 at week 12 with a statistically significant improvement can be seen at week 8 and week 12 (P=0.02 and P=0.001 respectively). Although the FrCO2 plus placebo side show a higher decline in percentage when comparing the FrCo2 plus astaxanthin side in every week, there was no statistically significant at any point. (Week4; P-value=1, Week8; P-value = 0.086, Week12; P-value=0.289) (Table 6.7)

Table 6.7: SCARS patient reported outcome score at week 4, week 8 and week12.

SCARS (%)	Laser + Astaxanthin	P-value[w]	Laser + Placebo	P-value[w]	P-Value[b]
Week 4	52.27 ± 15.76	Reference	52.27 ± 15.76	Reference	1
Week 8	49.33 ± 12.65	0.197	45.2 ± 12.31	0.020*	0.086
Week 12	43.6 ± 14	0.005*	41.27 ± 12.33	0.001*	0.289

The similar result can be seen in facial acne scar quality of life (FASQoL) tool. Patient rated a 10-questions based on how the atrophic acne scars bother their quality. Patient reported a decrease of percentage from 35.23 ± 17.42 at week 4 to 27.60 ± 12.31 at week 8 and to 26.6 ± 16.57 at week 12 in the FrCO2 plus astaxanthin group with a statistically significant improvement can be seen at week 8 and week 12 (P=0.002 and P=0.003 respectively). Likewise in the FrCO2 plus placebo group also show a drop of percentage from 35.9 ± 17.59 at week 4 to 26.73 ± 12.15 at week 8 and to 25.6 ± 13.61 at week 12 with a statistically significant improvement can be seen at week 8 and week 12 (P<0.001 both week). Although the FrCO2 plus placebo group show a higher decline in percentage in every follow up week, there was no statistically significant between the group at any point. (Week4; P-value=0.326, Week8; P-value = 0.130, Week12; P-value=0.231) (Table 6.8)

Table 6.8: Facial acne scar quality of life (FASQoL) at week 4 , week 8 and week 12.

FASQoL (%)	Laser + Astaxanthin	P-value[w]	Laser + Placebo	P-value[w]	P-Value[b]
Week 4	35.23 ± 17.42	Reference	35.9 ± 17.59	Reference	0.326
Week 8	27.6 ± 12.31	0.002*	26.73 ± 12.15	<0.001*	0.130
Week 12	26.6 ± 16.57	0.003*	25.6 ± 13.61	<0.001*	0.231

Lastly for the patient overall satisfaction score, patient were asked to rate their satisfaction of the outcome in terms of percentage of improvement of scars, wrinkles, post-inflammatory hyperpigmentation, pores and general facial improvement which were evaluated at 4th weeks, 8th weeks and 12th week after the first treatment.

In term of acne scar improvement, patients reported satisfaction with the acne scars improvement with percentage of 42.67 ± 17.8 at week 4, 52 ± 17.69 at week 8 and 56.67 ± 16.05 at week 12 for the astaxanthin side and 43.33 ± 18.45 at week 4, 56.67 ±

15.16 at week 8 and 58.67 ± 13.58 at week 12 for the side with the placebo. Both group were rated statistically significant improvement in acne scar as early as 8 weeks after the first treatment (P<0.001) and throughout the protocol. Though in each group shows higher percentage of satisfaction, there was no statistically significant between both group at any point of the follow up. (Week4; P-value=0.813, Week8; P-value = 0.109, Week12; P-value=0.476) (Table 6.9)

Table 6.9: Patient overall satisfaction score in term of improvement of acne scars

% Acne scar improvement	Laser + Astaxanthin	P-value[w]	Laser + Placebo	P-value[w]	P-value[b]
Week 4	42.67 ± 17.8	Reference	43.33 ± 18.45	Reference	0.813
Week 8	52 ± 17.69	<0.001*	56.67 ± 15.16	<0.001*	0.109
Week 12	56.67 ± 16.05	<0.001*	58.67 ± 13.58	<0.001*	0.476

In term of wrinkles improvement, patients reported satisfaction with the wrinkle improvement at 40 ± 18 at week 4, 45.33 ± 19.43 at week 8 and 52 ± 20.58 at week 12 for the astaxanthin side and 39.33 ± 16.39 at week 4, 48 ± 19.9 at week 8 and 51.33 ± 20.3 at week12 for the side with the placebo with equal statistically significant improvement in each group in week 8 and week 12 (P=0.043 and P=0.001 respectively). Though both groups shows higher percentage of satisfaction, there was no statistically significant between both group at any point of the follow up week. (Week4; P-value=0.745, Week8; P-value = 0.103, Week12; P-value=0.712) (Table 6.10)

Table 6.10: Patient overall satisfaction score in term of improvement of wrinkles

% Wrinkle improvement	Laser + Astaxanthin	P-value[w]	Laser + Placebo	P-value[w]	P-Value[b]
Week 4	40 ± 18	Reference	39.33 ± 16.39	Reference	0.745
Week 8	45.33 ± 19.43	0.043*	48 ± 19.9	0.043*	0.103
Week 12	52 ± 20.58	0.001*	51.33 ± 20.3	0.001*	0.712

In term of improvement of hyperpigmentation, patients reported satisfaction with the improvement of hyperpigmentation at 41.33 ± 19.43 at week 4, 48.67 ± 18.89 at week 8 and 56.67 ± 17.68 at week 12 for the astaxanthin side with statistically significant improvement can be seen at week 8 (P=0.019) and week 12 (P<0.001). For the placebo side, patients reported satisfaction with the improvement of hyperpigmentation at 42.67

± 18.56 at week 4, 53.33 ± 18.26 at week 8 and 54.67 ± 16.34 at week 12 with statistically significant improvement can be seen at week 8 (P<0.001) and week 12 (P=0.002). Though both groups shows higher percentage of satisfaction, there was no statistically significant between both group at week 4 and week 12 (Week4; P-value=0.489, Week12; P-value = 0.017), but a statistical significant difference were seen at week 8 (P=0.017) (Table 6.11)

Table 6.11: Patient overall satisfaction score in term of improvement of hyperpigmentation

% PIH improvement	Laser + Astaxanthin	P-value[w]	Laser + Placebo	P-value[w]	P-Value[b]
Week 4	41.33 ± 19.43	Reference	42.67 ± 18.56	Reference	0.489
Week 8	48.67 ± 18.89	0.019*	53.33 ± 18.26	<0.001*	0.017*
Week 12	56.67 ± 17.68	<0.001*	54.67 ± 16.34	0.002*	0.326

