THE POTENTIAL ROLE OF PLATELET-RICH PLASMA IN MELASMA TREATMENT

BY

MISS ARADA DANNARONGCHAI

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

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ENTITLED

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

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ABSTRACT

Background : Melasma is an acquired hyperpigmented skin disorder commonly found in Thailand. Despite several treatments for melasma, the results are variable success with certain complications.

Objective : This study aimed to assess the effectiveness of Platelet-Rich Plasma (PRP) in treatment of melasma.

Methods : Ten female patients with bilateral mixed-type melasma were enrolled in a split-faced, single-blinded prospective trial. PRP was randomly injected intradermally to one side of the face and normal saline to the other side every 2 weeks for 4 times. All patients were instructed to use only the specific moisturizer and cleanser with SPF50 sunscreen applying to both sides of the face in the morning. PRP was prepared by the centrifugation of 13.5 ml collected-blood in YCELLBIO Kit. Then, the injection of PRP 0.1ml/cm2 was performed to the entire melasma area on one side of the face. The subjects were afterwards examined by Mexameter, Modified MASI score, and Antera® 3D Analysis equipment. Patients' self-improvement score was assessed at baseline, 2nd, 4th, 6th week, and 1-month follow-up after the complete treatment protocol.

Results : mMASI score of PRP group was significantly changed from baseline to week 10 compared with that of control group, with the mean of 1.03 ± 0.44 (P = 0.042*). Meanwhile, the improved patients' self-assessment score from baseline was observed at week 2,4,6 and 10 with statistical significance. Though, a significant difference between both regimens did not reveal significant with regard to objective assessments, there are some trends in more reducing pigmentation from PRP than control.

Overall, the side-effects were mild and resolved spontaneously within a few days after the onset of symptoms.

Conclusions : Hence, the intradermal (ID) PRP could be an adjuvant therapy for melasma. However, larger and longer randomized, double-blinded, placebo-controlled trials are recommended for long-term efficacy and safety.

Keywords: Melasma, Melasma Treatment, Platelet-Rich Plasma (PRP)

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Miss Arada Dannarongchai

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
ACTH	Adrenocorticotrophic hormone
bFGF	Basic fibroblast growth factor
CBP	CREB-binding protein
CFTR	Cystic fibrosis transmembrane conductance regulator
CTGF	Connective tissue growth factor
CREB	cAMP responsive-element-binding protein
DCT	Dopachrome tautomerase
ECM	Extracellular matrix
EDN-1	Endothelin 1
EDNRB	Endothelin B receptor
EGF	Epidermal growth factor
ER	Estrogen receptor
Er:YAG	Erbium: Yttrium aluminum garnet
ERK	Extracellular signal-regulated kinase
ET-1	Endothelin-1
FGF	Fibroblast growth factor
GF	Growth factor
hr	Hour(s)
ID	Intradermal
IGF-1	Insulin-like growth factor 1
IL-1	Interleukin-1
IPL	Intense pulse light
LCM	Laser-treated keratinocyte-conditioned culture media
LEF-1	lymphocyte enhancer binding factor 1
MASI score	Melasma Area and Severity Index score
MC1R	Melanocortin-1 receptor

LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
MITF	Microphthalmia-associated transcription factor
mSCF	Membrane-bound stem cell factor
MSH	Melanocyte-Stimulating Hormone
NFAT	Nuclear factor of activated T cells
NGF	Nerve growth factor
NHE	Sodium–hydrogen exchanger
NO	Nitric Oxide
PAX3	Paired-box 3
PDGF	Platelet derived growth factor
PDL	Pulsed dye laser
PDZK1	PDZ domain protein kidney 1
PGE2	Prostaglandin E2
PIH	Post-inflammatory hyperpigmentation
PKA	Protein kinase A
PKC	Protein kinase C
PRP	Platelet-Rich Plasma
QS Nd: YAG	Q switched neodymium: Yttrium aluminium garnet
SCF	Stem cell factor
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TRP	Tyrosinase-related protein
TYK	Tyrosinase
TYR	Tyrosine kinase
UV	Ultraviolet
VEGF	Vascular endothelial growth factor

WB	Whole blood
WIF-1	Wnt inhibitory factor
μL	Microliter

CHAPTER 1
INTRODUCTION

1.1 Background and rationale

Melasma is an acquired pigmentary disorder characterized by symmetrical hyperpigmented macules and patches in the sun exposed area of the face, such as forehead, cheeks, lips, and nose. It is commonly found especially in women and people with darker skin (Fitzpatrick type IV-VI). Despite its still fully unknown pathogenesis, the common risk factors of melasma include genetic factor, UV light exposure, pregnancy, oral contraceptives, and drugs such as phenytoin.

Most recently, there have been evidences that dermal factors including increased number of mast cell, vascular growth factor (VEGF), solar elastosis, and basement membrane disruption could be involved in the pathogenesis of melasma (1). Besides, histopathological findings show an increased deposition of melanin and number of melanocytes (2).

Following several treatments for melasma such as topical agents, chemical peels, laser and light therapies, the treatment results are variable success with complications such as irritation, post-inflammatory hyperpigmentation (PIH), and rebound hyperpigmentation. Nonetheless, PRP is becoming to get attention in aesthetic medicine.

PRP is a plasma containing higher-than-normal platelet concentration, prepared by whole blood centrifugation (27). In alpha granules of platelets, there is an abundance of cytokines and growth factors affection to wound healing, collagen production and control homeostasis (21).

Hence, we are interested in PRP injection as an alternative melasma treatment due to the studies demonstrating that PRP injection could reduce the hyperpigmented lesions including melasma (18-20).

1.2 Research question

Our research question is whether the intradermal injection PRP could be efficacious for the reduction of hyperpigmented melasma, and if the efficacy of treatment is associated with the level of growth factor TGF-beta1.

1.3 Specific objective

Primary objective

- To evaluate the efficacy and safety of PRP in melisma treatment which is measured by mexameter and modified MASI score

Secondary objectives

- To identify the side effects of intradermal injections of PRP on melasma lesions.
- To analyze Patients' self- improvement score.
- To assess skin quality by Antera®.

1.4 Hypothesis

PRP could be a promising treatment with less side effects and rebound hyperpigmentation than other melasma treatments.

1.5 Keywords

Melasma
Melasma Treatment
Platelet-Rich Plasma (PRP)

1.6 Ethical consideration

The study protocol was approved by Thammasat University's Ethical Committee

1.7 Limitation

The limitation of this study was number of sample size.

1.8 Expected benefits and application

Melasma is commonly found in Thai women, with several alternative treatments for the incompletely cured melasma due to the impacts on self confidence and social quality of life. Thus, our study is expected to yield the efficacious outcome of PRP as an additional therapy of choice for melasma treatment with reduced side effects and rebound hyperpigmentation.

Table 1.1 Administration and time schedule.

	2016							2017					
	Jun	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Research proposal	■	■	■	■	■								
Research ethics			■	■	■	■							
Data collection						■	■	■	■				
Data analysis							■	■	■	■	■		
Manuscript preparation									■	■	■	■	
Thesis paperwork										■	■	■	■

CHAPTER 2
REVIEW OF LITERATURE

2.1 What is melasma ?

Melasma is an acquired pigmentary disorder commonly found especially in women and those having darker skin (Fitzpatrick type IV-VI), with symmetrical hyperpigmented macules and patches in the sun exposed area of the face such as forehead, cheeks, lips, and nose. Despite unknown pathogenesis, the common risk factors of melasma include genetic factors, UV light exposure, pregnancy, oral contraceptives, and drugs such as phenytoin (1-4).

2.1.1 Types of melasma

There are three types of melasma based on histopathological findings: 1) The epidermal type, with brown color and well-margin, characterized by increased melanin deposition in basal and suprabasal of epidermis; 2) The dermal type, with blue-gray color and fade margin, identified by increased melanophages in superficial and mid-dermis; and 3) The mixed type, marked by melanin deposition in both epidermis and dermis.