In term of pores improvement, patients reported satisfaction with the pores improvement at 41.33 ± 17.95 at week 4, 49.33 ± 18.56 at week 8 and 57.33 ± 18.56 at week 12 for the astaxanthin side with statistically significant improvement can be seen at week 8 (P=0.005) and week 12 (P<0.001). For the placebo side, patients reported satisfaction with pores improvement at 41.33 ± 17.17at week 4, 51.33 ± 18.89 at week 8 and 56 ± 17.54 at week 12 with statistically significant improvement can be seen at week 8 (P=0.001) and week 12 (P<0.001). At week 12, higher percentage of satisfaction of pores improvement is seen in the FrCO2 plus astaxanthin side with percentage of improvement of 57.33 ± 18.56. Throughout the follow up session of 4 weeks, 8 weeks and 12 weeks, there was no statistical significant at any point. (Week4; P-value=1, Week8; P-value = 0.184, Week12; P-value=0.489) (Table 6.12)

Table 6.12: Patient overall satisfaction score in term of improvement of pores

% Pores improvement	Laser + Astaxanthin	P-value[w]	Laser + Placebo	P-value[w]	P-Value[b]
Week 4	41.33 ± 17.95	Reference	41.33 ± 17.17	Reference	1
Week 8	49.33 ± 18.56	0.005*	51.33 ± 18.89	0.001*	0.184
Week 12	57.33 ± 18.56	<0.001*	56 ± 17.54	<0.001*	0.489

In term of general facial improvement, patients reported satisfaction with the facial improvement at 42 ± 18.64 at week 4, 54 ± 19.23 at week 8 and 60.67 ± 16.39 at week 12 for the astaxanthin side and 44 ± 18.31 at week 4, 56.67 ± 16.05 at week 8 and 60.67 ± 12.58 at week 12 for the side with the placebo. Both group were rated statistically significant overall improvement as early as 8 weeks after the first treatment (P<0.001) and throughout the protocol. By week 12, patient rated from their perspective that there was a statistical significant improvement in each side (P<0.001) but no significant difference between the two side. (P=1) (Table 6.13)

Table 6.13: Patient overall satisfaction score in term of general facial improvement

% Overall improvement	Laser + Astaxanthin	P-value[w]	Laser + Placebo	P-value[w]	P-Value[b]
Week 4	42 ± 18.64	Reference	44 ± 18.31	Reference	0.375
Week 8	54 ± 19.23	<0.001*	56.67 ± 16.05	<0.001*	0.293
Week 12	60.67 ± 16.39	<0.001*	60.67 ± 12.58	<0.001*	1

Regarding the side effect, both physician and patient were requested to fill out the adverse event assessment form to evaluate the symptom immediate after laser at day 0, day 3, week4, week 8 and week 12 from the first laser session. Moreover, patient were ask to fill out the universal pain assessment rated the pain score on a scale of 0 - 10 points.

At the follow-up check or safety check on day 3, from the physician perspective, level of erythema were rated at 34.17% ± 15.37 in the FrCO2 plus astaxanthin side and 37.5% ± 17.06 for the FrCo2 plus placebo side. Though percentage of level of erythema is lesser on the FrCO2 plus astaxanthin side, there was no statistical significant difference. (P=0.326) The same result can be seen for the level of edema with 25.83% ± 19.12 in the FrCO2 plus astaxanthin side and 28.33% ± 21.51 for the FrCo2 plus placebo side, with lower percentage on astaxanthin side but no statistical significant difference (P=0.476) For level of PIH, lower percentage can be seen in FrCO2 plus astaxanthin side with 16.67% ± 37.9 and 20% ± 40.68 for FrCo2 plus placebo side, no statistical significance. (Table 6.14)

Table 6.14: Physician's overall laser safety evaluations score at day 3

% Adverse event at day 3	Laser + Astaxanthin	Laser + Placebo	P-Value[b]
Level of Erythema	34.17 ± 15.37	37.5 ± 17.06	0.326
Level of Edema	25.83 ± 19.12	28.33 ± 21.51	0.476
Level of PIH	16.67 ± 37.9	20 ± 40.68	0.326

From the patient's perspective, after day 3 the first laser treatment, 1 patient (3.3%) rated sting, 1 patient (3.3%) rated edema, 15 patient (50%) rated erythema, 4 patient (13.3%) rated PIH and 9 patient (30%) rated desquamation for the FrCO2 plus astaxanthin side. For the FrCO2 plus placebo side, 1 patient (3.3%) rated sting, 1 patient (3.3%) rated edema, 15 patient (50%) rated erythema, 4 patient (13.3%) rated PIH and 8 patient (26.7%) rated desquamation. There was no statistically significant between the two group in any category of adverse events. (Table 6.15)

Table 6.15: Patient's overall laser safety evaluations score at day 3

Adverse event at day 3	Laser + Astaxanthin	Laser + Placebo	P-Value[b]
Sting	1 (3.3%)	1 (3.3%)	1
Edema	1 (3.3%)	1 (3.3%)	1
Erythema	15 (50%)	15 (50%)	1
PIH	4 (13.3%)	4 (13.3%)	1
Desquamation	9 (30%)	8 (26.7%)	0.317

These adverse events had cleared up by week 4, which can be explained as a normal inflammation associated with laser treatment. There was no reported of acne, bruise, milia, blister, dyschromia, bacterial or herpetic infection during the treatment protocol in both treated group. Also no patient reported any kind of skin rash or allergic contact dermatitis as a consequence of the application of topical astaxanthin and also no systemic side effect was found in this study.

CHAPTER 7
DISCUSSION

Acne scars can be mainly divided into atrophic scar and hypertrophic or keloid scar, most of which, acne scars are atrophic scar that is caused by the decreased of the net collagen resulted in a depression of the surface of the skin causing a serious cosmetic appearance problems. Many modalities have been used in treating atrophic acne scar such as chemical peels, dermabrasion, laser treatment, dermal grafting, tissue augmentation, needling and many more but combined therapies seems to provide the best result in treatment (3,6,7,8).

Laser treatment is widely known because for its high efficacy in treating generalized acne scar, especially the traditional ablative laser, which has high ability to resurfaces the skin. But due to its post laser treatment complication, fractional laser has been introduced in the field of lasers, to help aid in the post laser re-epithelialization. Among these, the carbon dioxide laser is very popular and is used for photo-rejuvenations and the trans-epidermal elimination of dermal content. Fractional carbon dioxide lasers offer advantages over the traditional ablative laser and are also highly effective while creating fewer adverse side effects (1,40,41).

Astaxanthin is an antioxidant which is highly active biologically in comparison to other carotenoids. As a consequence of its lipophilic and hydrophilic properties, which match those of cell membranes, it is able to thrive both inside and outside the cell, and hence it is around 550 times stronger than vitamin E, 880 times stronger than CoQ10 and 6,000 times stronger than vitamin C (5). It can also be used to prevent diabetes, serve as a nutritional supplement, act as an anti-cancer agent, and treat neurodegenerative disorders and cardiovascular diseases (19,22,27,32).

This controlled trial study was carried out to assess the efficacy of using fractional carbon dioxide combined with topical astaxanthin and to compare this with the outcomes from using fractional carbon dioxide alone in order to treat atrophic acne scars. The result showed that the combination of topical astaxanthin with the fractional carbon dioxide laser can effectively treat atrophic acne scars while creating minimal

adverse side effects, but this is not significantly different to the use of the fractional carbon dioxide laser alone.

According to table 6.1, this study had 30 patients in the age range between 20-40 years old of which 19 patients are male and 11 patients are female making percentage of 63.3% and 36.7% respectively. All of the patients are healthy and no difference in healing capacity. There were no patients of extreme age gap and with half of them has previous history of treating acne scar and another half did not. There is limitation in terms of the small sample size with 33 patients who meet the inclusion criteria but there was 3 patient who dropped out so the process due to poor compliance. So by the end, the study was left with a total of 30 patients. Recruiting more subjects could possibly alter the results.

According to figure 6.2 and table 6.3, by week 12, the skin texture for both groups showed a significant drop; $FrCO_2$ plus topical astaxanthin fell from 10.18 to 8.75 ($p<0.001$) and $FrCO_2$ plus placebo fell from 10.35 to 9.26 ($p<0.001$). Though FrCO2 plus topical astaxanthin group show a higher change in improvement of skin texture, there was no statistically significant difference between the two group. Similiarly, the scar volume for both groups showed a significant drop; $FrCO_2$ plus topical astaxanthin fell from 7.55 mm^3 to 5.43 mm^3 ($p<0.001$) and $FrCO_2$ plus placebo fell from 8.03 mm^3 to 6.48 mm^3 ($p<0.001$). Though FrCO2 plus topical astaxanthin group show a higher change in improvement of scar volume, there was no statistically significant difference between the two group. Likewise, Goodman and Baron quantitative score also show a similar result with significant decline in both group but no statistically significant difference when compare between two groups (Table 6.6).

Regarding the adverse events, the follow-up at day 3 revealed that every patient reported edema and erythema as well as itching sensation without rash on both side of the face, which could be explained as normal inflammation associated with laser treament. According to table 6.13, from physician's perspective the level of erythema, edema and post inflammatory hyperpigmentation is lower in the FrCO2 plus topical astaxanthin group but there was no statistical significant difference when compare to FrCO2 plus placebo side. From patient's perspective there was no difference side effect when compare between the two sides of the treatment. These symptoms cleared by week

4, and might be explained as the normal inflammation which is associated with laser treaments. No adverse effects were seen from the topical astaxanthin.

Early clinical studies on fractional ablative carbon dioxide laser resurfacing were performed mostly on lighter skin types. *Chapes et al.* treated 13 patients with moderate to severe acne scars at one to two months intervals for two to three treatment sessions using pulse energies of 70-100 mJ and total densities of 200-1,200 MTZ/cm2 were used. At 6 months after the first treatment session, significant improvement was obtained for acne scars with approximately mean level of improvement of 66.8% (34). Our study also provide slightly lower result with 62.7% of improvement in Goodman and Baron Quantitative acne scar score with the same number of treatment sessions but shorter period of follow up which is 3 months after the first treatment session. Moreover our study also use different laser setting with lower energy and lower density of 50mJ and 50 MTZ/cm2 respectively. The lower percentage of improvement could be explained by the laser setting and also a relatively short follow-up period of three months. This did not permit long-term results to be taken into consideration, whether beneficial or adverse. It is therefore suggested that future studies should involve more treatment sessions and a longer duration for the follow-up in order to validate the findings produced here.

Kar BR et al. conduct a clinical study using fractional carbon dioxide laser with topical platelet-rich plasma versus fractional carbon dioxide laser alone in treatment of atrophic acne scars; a split-face comparison trial also report that there is no significant difference between the two sides but downtime and inflammation associated with laser treatment gets significantly reduced on the PRP-treated side (2). Our study also report the same result in the improvement in acne scar that there was no significant different between the treated and un-treated side. On the contrary, our result shows that there was no statistically significant between the two side in downtime and inflammation associated with laser treatment, possibly due to their aggressive treatment of using energy of 200-250 mj per treatment session causing a deeper penetration of PRP to be delivered deeper in the skin.

Another study conducted by *Mohammed et al.* which evaluate the effect of injecting autologous platelet-rich plasma intradermally plus ablative fractional carbon dioxide laser in treating acne scars provide a statistically significant in acne scar improvement in the PRP injected side when comparing to the non-injected side (43).

When compare between *Kar BR et al* (2) and *Mohammed et al.* (43), my assumption is that fractional carbon dioxide laser in combination of injecting drug intradermally could provide a better result, therefore further study using astaxanthin could provide a better result by injecting astaxanthin intradermally.

Chan et al. conducted a study using fractional ablative carbon dioxide laser resurfacing for skin rejuvenation and acne scars in Asian using Fraxel Repair one treatment session with energy level ranging from 60-70 mJ, total energy of 6.80-10.28 kJ, percent coverage between 30% and 45% of the total pass, coverage between 8.75% to 11.25% per pass with 4 passes per one treatment session with subject of III-IV Fitzpatrick skin type. The result from their study shows that at 3 months after one laser treatment, there were improvement in term of texture, skin laxity, wrinkles, enlarged pores, and acne scars. Eighty percent of the subjects rated 20-40% improvement (mild improvement) in category of texture, skin laxity, wrinkles, enlarged pores, while 60% of the subjects rated 40-60% improvement (moderate improvement) in the category of acne scars. (25). Likewise, our study also provide similar result with satisfaction score of 50-60% improvement in wrinkle, irregular pigmentation, pores, overall satisfaction and acne scar but in our study we conduct more treatment sessions with lower number of passes per session.

When compare between the post-inflammatory hyperpigmentation at one month post procedure showed rate of 55.5% and 6.7% in *Chan et al* .study and our study, respectively. Much lower rate of post-inflammatory hyperpigmentation in our study could be explained by lower density, lower number of passes and possibly due to topical astaxanthin according to one investigation of astaxanthin indicated that it is able to inhibit ultraviolet-induced apoptosis in keratinocytes, which would imply that astaxanthin can prevent UV-induced inflammation and aging through decreasing iNOS and COX-2, which will restrict the apoptosis of keratinocytes (28).

In earlier studies it was confirmed that astaxanthin is an effective antioxidant which can bring cosmetic improvements such as the removal of crow’s feet, boosting elasticity of the skin, raising moisture content levels, blocking pigmentation, the generation of melanin, and the effects of aging due to light. It can also increase the rate of wound healing in mice (19,20,22,27).

Kumi et al performed a clinical studies in humans have shown that 6 mg of oral astaxanthin daily combined with 2 ml of topical astaxanthin daily can improve skin texture after 4 weeks and can improve the appearance of crow's feet and limit the size of age spots after 8 weeks (19). Another research from Seki revealed that within 2 weeks of the topical application of astaxanthin, moisture retention is improved along with the fine wrinkles on the outer canthi. Uniformity of the skin grain on the cheeks is also enhanced (27). A study conducted by Kumi similarly found that the condition of the skin is improved by the oral and topical application of astaxanthin in combination, with increased elasticity, reduction of wrinkles, and reduced transepidermal water loss (19). Our study suggest the same result with combination treatment of fractional carbon dioxide laser plus topical astaxanthin provide a statistical significant result of 45.3% improvement in wrinkle by week 8 (P=0.043) and 53% by week 12 (P=0.001), in patient's perspective (Table 6.10).