However, only the epidermal type of melasma is emphasized by Wood's light examination, with specifically best treatment response. Meanwhile, the mixed and the dermal types are respectively noted with partial and poor treatment responses.

2.1.2 Pathogenesis of melasma

Genetic factors, persistent UV exposure, and female hormones are main causes of melasma. In the meantime, inflammation could also be considered in melasma development (5,6)

2.1.2.1 Genetic factors

There have been no reports on the association of genetic polymorphism and melasma. Racial and positive family history are reported relating the melasma incidence.

For instance, Latin Americans and Hispanics and Asians with Fitzpatrick skin type III–V are likely to have pigmentary disorders such as melasma and PIH (5).

2.1.2.2 UV exposure

Exposing the sun for a long time and the area that easily exposed are the factors leading to the severity of melasma.

UV exposure stimulates melanogenesis with direct and indirect induces melanogenic factors releasing from melanocytes and keratinocytes. The direct effect of UV irradiation causes the formation of Endogenous 1,2-diacylglycerols' (DAGs) with protein kinase C-beta activation and nitric oxide (NO) production following cyclic guanylate monophosphate synthesis. Meanwhile, Keratinocyte-derived melanogenic factors such as ACTH, MSH, ET-1 indirectly affected by UV irradiation.

UV-induced melanogenesis is induced by the interaction of many paracrine secretion including POMC-derived peptides, ET-1 and stem cell factor (SCF).

The blinding of melanocortin to the MC-1 receptor acting as an MSH receptor, leads to the melanogenesis via cyclic AMP pathway and then activates protein kinase A (PKA) and MITF, especially MITF-M.

Moreover, keratinocytes also produce NO and cause the melanogenic effect after the UV radiation. Additionally, dermal fibroblasts release SCF, as direct exposure to UV, suggesting the communication between melanocytes and fibroblasts in UV-induced hyperpigmentation (5,6).

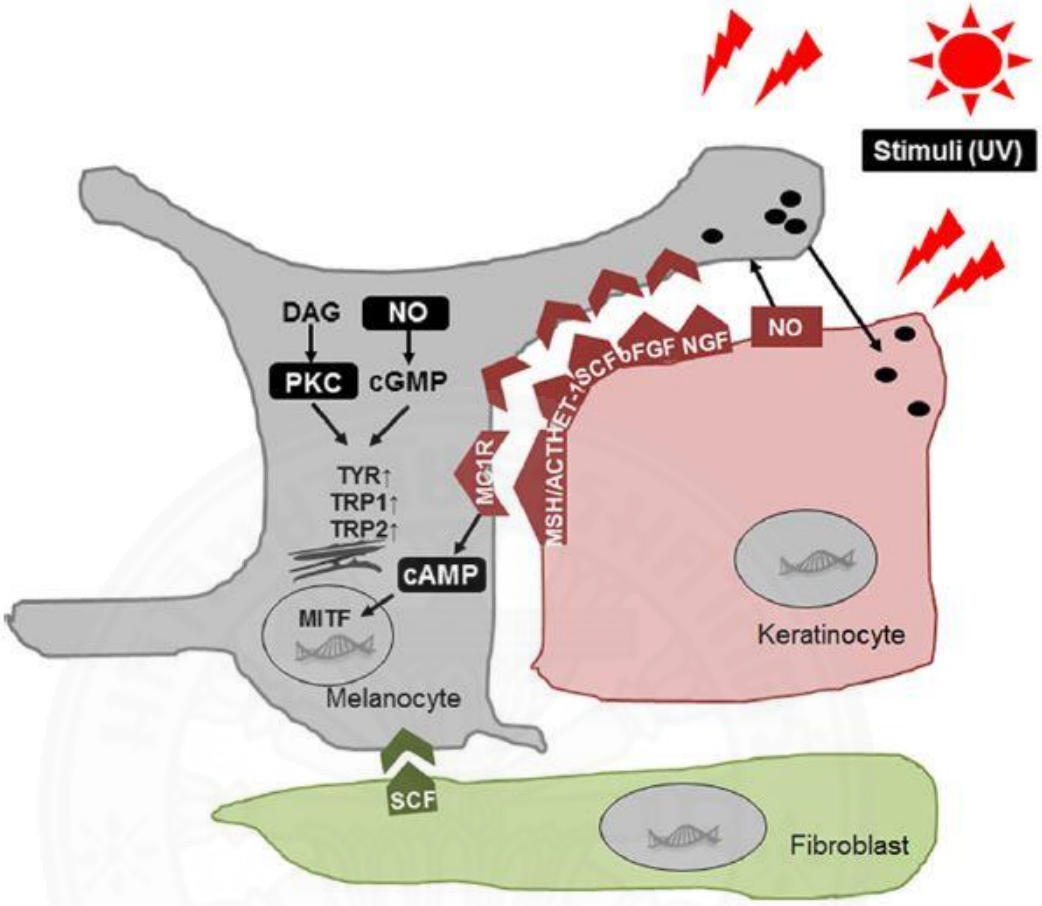


Figure 2.1 UV-induced melanogenesis pathway.

In melasma, DNA damages are however unspecified from the direct effect of UV photons or DAG/arachidonic acid pathways released from melanocyte membranes. In the meantime, both melanocytes and keratinocytes are considerably melanogenic mechanisms in melasma, similar to UV-induced melanogenesis.

The effect of some factors on onset and chronicity of melasma was surveyed in 324 women by Ortonne J et al. They reported risk of onset during pregnancy was associated with having spent more time outdoors. Post-pregnancy melasma significantly related to darker skin type. Most of the patients had a family history of melasma. A combination of these factors often triggers this disorder. The result showed a combination of genetic factor, Flitpatrick skin type and UV exposure

often triggers this disorder (7).

2.1.2.3 Female sex hormones

Besides differences in the prevalence of melasma among various ethnic groups and skin phototypes, the association of melasma and oral contraceptives during the women's reproductive lifespan can also be suggestive as another important factor affecting the prognosis and severity of melasma development. On the other hand, there is disagreement concerning the least effect of hormones on melasma.

Likewise, the increased estrogen receptor (ER) expression is revealed in the affected skin. Significantly, the immunohistochemical expression of ER-beta could only be found in the dermal melasma. By the binding of estrogen and ER, the induction of melanogenesis is occurred.

In addition, This event could be related to cAMP-PKA activation and melanogenic factors upregulation (5,6)(Fig. 2A).

PDZ domain protein kidney1 (PDZK1) expression is upregulated in lesional melasma skin which is detected by immunohistochemical and real-time PCR technique.

The PDZK1, a member of the sodium-hydrogen exchanger regulatory factor (NHERF), is founded overexpressed in melanocyte monocultures resulting in the overexpression of CREB, MITF and tyrosinase.

As a member of NHERF, PDZK1 interacts with ion exchangers such as NHE, cystic fibrosis transmembrane conductance regulator (CFTR) and SLC26A family with linking between ER- alpha and NHE3 or ER-beta and CFTR.

With the mediation of NHERF, PDZK1 could thus facilitate the estrogen interactions with other proteins including ion exchangers, resulting in the stimulation of melanogenesis and melanosome transfer in melasma patients (Figure 2B).

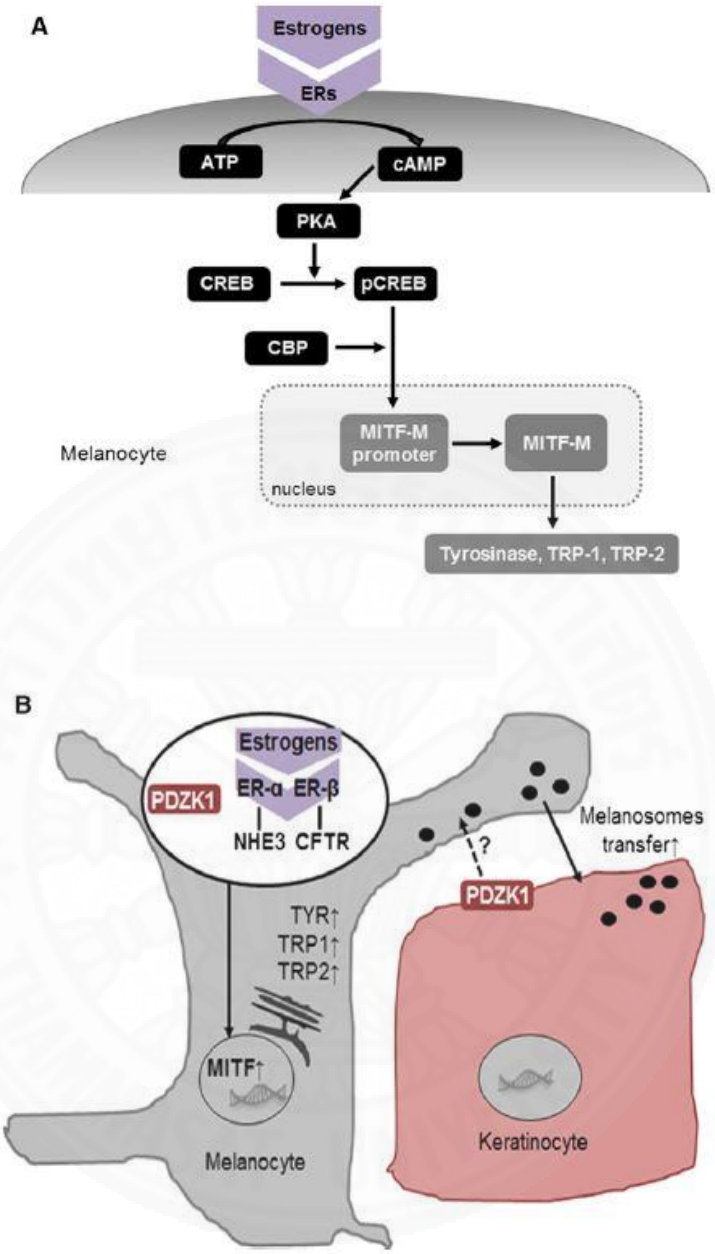


Figure 2.2 Estrogen-induced melanogenesis (A)
and role of PDZK1 in melanogesis (B)

2.1.2.4 Dermal factors

(1) Increased number of mast cell

Various studied showed even though the number of mast cells are increased in melasma lesions, mast cells do not have the exact role in melanogenesis.

(2) Role of WIF-1 in fibroblasts in melanogenesis

Chronic sunlight exposure results in the SCF secretion from fibroblasts increasing pigmentation afterwards.

According to the immunohistochemistry data from melasma patients, a role of increased SCF is suggestive in the dermis with increased c-KIT in the epidermis in pigmentation. Meanwhile, a role of reduced Wnt inhibitory factor-1 (WIF-1) expression involed the activation of melanogenesis and melanosome transfer (6,8).

WIF-1 inhibits both the canonical and the non-canonical Wnt pathways. WIF-1 is expressed in both cultured normal human keratinocytes and fibroblasts, but not in melanocytes. The decreased expression of WIF-1 in keratinocytes and fibroblasts reduces the binding of WIF-1 to Wnts in melanocytes. This exerting action results in the MITF upregulation and the translocation of nuclear factor of activated T cells (NFAT) to nucleus through the canonical Wnt/beta-catenin and the non-canonical pathways. (Figure 2.3)

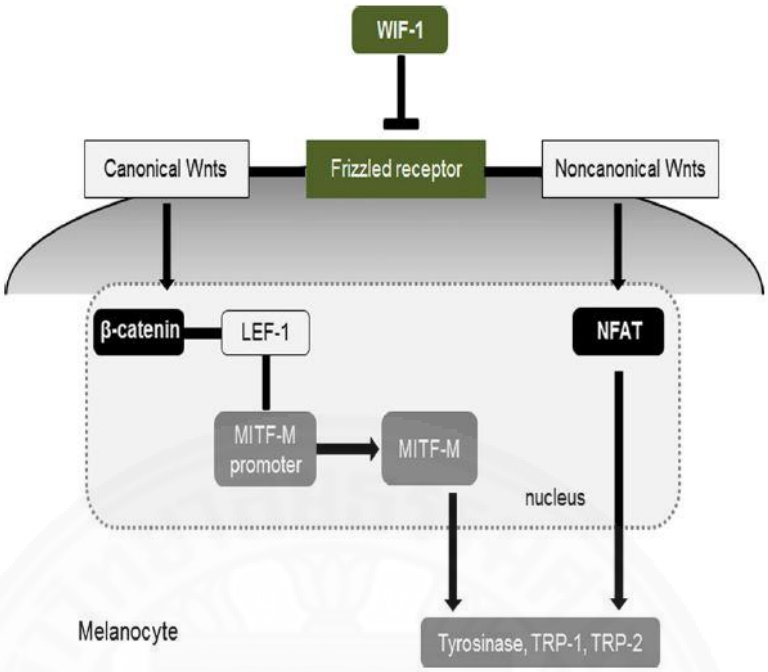


Figure 2.3 WIF-1 inhibits melanogenesis.

(3) Increased vascularization

Altered dermal vasculature has been found in both melasma and UV-damaged skin. Moreover, blood vessel and vascular endothelial growth factor (VEGF) are increased in dermal melasma. However, the VEGF role in melanogenesis is still not clear.

(4) Solar elastosis

Abnormalities of extracellular matrix are commonly observed in dermal melasma. The accumulation of abnormal elastic tissued is observed in the sun-exposed skin and also known as solar elastosis.

Despite the variations in the higher level of solar elastosis, it is still suggestive that photoaging plays a crucial role in melasma development.

After UVB exposure, the secretion of SCF, bFGF interleukin-1, endothelin-1, inducible nitric oxide synthase, MSH and PGE2 by keratinocytes is occurred. This event may not only induce dermal pigment but also epidermal hyperpigmemtation.

(5) Basement membrane disruption

This abnormality has been reported in many studies. This could be the consequence of long time UV exposure leading to the occurrence of melasma.

During chronic UV irradiation, the expression of collagen degradating proteins (MMP-2 and MMP-9) is upregulated. This event leads to basement membrane disruption and facilitates the descent of melanocytes and melanin into the dermis (5,6). Refractory melasma incidence could be explained by this theory.

2.1.2.5 MicroRNAs and their targets

Tyrosinase and melanogenic factors are less activated when miR-675 expression is increased. Whilst, miR-675 expression is reduced in lesional melasma skin. miR-675 releasing from keratinocytes to melanocytes via exosomes.

2.2 Treatments of melasma

2.2.1 Avoidance of exacerbating factors

This is very essential way to improve melasma. The patients should avoid exacerbating factors including oral contraceptive drugs, melasma-induced drugs and sun exposure.

2.2.2 Photoprotection

Patients should avoid the sun by using at least SPF30 sunscreen and reapply every 2 h. There are many evidences shown in studies that visible and UV light can induce skin pigment in all skin types.

The study of Lakhdar H et al. demonstrated that the percentage of women who developed chloasma had been decreased by the application of SPF50 sunscreen every 2 h at day time.

Another study found that pigmentation induced by VL was darker and more sustained than that induce by UVA-1. The group that used UV-VL sunscreen showed 28% greater improvements than the UV-only group in colorimetric values in one study (9).

2.2.3 Topical treatments

2.2.3.1 Hydroquinone

Hydroquinone is a hydroxyphenolic chemical that inhibits the conversion of DOPA to melanin by inhibiting tyrosinase. It has been commonly used to treat melasma for decades. It is commonly used in 2-5% and the better improvement will be shown when used with other whitening agents such as tretinoin, topical steroids and kojic acid. Adverse effects of hydroquinone include irritation, erythema, stinging, irritant or allergic contact dermatitis, milia, PIH and exogenous ochronosis.