One study, *Meephansan et al.,* examining cutaneous wound healing in mice revealed that topical astaxanthin can potentially accelerate the process by upregulating the basic fibroblast growth factor (bFGF), collagen 1A1, and pro-collagen which decline as a natural part of inflammation (22). The concentration of the topical astaxanthin that were used to conducted the study was 78.9 μM topical astaxanthin and result showed that it can potentially accelerate the cutaneous wound healing in mice. The biopsy from the back of the mice shows the upregulation in basic fibroblast growth factor (bFGF), collagen 1A1, and pro-collagen (22). This study also conduct using 78.9 μM topical astaxanthin but it is conducted in human subjects. So one possible explanation could be that this concentration of 78.9 μM topical astaxanthin is relatively low in order to accelerate wound healing in human. Another possible assumption is that the improvement of acne scars was rather low that it could only be seen under microscopic view. So it is suggested that further study should conducted with higher concentration than 78.9 μM of topical astaxanthin in human and skin biopsy should be done to evaluate and compare healing factors between astaxanthin treated side and untreated side.

Table 7.1: Comparison between the studies

	P	I	C	O	T	Conclusion
	30 Subjects Mod-severe acne scars	FrCO2 +Topical astaxanthin Energy: 50mJ Density: 50 spots/cm2 (%Coverage=3.2%) No. pass: 1 Overlapping: 10% Power: 30W	FrCo2+Placebo	Acne scars quality 12 weeks: 15% improvement in acne scar index 62.7% improvement in Goodman Baron quantitative acne scar score Skin quality improvement 12 weeks: 53% wrinkle improvement 56.67% dyspigment imp. 57.33% pores size imp. 60.67% overall imp. Decrease downtime and inflammation associated with laser treatment 4 weeks: PIH rate 6.7% 12 weeks: PIH rate 3.3%	12 Wks (3 sess.)	
Chapas et al.	13 Subjects Mod- severe acne scars	FrCO2 alone E: 70-100 mJ D: 200-1,000 MTZ/cm	Whole face comparison	Acne scars quality 6 months: 66.8% mean level of improvement	4-8 Wks (2-3 sess.)	Involve more treatment sessions Longer duration of follow up

	P	I	C	O	T	Conclusion
Nicola et al.		FrCO2 alone (Fraxel) E: 60-70 mJ %Coverage: 8.75-11.25% No. pass: 4	Whole face comparison	Skin quality 3 months: 60%= Moderate improvement in acne scars 80%=Mild improvement in texture, skin laxity, wrinkles and enlarged pores Decrease downtime and inflammation associated with laser treatment 1 month: PIH rate 55.5%	1 sess.	Lower energy and lower number of passes cause lower PIH
KRB et al.	30 Subjects Mod- severe acne scars	FrCO2 + Topical PRP E: 200-250mJ	FrCo2+Placebo	Acne scars quality 3 months: No significant difference Decrease downtime and inflammation associated with laser treatment 3 months`: Significant difference		Need higher energy setting Longer duration of follow up

	P	I	C	O	T	Conclusion
Ahmed et al.	30 Subjects Mod-severe acne scars	FrCO2 + PRP ID E: 15 watt 600µs dwell time 700 µs spacing Smart stack level 3	FrCo2 + NSS ID	<u>Acne scars quality</u> Excellent improvement in 13 % of the patient in the treated side No excellent improvement in the placebo side	1 sess.	Injecting drug provide better result
Kumi et al.	30 Subjects Healthy adults	6 mg oral astaxanthin (AstaReal Oil 50 F) + 2 ml topical astaxanthin (78.9µM Astaxanthin, AstaTROL - HP	Whole face comparison	<u>Skin quality</u> 4 weeks: Improve in skin texture, appearance of crow's feet, reduces trans-epidermal water loss 8 weeks: Limits the size of age spots after 8 weeks	8 weeks	Astaxanthin can reduce fine lines wrinkles
Seki et al.	45 Subjects Healthy adults	Topical astaxanthin (AstaReal Oil 50 F)	Whole face comparison	<u>Skin quality</u> 2 weeks: Moisture retention is improved along with fine wrinkles on the outer canthi and uniformity of the skin grain on the cheeks is also enhanced		Astaxanthin can reduce fine lines wrinkles

	P	I	C	O	T	Conclusion
Meephansan et al.	36 Mice	Full thickness dermal wound + topical astaxanthin (78.9μM Astaxanthin)	Full thickness dermal wound	Wound healing: Day3: Wound contraction Day9: Fully epithelialization and lost eschar RT-PCR: -Increase: bFGF, Col1A1 and pro-collagen -Decrease: iNOS	Twice daily 15 days	Conduct with higher concentration in human study Skin biopsy or RT-PCR should be obtained

CHAPTER 8
CONCLUSION AND RECOMMENDATIONS

8.1 Conclusion

It can be concluded that the treatment using topical astaxanthin in combination with the fractional carbon dioxide laser is both effective and safe when used to treat acne scars. Within twelve weeks of treatment, the acne scar index values had improved. Meanwhile the patient perceptions indicated a 55-60% improvement from the three session of treatment, while the side effects of the use of the laser could easily be tolerated. This study revealed no significant difference in efficacy when comparing the outcomes from $FrCO_2$ alone with the results of $FrCO_2$ in combination with topical astaxanthin, although this may be due to the small number of patients tested, the use of a three treatment session, the limited follow-up duration, low concentrations of topical astaxanthin used and relatively low parameter of laser setting.

8.2 Recommendations

8.2.1 Further studies should involve multiple treatment sessions.

8.2.2 Further studies with a longer duration of follow-up in order to validate the findings produced here.

8.2.3 Further studies with a larger number of participants may provide the better precision and accuracy.

8.2.4 Further study with higher concentration of astaxanthin could be done to optimize efficacy of the treatment.

8.2.5 Further study with other laser parameter setting could possibly alter the result.

8.2.6 Further study should involve other route of administration of astaxanthin such as oral form or intradermal form.

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APPENDICES

APPENDIX A
OVERALL SATISFACTION SCORE

แบบสำรวจความพึงพอใจโดยรวม (Overall Satisfaction Score)

รหัสคนไข้ _____

กรุณากากบาทในกล่องสี่เหลี่ยมที่อธิบายถึงสภาพผิวบนใบหน้าของท่านหลังจากทำเลเซอร์แฟรคชั่นแนลคาร์บอนไดออกไซด์ร่วมกับสารสกัดแอสต้าแซนทินได้ดีที่สุด โดยประเมินแยกใบหน้าฝั่งซ้ายและฝั่งขวา

1. คุณรู้สึกว่า ” หลุมสิว” บนใบหน้าของคุณดีขึ้นมากน้อยเพียงใด

หน้า	น้อยมาก (0-20%)	น้อย (20-40%)	ปานกลาง (40-60%)	มาก (60-80%)	มากที่สุด (>80%)
ขวา					
ซ้าย					