Histological characteristic of exogenous chronosis is banana-shaped deposits in the dermis and clinical characteristic is bluish black discoloration in the treated areas of melasma. It is mainly reported when using in high concentration hydroquinone by dark skin patients.

SBP Ennes and colleagues studied the efficacy and tolerability of hydroquinone 4% cream with sunscreens, compared with placebo. A total of 48 patients were randomly chosen into two groups. Group A was treated with only hydroquinone 4%, Group B with placebo. Both groups were instructed to use and apply additional sunscreen. Photographs was taken every 3 weeks for 4 times. The result showed 30% better improvement of group A than placebo group (10).

2.2.3.2 Retinoids

Various topical retinoids have proven to be efficacious in melasma therapy. Its mechanisms of action involes skin lightening by decreasing melanosome transfer, inhibiting tyrosinase transcription, and interrupting the synthesis of melanin.

Topical tretinoins are commonly used as combination therapy in order to reduce their side effects such as erythema and desquamation.

Another study showed that 41 % reduction of MASI score in the 0.1% adapalene group compared with 37% in the 0.05% tretinoin group. The adverse effects of 0.1% adapalene was less than 0.05% tretinoin group.

2.2.3.3 Tranexamic acid

Tranexamic acid, a plasmin inhibitor, has recently obtained in popularity in melasma treatment. Moreover, it has been reported to show a decrease in hyperpigmentation effect (2)**.** The Split-faced studies by Laothaworn V have shown a

reduction in melasma measured by mexameter at baseline, 2nd, 4th and 8th week in both 1064 nm QS Nd: YAG laser plus topical tranexamic acid side and 1064 nm QS Nd: YAG laser alone side. Though, both regimens did not reveal significant difference results (11).

2.2.3.4 Kojic acid

Kojic acid reduced hyperpigmentation by inhibiting tyrosinase enzymes. The variable results have been shown in using kojic acid.

When 2% kojic acid in a gel containing 10% glycolic acid and 2% HQ was compared with the same application but without kojic acid in one split-face study, a 60% and 47.5% reduction in clinical evaluation score. Redness, stinging, and exfoliation were found in both groups.

2.2.3.5 Combinations

(1) Kligman 's Formula

The Kligman–Willis formula is 5% hydroquinone, 0.1% tretinoin and 0.1% dexamethasone combination. It was developed for over 30 years to reduce the hyperpigmentation by applying twice daily for three weeks. The benefits of this regimen are irritancy reduction, effect lengthening, HQ oxidation prevention and penetration improvement.

(2) Triple combination cream

Triple Combination Cream (TCC) is the well-known combination therapy in the world containing 4% hydroquinone, 0.05% tretinoin, and 0.01% fluocinolone acetonide (3).

Ferreira Cestari T et al. performed a study to assess the efficacy and safety of a TCC, compared to topical hydroquinone in patients with moderate to severe facial melasma (12).120 subjects were randomized to receive TCC once daily or HQ cream twice daily for 8 weeks. Improvement of more than 75% of global severity score was achieved by 73% of TC cream patients and less than half of HQ cream patients. Side effects seen were erythema, burning sensation, and desquamation that occured in both groups. No patient dropped out of the study because of drug-related adverse events. TC cream was more effective than the HQ cream for the treatment of moderate to severe facial melasma. Both products had similar safety profiles.

2.2.4 Chemical peels

2.2.4.1 Glycolic acid (GA)

GA is alpha hydroxyl acid which is most commonly used. GA with 20-70% concentrations have been used in many studies (13). A split-faced study shown a same improvement in melasma when using 20-70% GA compared with topical hydroquinone. In another study, 70% GA peels found no statistical difference in MASI scores comparing to 1% tretinoin. Moreover, The result was also the same when compared with fruit acid peels (4). The MASI scores reduction was noted by using 50% GA peels monthly for three times in Indian melasma patients. In addition, GA as a combination tended to increase the melasma improvement in one study. However, higher concentrations of GA should be used with caution especially in darker skin. PIH was reported as a side effect in many studies.

2.2.4.2 Lactic acid (LA)

In 2005, an initial study demonstrated that after applying 92% LA peels six times, 56% decrease in MASI in twelve melasma patients. Another split-face study showed similar results between LA and Jessner's solution. LA should also be used with caution in skin of colour like GA.

2.2.4.3 Salicylic acid (SA)

SA is a beta hydroxy acid peel. SA decreases hyperpigmentation by its anti-inflammatory and diffuse whitening effect. A pilot study by Grimes et al. showed a moderate improvement in dark-skinned melasma patients with mild side effects.

Another split-face study by Kodali S et al. compared only 4% HQ and a combination of 4% HQ with SA peels. This study found both of them improved bilateral melasma lesions in twenty Latin American women.

2.2.4.4 Trichloroacetic acid (TCA) peels

TCA peels are commonly used with 10-20% in lighter skin types and should be used with caution in darker skin types due to the risk of PIH. Kumari et al. showed the similar MASI scores reduction between 10-20% TCA and 10-35% GA peels. In addition, TCA peels was associated with higher side effects that GA.

2.2.4.5 Tretinoin peel

Tretinoin reduces pigmentation by dispersing pigment

granules, interfering melanosome transfer and accelelerating epidermal turnover. TCA concentration that generally applied is 0.025 – 0.1%.

In a split face pilot study in which the patients applied 70% GA on one half of the face and 1% tretinoin peels on the other weekly, there were significant reduction in MASI scores at week 6 and week 12 in both groups but the results between group were not significantly different. The tretinoin group were less experienced side effects such as irritation.

2.2.4.6 Other peels

A number of new agents have been produced including pyrivic acid, mandelic acid, phytic acid, obagi blue and amino fruit acid peels. Their evidences supporting day-to-day use are too few. All chemical peels should be used as adjunct therapy and must be used with caution due to the risk of PIH and scarring.

2.2.5 Lasers and light therapies

2.2.5.1 Intense Pulsed Light (IPL)

IPL is a non-coherent, broad spectrum light ranging from 500 nm to 1200 nm(500–1200 nm) makes it useful for many cutaneous lesions and conditions. It is effective for epidermal melasma.

Wang et al. compared IPL with 4%HQ by using setting IPL 26-33 J/cm2, double ode, 3-5 ms. According to Wang et al., the patients treated with IPL showed 28%improvement in the melanin index more than that of HQ group at week 16. However, recurrence was occurred after the last treatment 24 weeks.

Because of its ability to induce PIH, IPL should be avoided in dark skin types. The patients should strict with pre and post treatment with sucscreen.

2.2.5.2 Q-switched lasers

QS Nd:YAG, QS alexandrite, and QS ruby lasers target melanosomes with pulse durations in the nanosecond range (2).These short pulses target the small chromophore of melanin as well as generating the photoacoustic effect that causes melanin destruction.

(1) Q-switched Ruby laser

The QS ruby laser (QSRL), at 694 nm, is more selective for melanin than the 1064-nm QS Nd:YAG laser (2). However, little improvement and/or recurrence in the posttreatment have been mostly reported. Taylor et al. treated

eight patients with melasma and PIH and found it to be ineffective. By contrast, a study by Jang et al. of 15 Korean women (Fitzpatrick skin types III–IV) using the 694-nm QS ruby fractional with 6 low-dose treatments at 2-week intervals concluded that a low-dose fractional QSRL may be an effective strategy for the treatment of dermal or mixed-type melasma. Melasma Area and Severity Index (MASI) before and after each sessionwere evaluated, including at weeks 4 and 16 following the final treatment. Mean MASI score decreased from 15.1 at before treatment to 10.6 at 16 weeks after the final treatment (2, 16).

Tse et al. reported that the QS ruby laser showed no effect in the investigation of 20 patients, as well as little effect with the QS Nd:YAG with the parameters used. In contrast to Tse et al. and Taylor et al., Jang et al. the applied lower doses of energy and a fractional mode for treatments at 2 - week intervals. Thus, the efficacy of the QSRL for melasma remains controversial.

(2) Q-switched Nd:YAG

Polnikorn suggested a new QS Nd:YAG using technique for melasma treatment in his study which was the lower than 5J/cm2 energy. Polnikorn proposed that delivering subphotothermolytic fragments and disperses melanin granles into the cytoplasm wiothout cellular destruction. This modality is believed causes only melanosome destruction without cell damage (1-4).

Collimated low fluence Q-switched Nd. YAG Laser has been used in recent years. " Laser toning" technique consists of large spot size and low fluence until the skin looked mild erythema. The pros of this protocol are minimal thermal damage, less damaged to normal skin and less downtime. However,Common side effects of laser toning than commonly found are rash, temporary stinging. Moreover, hypopigmented macules are found rarely.

2.2.5.3 Ablative lasers

The 2940-nm Er:YAG laser is ablative and highly absorbed by water, reducing thermal skin damage. A pilot study in 1999 by Nouri et al. used a 10,600-nm CO2 laser to treat melasma. Eight patients (Fitzpatrick skin phototypes IV–VI) were pretreated with Kligman's formula for 14 days (2). Thereafter, the subjects were randomly assigned into CO2 laser alone group versus first-pass CO2 laser followed by a second-pass QS 755-nm alexandrite laser (QSAL). Nouri et al. concluded that combination laser (CO2 and QSAL) was highly effective in treating

hyperpigmentation with maintenance of results at 24-week follow-up (3,4).

One study found side effects including erythema and PIH in patients with darker skin phototypes. Another study also showed no effective of Er :YAG for melasma improvement . Moreover, recurrences were seen in all cases.

2.2.5.4 Non-ablative 1550 nm fractional laser

Among the many lasers used for the treatment of melasma are the nonablative fractionated lasers. Such“resurfacing” devices generally avoid many of the side effects of ablative systems. Fractional photothermolysis creates microthermal zones and leaves the majority of the treatment area intact (2). The intact skin aids in the healing process via the extrusion of necrotic debris and migration of keratinocytes.

In 2005, a pilot study described ten women (Fitzpatrick skin types III–V) who received 4–6 treatments with nonablative fractionated 1550-nm erbium fiber and 1535-nm lasers at 1–2 week intervals. Six subjects demonstrated 75–100% clearance and only one patient developed PIH (16). However, since this pilot study, several other studies have shown therapeutic efficacy that did not differ significantly from conventional treatment with topical bleaching agents. Kroon et al. performed a randomized clinical trial assessing twenty female patients (Fitzpatrick skin types II–V) for moderate to severe melasma comparing the nonablative 1550-nm fractional laser versus daily triple topical therapy for 8 weeks. The mean treatment satisfaction were significantly higher in the laser group at 3 weeks.

2.2.5.5 Fractional 1927 laser

Massaki et al. gave 10-20 mJ/cm2 with 60-70% coverage fraction 1927 laser for twenty melasma patients and then 4%HQ applied one month after the treatment. The MASI score

A 54% reduction (P = 0.004) in the MASI scores was noted at follow up session in the eight subjects. However, two skin-typed IV patients experienced temporary PIH. The result of another single-blinded study was quite similar in the non-ablative fraction laser-treated group and only sunscreen group. None of benefit of this kind of laser for melasma treatment was the conclusion of many recent studies (16).

2.2.5.6 Radiofrequency

Radiofrequency (RF) devices are increasingly used in cosmetic dermatology and come in various configurations. Cameli et al. used a monopolar RF device to improve transdermal delivery of a topical depigmenting kojic acid product. Fifty subjects (Fitzpatrick skin types II–IV) with melasma underwent sessions of treatment at 1-week intervals. Hyperpigmentation was significantly reduced compared with baseline. No side effects were observed or reported. This study illustrates the successful combination of energy-based device and topical treatment for safe, tolerable, and effective treatment of melasma (16).

2.2.5.7 Light-emitting diode (LED)

Many studies showed that LED emitting 633 and 830 nm light could be beneficial in melasma in darker skin types due to none of thermal effect. It also improved the fine lines and wrinkles, smoothtened skin textures and pores, and decreased the number of porphyrins (16).

Kim et al evaluated the effect of seven different LED wavelengths on melanogenesis. LED irradiation at 830 nm (dose-dependent, from 1 to 20 J/cm2) and 850 nm (1 J/cm2) significantly reduced melanin production and tyrosinase expression. These results indicate that LEDs could potentially be used to treat melanin-overproducing skin conditions.

Mpofana et al. evaluated six women with skin type VI between the ages of 35 and 54 years with either dermal or each participant was exposed to a 633-nm LED treatment of 20 minutes, followed by another exposure to 830-nm LED light treatment after two days. The light panels of 633-nm and 830-nm wavelengths were positioned at a distance of about three fingers from the participants' face. 84% of patients are satisfied a better skin tone and texture and less fine lines and wrinklkes. 633-nm and 830-nm LED light treatment is not only lighten melasma lesions but also reduce several signs of aging.

2.2.6 Systemic treatments

The possible mechanism of action of oral transnexemic acid that reduce hyperpigcmentation is tyrosinase inhibitor. This drug is traditionally used for stop prolonged bleeding in menorrhagia and bleeding disorders (2). The success rate for melasma treatment was variable. Moreover, the side effects such as headaches,

menstrual irregularity were commomly occurred. The rare dangerous consequence are vision changes and serious blood clot problems. Therefore, strict monitoring is required while using the drug and It should be used temporary (less than six months).

2.2.7 Combinations

There is a wide variety of treatments including whitening agents, chemical peeling, lasers and light modalities. All of treatment options must include a broad-spectrum sunscreen and sun avoidance.

2.3 Platelet-Rich Plasma (PRP)

PRP is a plasma processed to increase levels of bioactive factors. Autologous PRP has been used extensively in oral and maxillofacial surgery, orthopedics and sports medicine, plastic surgery, ear-nose-throat surgery, neurosurgery, gynecology, ophthalmology and cosmetic surgery for over 30 years. Recently, PRP has been used in various dermatologic conditions including alopecia, wound healing, atrophic scar, striae distensae, skin rejuvenation and hyperpigmented lesions (22-24).

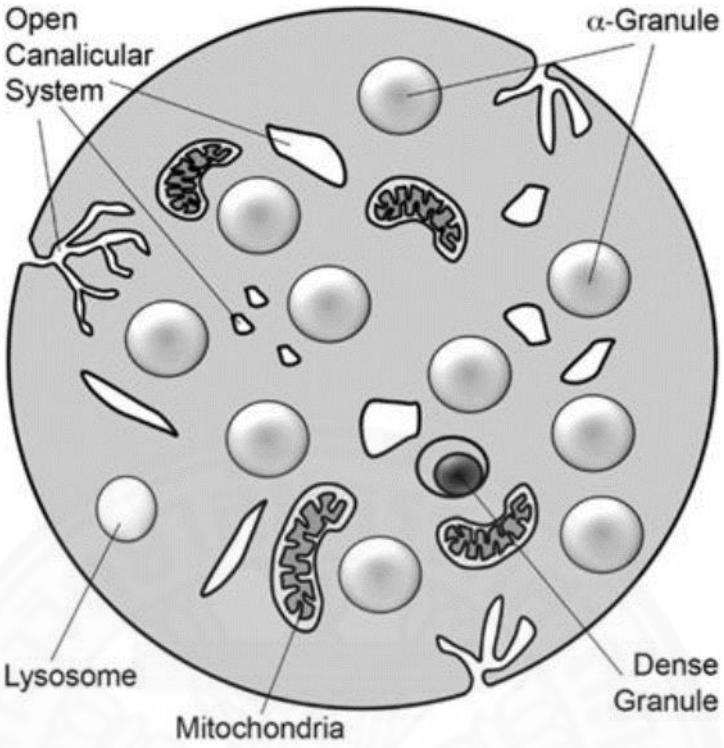


Figure 2.4 Platelet and its component (25)

Platelets are cytoplasmic fragments of megakaryocytes, formed in the marrow and 2 mm in diameter. They contain α-granules, dense granules, lysosomes and mitochondria (25).