2. คุณรู้สึกว่า “ริ้วรอย” บนใบหน้าของคุณดีขึ้นมากน้อยเพียงใด

หน้า	น้อยมาก (0-20%)	น้อย (20-40%)	ปานกลาง (40-60%)	มาก (60-80%)	มากที่สุด (>80%)
ขวา					
ซ้าย					

3. คุณรู้สึกว่า “รอยดำ” บนใบหน้าของคุณดีขึ้นมากน้อยเพียงใด

หน้า	น้อยมาก (0-20%)	น้อย (20-40%)	ปานกลาง (40-60%)	มาก (60-80%)	มากที่สุด (>80%)
ขวา					
ซ้าย					

4. คุณรู้สึกว่า “รูขุมขน” บนใบหน้าของคุณดีขึ้นมากน้อยเพียงใด

หน้า	น้อยมาก (0-20%)	น้อย (20-40%)	ปานกลาง (40-60%)	มาก (60-80%)	มากที่สุด (>80%)
ขวา					
ซ้าย					

5. คุณรู้สึกว่า “โดยรวม” ใบหน้าของคุณดีขึ้นมากน้อยเพียงใด

หน้า	น้อยมาก (0-20%)	น้อย (20-40%)	ปานกลาง (40-60%)	มาก (60-80%)	มากที่สุด (>80%)
ขวา					
ซ้าย					

APPENDIX B
THESIS CASE RECORD FORM

<u>แบบบันทึกระหว่างการทำวิจัย</u> <u>(THESIS CASE RECORD FORM)</u>

รหัสคนไข้ _____

(ประเมิน ณ วันแรกที่เข้าร่วมโครงการ)

รหัสคนไข้:	เพศ:	วันเกิด:
เชื้อชาติ:	ส่วนสูง: น้ำหนัก:	อาชีพ:
ที่อยู่:		อีเมล์:
อำเภอ/เขต:	จังหวัด: รหัสไปรษณีย์:	เบอร์ติดต่อฉุกเฉิน:
เบอร์ติดต่อ:		ชื่อผู้ติดต่อฉุกเฉิน:
ข้อมูลสุขภาพทั่วไป		
1. ระดับความเครียดหรือความกังวลของคุณ (5=มากที่สุด, 1=น้อยที่สุด) 5 4 3 2 1		
2. ท่านกำลังตั้งครรภ์หรือให้นมบุตรหรือไม่? ใช่ ไม่ใช่		
3. ท่านสูบบุหรี่หรือไม่? ไม่สูบ เคยสูบ (ระยะเวลาที่เลิก……. ปี ระยะเวลาที่สูบ……. ปี เคยสูบบุหรี่……..มวนต่อวัน) ปัจจุบันสูบ (ระยะเวลาที่สูบ……. ปี สูบบุหรี่……..มวนต่อวัน)		
4. ท่านดื่มเครื่องดื่มแอลกอฮอล์หรือไม่? ไม่ใช่ ใช่ (นานๆครั้ง เป็นประจำ)		
5. กรุณาระบุหากท่านได้ประสบอุบัติเหตุหรือรับการผ่าตัดในช่วง 6 เดือนที่ผ่านมา:		
6. กรุณาระบุยา,สมุนไพร หรืออาหารเสริมที่ท่านรับประทานอยู่ในปัจจุบัน		
ยาที่แพทย์สั่ง	ยาซื้อรับประทานเอง	
ประวัติสุขภาพ(กรุณาระบุระยะเวลาหลังโรคที่ท่านเป็น)		
เช่น โรคเบาหวาน โรคงูสวัด โรคเริม โรคการแข็งตัวของเลือดผิดปกติ โรคตับแข็ง โรคผื่น ชาบริเวณใบหน้า โรคซึมเศร้า		
อื่นๆ (โปรดระบุ):		
ประวัติการรักษาสิวและหลุมสิว		
1. อายุที่ท่านเริ่มมีสิว/หลุมสิว: น้อยกว่า10ปี 10-15ปี 15-20ปี 20-25ปี 25-30ปี 30-35ปี 35-40ปี มากกว่า40ปี		
2. ระยะเวลาที่ท่านมีสิว: น้อยกว่า1ปี 1-5ปี 5-10ปี 15-20ปี มากกว่า20ปี		
3. ระยะเวลาที่ท่านมีหลุมสิว: น้อยกว่า1ปี 1-5ปี 5-10ปี 15-20ปี มากกว่า20ปี		
4. หลุมสิวส่งผลกระทบต่อชีวิตประจำวัน: ไม่ส่งผล น้อยมาก น้อย ปานกลาง มาก		
5. ท่านเคยรักษาสิวด้วยการรับประทานยา: ไม่ใช่ ใช่ (โปรดระบุ):		
6. ท่านเคยรักษาสิวด้วยการทายา: ไม่ใช่ ใช่ (โปรดระบุ):		
7. ท่านเคยรักษาสิวด้วยหัตถการดังต่อไปนี้: Needling (การรักษาด้วยเข็มเล็ก) Punch techniques (การรักษาโดยการผ่าตัดหลุมสิว) Chemical peels/TCA (การรักษาด้วยการผลัดเซลล์ผิวด้วยน้ำยาเคมีและกรดไทรคลอโรอะซีติค) Laser(การรักษาโดยการเลเซอร์) Dermabrasion/Microdermabrasion(การรักษาด้วยการกรอผิว) Fillers(การรักษาโดยการใช้สารไฮยาลูรอนิคเอซิดเติมเต็ม)		
การดูแลผิวหน้า		
1. ท่านอยู่ภายใต้การดูแลของแพทย์เฉพาะทางผิวหนัง? ใช่ ไม่ใช่		
2. ท่านได้รับประทานยาที่เกี่ยวกับผิวหน้า: ไม่ใช่ ใช่ (โปรดระบุ):		
3. ท่านเคยทำหัตถการบนผิวหน้า: การลอกผิวด้วยสารเคมี การกรอผิว โบทอก เลเซอร์ อื่นๆ:		
4. ท่านได้ใช้ผลิตภัณฑ์ใดในต่อไปนี้ในปัจจุบันหรือไม่: กรดไกลโคลิค กรดแลคติก กรดไฮดรอกซี วิตามินเอ		
5. ท่านมีประวัติแพ้ยาหรือสารใดบนผิวหน้าหรือไม่? ไม่ใช่ ใช่ (โปรดระบุ):		
6. ลักษณะผิวของท่าน: ผิวมัน ผิวแห้ง ผิวแพ้ง่าย ผิวมีสิว ผิวมีผดผื่น ผิวขรุขระ/มีหลุมสิว		
7. ผลิตภัณฑ์ที่ท่านใช้: สบู่ล้างหน้า ครีม/โฟมล้างหน้า โลชั่นเช็ดผิว(โทนเนอร์) ครีมบำรุงผิว ครีมผลัดผิว ครีมกันแดด		
8. เป้าหมายผิวหน้าของคุณ?		

APPENDIX C
PATIENT'S RECORD FORM

แบบบันทึกข้อมูลสำหรับคนไข้ (PATIENT'S RECORD FORM)

รหัสคนไข้ _____

(ประเมิน ณ วันแรกที่เข้าร่วมโครงการ)

เกณฑ์พิจารณาการเข้าร่วมวิจัย

ท่านมีคุณสมบัติดังนี้หรือไม่ โปรดทำเครื่องหมาย / ในช่อง □ ที่ตรงกับท่าน

	ใช่	ไม่ใช่
1. อายุระหว่าง 20-40 ปี	□	□
2. เป็นแผลเป็น/หลุมสิวมานานกว่า 6 เดือน	□	□
3. ไม่ได้รับการรักษาแผลเป็นด้วยวิธีใดๆ มาอย่างน้อย 6 เดือน	□	□
4. ยินยอมปฏิบัติตามข้อกำหนดและคำแนะนำของแพทย์ตลอดระยะเวลาการวิจัย	□	□

คุณสมบัติต้องห้ามในการเข้าร่วมงานวิจัย

ท่านมีคุณสมบัติดังนี้หรือไม่ โปรดทำเครื่องหมาย / ในช่อง □ ที่ตรงกับท่าน	ใช่	ไม่ใช่
1 .ท่านมีการติดเชื้อ ผิวหนังอักเสบ ผื่นแพ้แสง หรือ โรคผิวหนังอื่นๆ บริเวณหลุมสิวที่จะทำการวิจัย	□	□
2. ท่านได้รับยารับประทานรักษาสิวเช่น Isotretinoin / Acnotin ในช่วง 6 เดือนที่ผ่านมา	□	□
3. ท่านได้รับการรักษาผิวหน้าด้วยเลเซอร์ในช่วง 4 เดือนที่ผ่านมา	□	□
4. ท่านมีประวัติเคยเป็นเริมหรืองูสวัด	□	□
5. ท่านแพ้ยาชาหรือยาทาที่มีสารประกอบของ Astaxanthin (แอสตาแซนธิน)	□	□
6. ท่านมีคนในครอบครัวมีแผลเป็นนูน (keloid)	□	□
7. ท่านมีโรคประจำตัว ต่อไปนี้ เบาหวาน, โรคเลือดและหลอดเลือด	□	□
8. ปัจจุบันท่านมีรับประทานยากดภูมิคุ้มกัน	□	□
9. ท่านอยู่ระหว่างการตั้งครรภ์หรือให้นมบุตร	□	□

APPENDIX D
GOODMAN AND BARON SCAR GRADING SYSTEM

แบบประเมินหลุมสิว

(Goodman and Baron Scar Grading System)

รหัสคนไข้ _____

Goodman and Baron Quantitative Scar Grading System

Grade or Type	Number of lesions 1 (1-10)	Number of lesions 2 (11-20)	Number of lesions 3 (>20)
Milder scarring (1 point each) Macular erythematous pigmented Mildly atrophic dish-like	1 point	2 points	3 point
Moderate scarring (2 points each) Moderately atrophic, dish like Punched out with shallow bases small cars (<5 mm) Shallow but broad atrophic areas	2 points	4 points	6 points
Severe scarring (3 points each) Punched out with deep but normal bases, small scars (<5 mm) Punched out with deep but abnormal bases, small scars (<5 mm) Linear or troughed dermal scarring Deep, broad atrophic areas	3 points	6 points	9 points
Hyperplastic Papular scars Keloidal/Hypertrophic scars	2 points Area<5 mm 6 points	4 points Area 5-20 mm2 12 points	6 points Area>20 cm2 18 points

Table 2: (b) Assessment of improvement using Goodman and Baron's quantitative acne scar grading system

Grades	Improvement status
0-5	Minimal reduction in GSGS scores
5-10	Moderate reduction in GSGS scores
10-15	Good reduction in GSGS scores
>15	Very good reduction in GSGS scores

Goodman and Baron Qualitative Scar Grading System

TABLE 1. Grades and Examples of Postacne Scarring

Grade	*Level of disease*	*Characteristics*	*Examples of scars*
1	Macular disease	Erythematous, hyper- or hypopigmented flat marks visible to patient or observer irrespective of distance.	Erythematous, hyper- or hypopigmented flat marks
2	Mild disease	Mild atrophy or hypertrophy that may not be obvious at social distances of 50 cm or greater and may be covered adequately by makeup or the normal shadow of shaved beard hair in males or normal body hair if extrafacial.	Mild rolling, small soft papular
3	Moderate disease	Moderate atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in males or body hair if extrafacial, but is still able to be flattened by manual stretching of the skin.	More significant rolling, shallow ''box car,'' mild to moderate hypertrophic or papular scars
4	Severe disease	Severe atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in males or body hair (if extrafacial) and is not able to be flattened by manual stretching of the skin.	Punched out atrophic (deep ''box car''), ''ice pick'', bridges and tunnels, gross atrophy, dystrophic scars significant hypertrophy or keloid

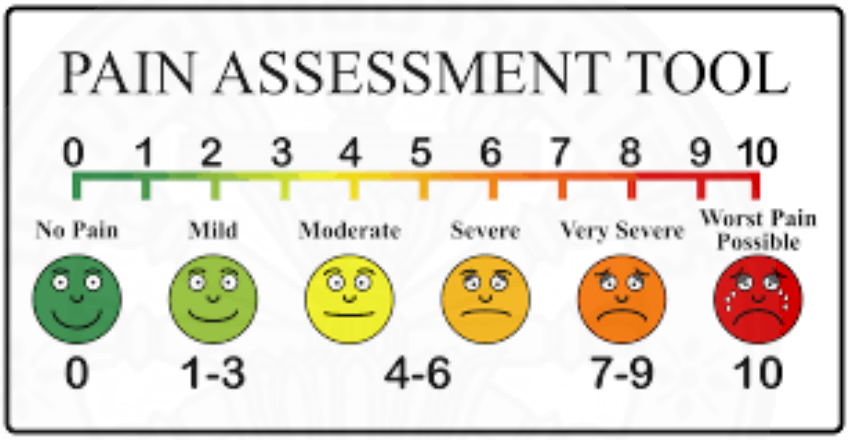
APPENDIX E
PATIENT'S UNIVERSAL PAIN ASSESSMENT TOOL

แบบประเมินระดับความเจ็บปวดหลังการทำเลเซอร์

(PATIENT'S UNIVERSAL PAIN ASSESSMENT TOOL)

รหัสคนไข้ _____

กรุณาประเมินความเจ็บปวดระหว่างการทำเลเซอร์รักษาหลุมสิวจาก 0 **ถึง** 10 **คะแนน โดยที่** 0 **คือไม่เจ็บปวดเลย และ** 10 **คือเจ็บปวดมากที่สุดในชีวิต**



APPENDIX F
PATIENT'S LASER SAFETY EVALUATION FORM

<u>แบบสอบถามผลข้างเคียงจากผู้ป่วยหลังการทำเลเซอร์</u>

<u>(PATIENT'S LASER SAFETY EVALUATION FORM)</u>

รหัสคนไข้ _____

ผลข้างเคียงใบหน้าครึ่งขวา

☐ ไม่มี ☐ มี โปรดระบุ ☐ แสบร้อน ☐ ผิวหนังบวม ☐ ผิวหนังแดง ☐ มีตุ่มน้ำ
☐ มีรอยไหม้ ☐ มีรอยดำคล้ำ ☐ ผิวลอก
☐ อื่นๆ โปรดระบุ .....