Contents of α-granules include IGF-1, PDGF, TGFβ, platelet factor 4 and other clotting proteins (such as thrombospondin, fibronectin, factor V, and von Willebrand factor). While the dense granules of human platelets contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), ionized calcium, histamine and serotonin.

2.3.1 Mechanism of actions

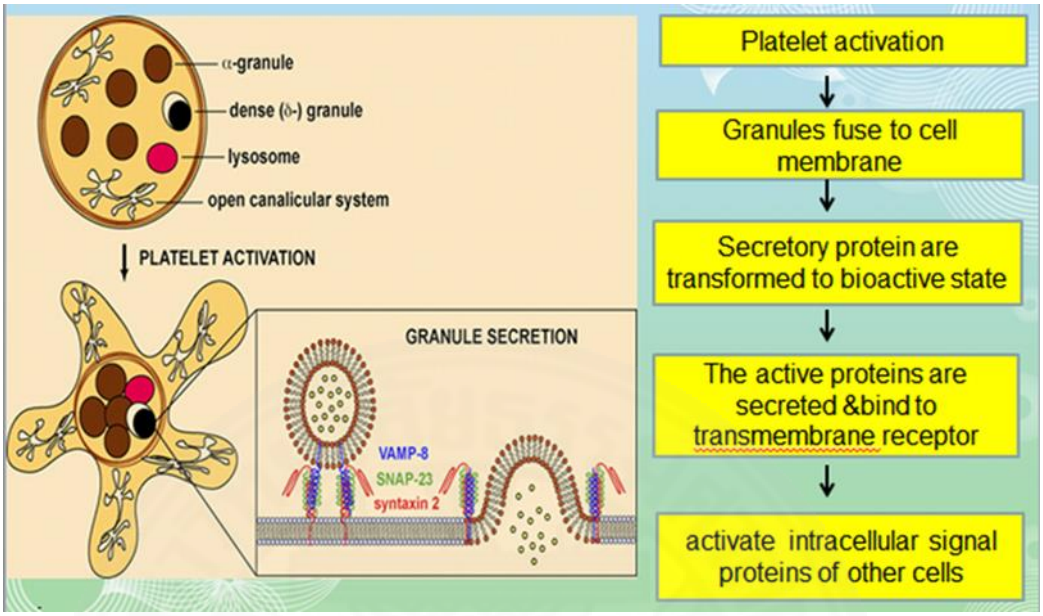


Figure 2.5 Platelet degranulation (26)

After PRP contact the collagen, the granules inside platelets will be activated and then fuse to cell membrane to release the growth factors such as PDGF, EGF, TGF-beta. These secretory proteins are induced to an active state by the addition of histones and carbohydrate side chains. The secretion of these active proteins are followed by the binding to transmembrane receptors affective many cell types. The binding causes the expression of a gene sequence controlling many functions including cellular proliferation, collagen synthesis and tissue regeneration.

Within 10 min after activation, these growth factors are secreated by platelets and it should be used within 1 hr (27).

Calcium chloride is a substance that can delay the release of growth factors by initiation the formation of thrombin. This delay could be up to 7 days.

2.3.2 What is PRP?

PRP is a plasma containing highter-than-normal platelet concentrations which is prepared by centrifugation (16). More than 1 million/ μL platelet concentration is believed to be enough therapeutic effect (28,29).

2.3.3 PRP preparation

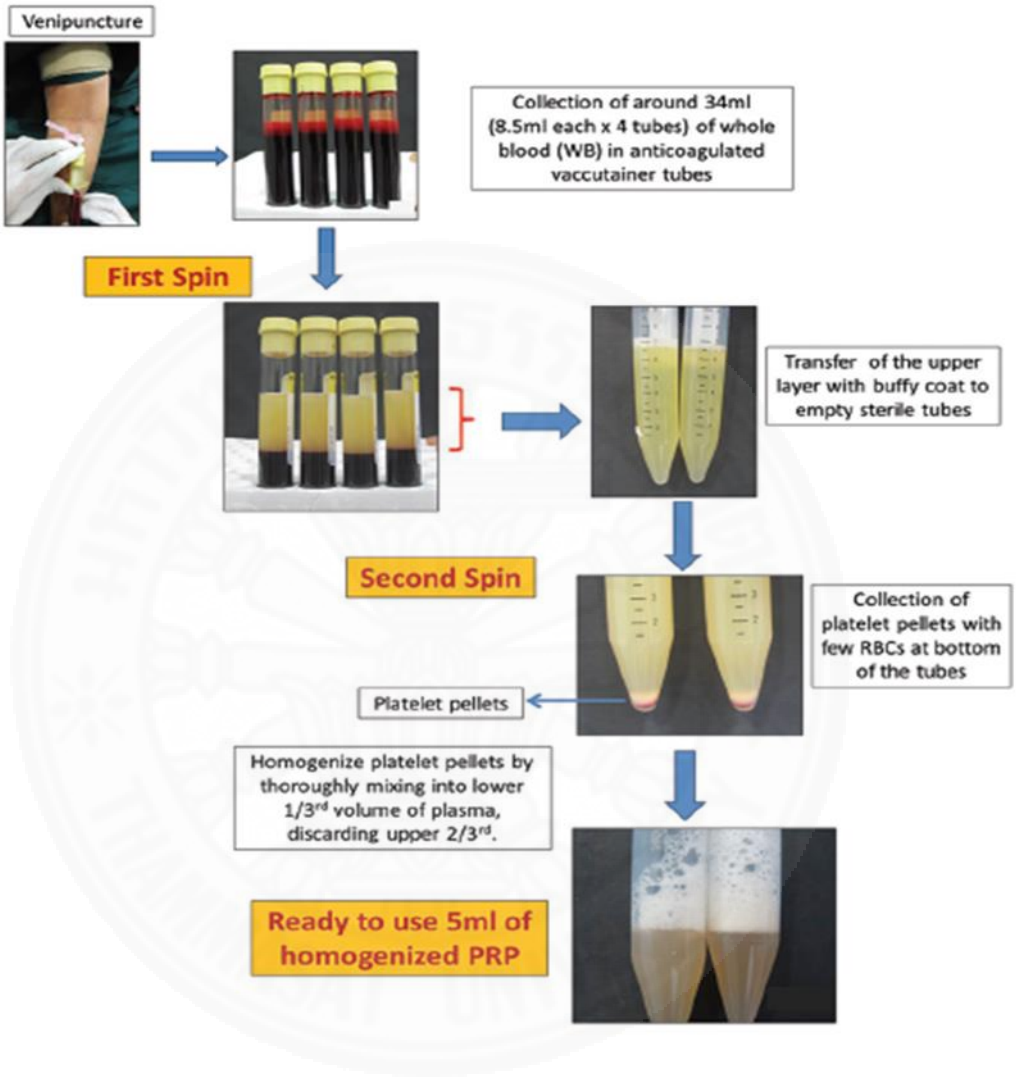


Figure 2.6 A double centrifugation process of PRP (29)

The consent form is informed before taking blood from patients. 30cc whole blood is collected in tubes with anticoagulants. After centrifugation, this whole blood will give 3-5 cc of PRP. The amount of PRP depends on the centrifugation device, the technique and the baseline platelet count of an individual.

RBCs are separated from whole blood by the first centrifugation. There are three layers in tube consisting of platelet-poor plasma (PPP) at the upper layer, buffy coat with white blood cells (WBC) at the intermediate layer and red blood cells at the bottom layer.

Figure 2.7 Three layers of whole blood (29)

PRP content is in the buffy coat layer so that PPP and superficial buffy coat are removed in order to produce pure PRP.