ผลข้างเคียงใบหน้าครึ่งซ้าย

☐ ไม่มี ☐ มี โปรดระบุ ☐ แสบร้อน ☐ ผิวหนังบวม ☐ ผิวหนังแดง ☐ มีตุ่มน้ำ
☐ มีรอยไหม้ ☐ มีรอยดำคล้ำ ☐ ผิวลอก
☐ อื่นๆ โปรดระบุ .....

APPENDIX G
PHYSICIAN'S LASER SAFETY EVALUATION FORM

แบบสอบถามผลข้างเคียงจากผู้สำรวจหลังการทำเลเซอร์

(PHYSICIAN'S LASER SAFETY EVALUATION FORM)

รหัสคนไข้ _____

ผลข้างเคียงใบหน้าครึ่งขวา

Erythema	None	0	Edema	None	0
	Trace	1		Trace	1
	Mild	2		Mild	2
	Moderate	3		Moderate	3
	Severe	4		Severe	4
Dyschromia	None	0	Petechiae	Yes	No
	Trace	1	Oozing	Yes	No
	Mild	2	Crusting	Yes	No
	Moderate	3	Rash	Yes	No
	Severe	4	PIH	Yes	No

ผลข้างเคียงใบหน้าครึ่งซ้าย

Erythema	None	0	Edema	None	0
	Trace	1		Trace	1
	Mild	2		Mild	2
	Moderate	3		Moderate	3
	Severe	4		Severe	4
Dyschromia	None	0	Petechiae	Yes	No
	Trace	1	Oozing	Yes	No
	Mild	2	Crusting	Yes	No
	Moderate	3	Rash	Yes	No
	Severe	4	PIH	Yes	No

APPENDIX H
PATIENT'S SATISFACTION FORM
SCARS PATIENT REPORTED OUTCOME TOOL I

แบบสำรวจความพึงพอใจของคนไข้ (PATIENT'S SATISFACTION FORM)

SCARS patient reported outcome tool

รหัสคนไข้ _____

แบบประเมินหลุมสิว **SCARS** ส่วนที่**1:**

หมายเหตุ**: 1.**กรุณาล้างเครื่องสำอาง ถอดเครื่องประดับ และ มัดผมก่อนทำแบบสอบถามนี้

2.แบบสอบถามด้านล่างจะทำให้ท่านเข้าใจข้อแตกต่างระหว่างสิวและหลุมสิว

คำถามต่อไปนี้จะช่วยให้ท่านแยกระหว่างหลุมสิวกับสิว

ก. -สิว รวมถึง สิว สิวเสี้ยน สิวอุดตัน **(zits, breakouts, pimples, whiteheads and blackheads)**

-เมื่อคุณมองหน้าตัวเองในกระจกตอนนี้คุณเห็น"สิว"บนใบหน้าของคุณ**?**

ใช่ / ไม่ **(**โปรดวงกลม**1**คำตอบ**)**

-ถ้าใช่ โปรดให้คะแนนความรุนแรงของปริมาณสิวบนใบหน้าโดยการใส่เส้นตรงลงบริเวณแถบประเมินด้านล่าง

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ไม่มีสิว สิวรุนแรง

คำถามต่อไปนี้จะถามเกี่ยวกับหลุมสิวเท่านั้น "**ไม่**" รวมถึงสิวเสี้ยน สิวอุดตัน รอยดำและรอยแดง

ข. หลุมสิว คือ บริเวณที่มีการยุบตัวของผิวหนังที่ก่อนหน้านี้เคยมีสิวมาก่อน

(ไม่ร่วมถึงแผลเป็นจากอุบัติเหตุ การแคะ หรือ เกา**)**

เมื่อคุณมองหน้าตัวเองในกระจกตอนนี้คุณเห็น"หลุมสิว"ที่เกิดจากสิวบนใบหน้าของคุณ**?**

ใช่ / ไม่ **(**โปรดวงกลม**1**คำตอบ**)**

-ถ้าไม่ใช่ คุณได้ทำแบบสอบถามชุดนี้จบแล้ว ขอบคุณที่สละเวลา

-ถ้าใช่ โปรดให้คะแนนความรุนแรงของปริมาณหลุมสิวบนใบหน้าโดยการใส่เส้นตรงลงบริเวณแถบประเมินด้านล่าง

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ไม่มีหลุมสิว หลุมสิวรุนแรง

APPENDIX I
PATIENT'S SATISFACTION FORM
SCARS PATIENT REPORTED OUTCOME TOOL II

แบบสำรวจความพึงพอใจของคนไข้ (PATIENT'S SATISFACTION FORM)

SCARS patient reported outcome tool

แบบประเมินหลุมสิว **SCARS** ส่วนที่**2:** รหัสคนไข้ _____

หมายเหตุ**: 1.**การทำแบบสอบถามนี้ ให้พิจารณาเฉพาะ แผลเป็นชนิดหลุมสิว เท่านั้น

2.แบบสอบถามนี้**"**ไม่**"**รวมถึงสิว สิวเสี้ยน สิวอุดตัน รอยดำและรอยแดง

กรุณา กากบาท ในกล่องสี่เหลี่ยม ที่อธิบายถึงหลุมสิวบนใบหน้าของคุณได้ดีที่สุด ณ ปัจจุบัน

1. เมื่อคุณส่องกระจกตอนนี้ ใบหน้าของคุณมีหลุมสิวเป็นบริเวณเท่าใดของใบหน้าคุณ

หน้า	น้อยมาก (0-20%)	น้อย (20-40%)	ปานกลาง (40-60%)	มาก (60-80%)	มากที่สุด (>80%)
ขวา					
ซ้าย					

2. เมื่อคุณส่องกระจกตอนนี้ คุณรู้สึกว่าหลุมสิวในแต่ละหลุมมีขนาดเล็กหรือใหญ่เพียงใด

หน้า	เล็กมาก (0-20%)	เล็ก (20-40%)	ปานกลาง (40-60%)	ใหญ่ (60-80%)	ใหญ่มาก (>80%)
ขวา					
ซ้าย					

3.เมื่อคุณส่องกระจกตอนนี้ คุณเห็นหลุมสิวมากน้อยเพียงใด

หน้า	น้อยมาก (0-20%)	น้อย (20-40%)	ปานกลาง (40-60%)	มาก (60-80%)	มากที่สุด (>80%)
ขวา					
ซ้าย					

4. เมื่อคุณส่องกระจกตอนนี้ หลุมสิวของคุณมีความลึกมากเพียงใด

หน้า	ตื้นมาก (0-20%)	ตื้น (20-40%)	ปานกลาง (40-60%)	ลึก (60-80%)	ลึกมาก (>80%)
ขวา					
ซ้าย					

5. เมื่อคุณส่องกระจกตอนนี้ มีหลุมสิวที่มองเห็นได้ชัดเจนมากน้อยเพียงใด

หน้า	ไม่เห็น (0-20%)	เห็นน้อย (20-40%)	เห็นปานกลาง (40-60%)	เห็นชัดเจน (60-80%)	เห็นชัดเจนมาก (>80%)
ขวา					
ซ้าย					

APPENDIX J
PATIENT'S SATISFACTION FORM
FACIAL ACNE SCAR QUALITY OF LIFE(FASQoL)

<u>แบบสำรวจความพึงพอใจของคนไข้</u> (PATIENT'S SATISFACTION FORM)

<u>Facial acne scar quality of life (FASQoL)</u>

รหัสคนไข้ _____

<u>แบบประเมินหลุมสิวส่วนที่**2:**</u>

หมายเหตุ**: 1.**แบบสอบถามเกี่ยวกับแผลเป็นชนิดหลุมสิวมีผลอย่างไรกับชีวิตประจำวัน (โดยต้องเกิดจากการเป็นสิวมาก่อน)

2.การทำแบบสอบถามนี้ ให้พิจารณาเฉพาะ แผลเป็นชนิดหลุมสิว เท่านั้น

กรุณา กากบาท ในกล่องสี่เหลี่ยม ที่อธิบายถึงผลกระทบจากแผลเป็นชนิดหลุมสิวต่อตัวคุณได้ดีที่สุด ในช่วง7วันที่ผ่านมา

1. ในช่วง 7 วันที่ผ่านมา คุณรู้สึกประหม่าเวลาพบปะผู้อื่น เนื่องจากหลุมสิวบนใบหน้าของคุณ

หน้า	ไม่รู้สึก (0-20%)	รู้สึกเล็กน้อย (20-40%)	รู้สึกปานกลาง (40-60%)	รู้สึกมาก (60-80%)	รู้สึกมากที่สุด (>80%)
ขวา					
ซ้าย					

2. ในช่วง 7 วันที่ผ่านมา คุณรู้สึกมีเสน่ห์ลดลง เนื่องจากหลุมสิวบนใบหน้าของคุณ

หน้า	ไม่รู้สึก (0-20%)	รู้สึกเล็กน้อย (20-40%)	รู้สึกปานกลาง (40-60%)	รู้สึกมาก (60-80%)	รู้สึกมากที่สุด (>80%)
ขวา					
ซ้าย					

3. ในช่วง 7 วันที่ผ่านมา คุณรู้สึกรำคาญ เนื่องจากหลุมสิวบนใบหน้าของคุณ

หน้า	ไม่รู้สึก (0-20%)	รู้สึกเล็กน้อย (20-40%)	รู้สึกปานกลาง (40-60%)	รู้สึกมาก (60-80%)	รู้สึกมากที่สุด (>80%)
ขวา					
ซ้าย					

4. ในช่วง 7 วันที่ผ่านมา คุณรู้สึกกังวล เนื่องจากหลุมสิวบนใบหน้าของคุณ

หน้า	ไม่รู้สึก (0-20%)	รู้สึกเล็กน้อย (20-40%)	รู้สึกปานกลาง (40-60%)	รู้สึกมาก (60-80%)	รู้สึกมากที่สุด (>80%)
ขวา					
ซ้าย					

5. ในช่วง 7 วันที่ผ่านมา คุณรู้สึกเศร้า เนื่องจากหลุมสิวบนใบหน้าของคุณ

หน้า	ไม่รู้สึก (0-20%)	รู้สึกเล็กน้อย (20-40%)	รู้สึกปานกลาง (40-60%)	รู้สึกมาก (60-80%)	รู้สึกมากที่สุด (>80%)
ขวา					
ซ้าย					

6. ในช่วง 7 วันที่ผ่านมา คุณรู้สึกไม่พอใจจากคำวิจารณ์แง่ลบของผู้อื่น เนื่องจากหลุมสิวบนใบหน้าของคุณ

หน้า	ไม่รู้สึก (0-20%)	รู้สึกเล็กน้อย (20-40%)	รู้สึกปานกลาง (40-60%)	รู้สึกมาก (60-80%)	รู้สึกมากที่สุด (>80%)
ขวา					
ซ้าย					

7. ในช่วง 7 วันที่ผ่านมา คุณหลีกเลี่ยงจะไปพบผู้อื่น เนื่องจากหลุมสิวบนใบหน้าของคุณ

หน้า	ไม่รู้สึก (0-20%)	รู้สึกเล็กน้อย (20-40%)	รู้สึกปานกลาง (40-60%)	รู้สึกมาก (60-80%)	รู้สึกมากที่สุด (>80%)
ขวา					
ซ้าย					

8. ในช่วง 7 วันที่ผ่านมา คุณรู้สึกรำคาญในการต้องปกปิดหลุมสิวบนใบหน้าของคุณ

หน้า	ไม่รู้สึก (0-20%)	รู้สึกเล็กน้อย (20-40%)	รู้สึกปานกลาง (40-60%)	รู้สึกมาก (60-80%)	รู้สึกมากที่สุด (>80%)
ขวา					
ซ้าย					

9. ในช่วง 7 วันที่ผ่านมา หลุมสิวบนใบหน้าของคุณส่งผลต่อความสัมพันธ์ของคุณและผู้อื่น เช่น เพื่อน ครอบครัว หรือแฟน

หน้า	ไม่รู้สึก (0-20%)	รู้สึกเล็กน้อย (20-40%)	รู้สึกปานกลาง (40-60%)	รู้สึกมาก (60-80%)	รู้สึกมากที่สุด (>80%)
ขวา					
ซ้าย					

10. ในช่วง 7 วันที่ผ่านมา หลุมสิวบนใบหน้าของคุณส่งผลต่อการทำงานหรือการเรียน

หน้า	ไม่รู้สึก (0-20%)	รู้สึกเล็กน้อย (20-40%)	รู้สึกปานกลาง (40-60%)	รู้สึกมาก (60-80%)	รู้สึกมากที่สุด (>80%)
ขวา					
ซ้าย					

BIOGRAPHY

Name	Miss Chanya Sueverachai
Date of Birth	March 20, 1992
Educational Attainment	Academic Year 2010: Doctor of Medicine, Ramathibodi hospital, Mahidol University, Thailand Academic Year 2017: Master of Science (Dermatology), Chulabhorn International College of Medicine, Thammasat University, Thailand
Work Experiences	General Practice at Yasothon hospital