Calcium chloride leads to the activation of the PRP while nonactivated form gets activated after coming in contact with collagen of the tissues.

2.3.4 Classification of PRP

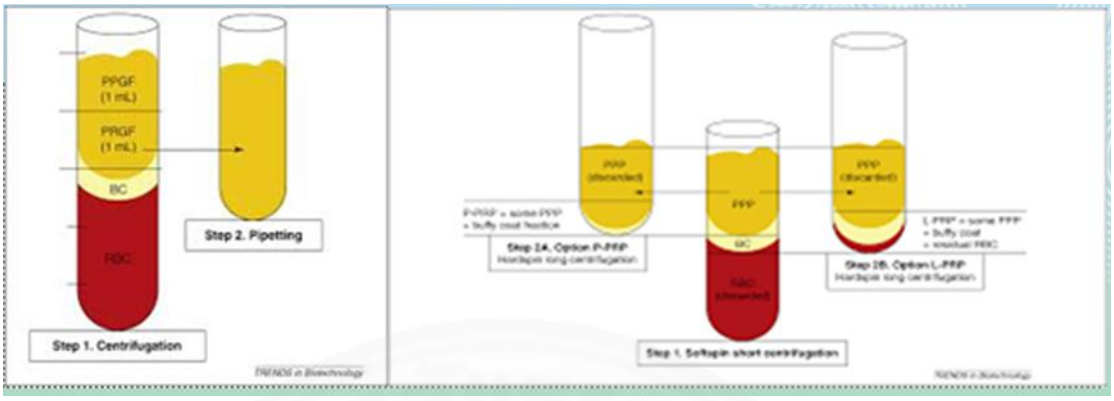


Figure 2.8 Classification of PRP (29)

Ehrenfest et al. have classified PRP into four classes in 2009. They are defined by their WBC content and fibrin network existence.

Table 2.1 Classification of PRP (29)

	Pure Platelet-Rich Plasma (P-PRP)	Leucocyte PRP (L-PRP)	Pure Platelet-Rich Fibrin (P-PRF)	Leucocyte Platelet-Rich Fibrin (L-PRF)
With WBC	-	√	-	√
High density fibrin network	-	-	√	√

1. Pure Platelet-Rich Plasma (P-PRP) or leucocyte-poor PRP products are PRP without WBC and fibrin network. This kind of PRP is formed in liquid or gel form and helps wound repair.

2. Leucocyte- and PRP (L-PRP) products are PRP with WBC and without fibrin network. This kind of PRP is formed in liquid or gel form.

3. Pure platelet-rich fibrin (P-PRF) or leucocyte-poor platelet-rich fibrin preparations are PRP without WBC and with a high-density fibrin network. These products are formed in only gel form.

4. Leucocyte- and platelet-rich fibrin(L-PRF) or second-generation PRP products are PRP with WBC and fibrin network.

In this study, we choose P-PRP because it has neither WBC which can promote inflammation in melasma lesion nor fibrin which is not necessary in melasma treatment.

Table 2.2 Growth factors found in ∝-granule (30-32)

Growth Factor	Biological Activity
EGF (Epidermal Growth Factor) was discovered in 1962 by Cohen	Enhance wound healing Accelerate epidermal regeneration Cell differentiation Cell mitosis
PDGF (Platelet derived Growth Factor) was discovered in 1974	Cellular migration Control cell membrance receptos Enhance the motility of fibroblasts, endothelial cells and neurons
IGF-1 (Insulin-like Growth Factor1)	Angiogenesis Cell differentiation
TGFβ-1 (Transforming Growth Factor-Beta1)	Mitogen for fibroblasts, smooth muscle cells, and osteoblasts Promote angiogenesis and extracellular matrix production
VEGF(Vascular Endothelial Growth Factor)A,B,C	Induce angiogenesis Alter vascular physiology and permeability
FGF1 (Fibroblast Growth Factor)	Induce fibroblast proliferation and angiogenesis

2.3.5 PRP in Clinical Practices

2.3.5.1 PRP with androgenic alopecia

Parul Singhal et al. injected PRP every 2 weeks for four sessions and observed that there were clinical improvement in the hair counts, hair thickness, hair root strength (33).

Betsi E. E. et al. showed that PRP injections for the treatment of alopecia had the better results and lasting improvement in patients with an early stage of alopecia (34).

Growth factors from PRP have the hair regrowth quality by inducing the proliferation of dermal papilla cells.

The main growth factors involved in the hair lenghtening are VEGF, EGF, IGF-1, and FGF. Moreover, beta-FGF is reported to promote the in vitro proliferation of papilla cells and hereby playing a key role in elongating the hair shaft. To sum up, the method is cheap, safe and minimal morbidity and it is an effective adjuvant treatment of AGA.

2.3.5.2 PRP with rejuvenation

Min-Kyung Shin et al. investigation demonstrated that the combination of nonablative fractional laser and increased subject satisfaction and skin elasticity and decreased the erythema index. Biopsies also showed that PRP increased the length of the dermo-epidermal junction, the amount of collagen and the number of fibroblasts (35).

The result of PRP on general appearance, kin firmness-sagging, wrinkle state and the pigmentation evaluated by Esra Pancar Yuksel et al. was only statistically significant increase in the skin firmness-sagging than before treatment.

MMP-1 and MMP-3 proteins were increased after PRP treatment. MMP-1 induce removal of damaged collagen fragments, thus facilitating deposition of new collagen in photo-aged skin. Topical application of PRP or its direct injection into the skin produces ECM remodelling and induces the synthesis of new collagen by fibroblasts.

2.3.5.3 PRP with wound healing

The comparative study conducted by Simran Chawla showed that microneedling combined with PRP were better improve in firmness and smoothness of skin than microneedling with vitamin C for the treatment of atrophic acne scars (36).

Another study by Jung-Im Na et al. demonstrate that when apply PRP after ablative fractional Co2 laser, there were more rapid healing and reduced erythema compared with normal saline application (37).

Mechanism of PRP to enhance wound healing was confirmed by Law Jia Xian et al. study. They investigated the wound healing effects of 10% v/v PRP and 20% v/v PRP by fibroblasts and keratinocytes coculture.

They had different results which 10% PRP with wound remodeling and 20% PRP with inflammation enhancer and collagen deposition (38).

2.3.5.4 PRP with dark eye circles

Salah Hashin Al-shami injected autologous PRP intradermally into the whole face monthly for 3 sessions (39). The improvement scale assessed by dermatologists was so obvious improved of 38% moderate level from first month to six months after the treatments.

Another assessment of the efficacy of PRP on dark eye circles was done by Pedram Mehryan et al. Ten participants were treated with intradermal injections of 1.5 mL PRP one time into tear trough area and crow's feet wrinkles on each side (40).

The improvement in infraorbital color homogeneity was statistically significant (P = 0.010), but no statistically significant changes were observed in melanin content, stratum corneum hydration, wrinkle volume, and visibility index.

2.3.5.5 PRP with melasma

Yew CH et al. reported the significant reduction of MASI score in 2 patients with refractory dermal melasma. In this study, they administered intradermal PRP monthly for 2 sessions as an adjunct to a monthly Q-switched Nd:Yag laser treatment and topical alpha arbutin application (18). At the follow up of the 3rd month, the MASI score was reduced by mean 33.5% for case 1 and 20% for case 2. However, recurrence of melasma was found in case 2 by a worsening of the MASI score mean to 53% at the 6th month follow up. The side effects observed in both cases was minimal such as mild erythema, edema and small bruises for the first few days after the injection. They discussed less improvement observed in case 2 could be due to a higher Fitzpatrick skin type and a mixed type melasma with telangiectasia.

A case reported by Mutlu Çayırlı et al showed that more than 80% reduction in epidermal hyperpigmentation after injected PRP biweekly for 3 sessions and there has been no recurrence of melasma for 6 months (19).

Although the exact pathogenesis of melasma is unknown, it is hypothesized that following exposure to UV irradiation (or another inducer), hyperfunctional melanocytes within involved skin produce increased amounts of melanin. Increased expression of c-KIT and SCF within the lesional epidermis and dermis, respectively, may play a role in the hyperpigmentation of melasma (41).

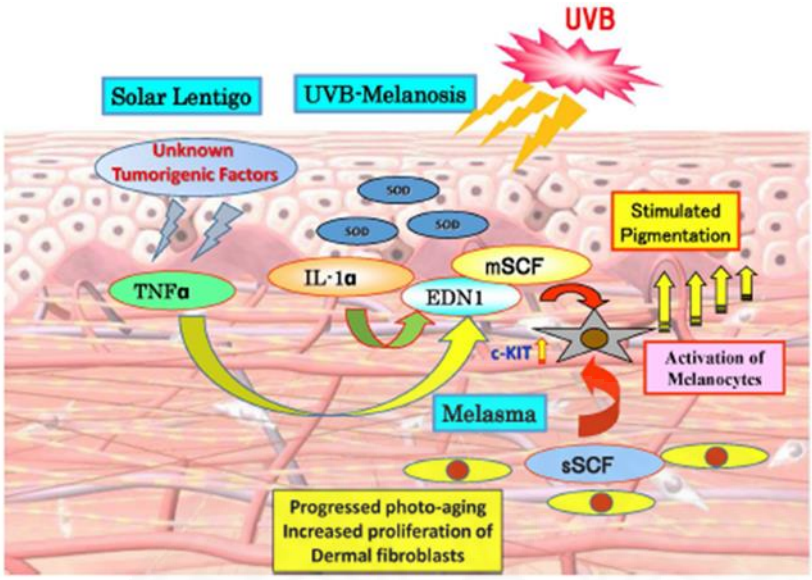


Figure 2.9 UVB-induced mechanisms in UVB-melanosis, solar lentigo and melasma (41).

The upregulation of soluble SCF founded in dermal melasma facilitates its ascent into the epidermis and then activates epidermal melanocytes.

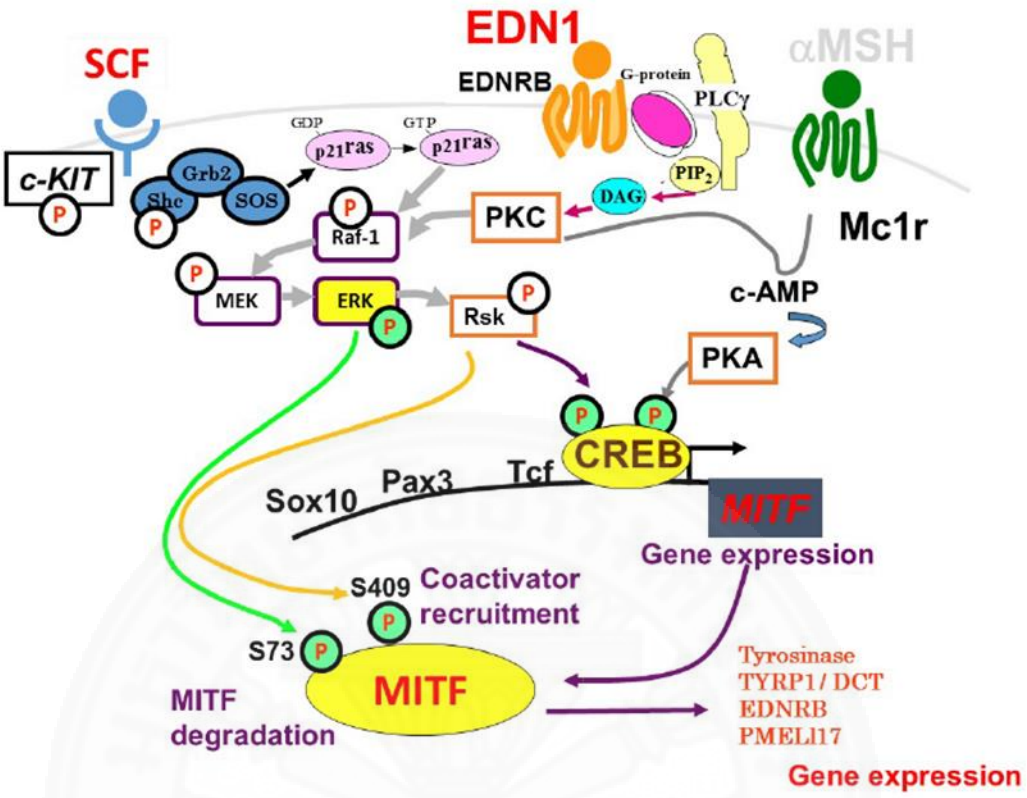


Figure 2.10 EDN1, SCF and MSH role in MITF activation (41).

Kim et al. used mouse melanocyte cell line to study the effect of TGF-beta1 on melanogenesis. The result from their study showed that TGF-beta1 reduced the activity of tyrosinase and MITF promoter and also decreased tyrosinase-related protein production. Moreover, TGF-beta delayed the activation of extracellular signal-regulated kinase (ERK) at 6 hr instead of in minutes like other growth factors (42). Lastly, TGF-β also can inhibit the expression of paired-box homeotic gene (PAX 3), which is a key regulator of UV-induced melanogenesis (43).

Yun et al. found that EGF decreased melanogenesis by inhibiting PGE2 and tyrosinase in a laser-treated keratinocyte-conditioned culture media (LCM).They suggested that EGF could be used as skin whitening and PIH prevention (44).

Another study showed that PDGF associated with the increase in skin volume by enhance the formation of blood vessel, collagen and components of the extracellular matrix, including hyaluronic acid. When the volume of the skin is

increased, the hyperpigmented lesions are looked more glowing (45).

Some mild bruises has been reported as an adverse effect of PRP injection and it has been resolved in a few days.

The side effects after PRP injections were minimal pain, redness at the time of injections and pinpoint bleeding. Being an autologous preparation, PRP has not been involved in the infections such as hepatitis B or C and Human immunodeficiency virus (HIV).

The PRP method is cheap, safe and minimal morbidity and holds a promising future treatment and a useful adjuvant therapeutic option in the treatment of melasma.

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Materials

3.1.1 Study sample

Female patients with symmetrical mixed-type melasma on the face and aged 18-65

3.1.1.1 Sample size

Calculated by G* power : ANOVA, repeated measures within between interaction N=10

Program G* Power 3.1.7 (163) :

Effect size f = 0.5

α error probability = 0.05

Power (1-β error probability) = 0.80

Number of groups = 2

Total sample size = 10

3.1.1.2 Inclusion criteria

Female patients with symmetrical mixed-type melasma

Age 18-65

Ability and willingness to comply with the requirements of the protocol

3.1.1.3 Exclusion criteria

Patients who can not cooperate with the protocal

History of malignancies, anemia or bleeding disorders

History of Hepatitis B or C, HIV

Local inflammation at the site of blood taking or treatment area

Women who are pregnant or breastfeeding

Patients on consistent use of anti-coagulateds or NSAID within 48 hours of procedure

Concomitant use of isotretinoin or hormonal therapy

Parcipitated in major outdoor activities Known history or clinically relevant allergy to components of the sunscreen or topical anaesthetic used in this trial. Use of topical retinoids or topical whitening cream within 1 months prior to study entry.

Use of topical hydroquinone within 6 months prior to study entry. Use of topical corticosteroids within 1 month or systemic corticosteroids withing 2 weeks prior to study entry. Laser surgery procedures in the treatment regions within 6 months prior to study entry.

Use photo-sensitizing medication (e.g., tetracyclines, gold).

3.1.1.4 Discontinuation criteria

Patients' refusal to participate the study

Patient suffering serious adverse effect of PRP injection

Unreliable and poor compliance patient

3.1.2 PRP preparation

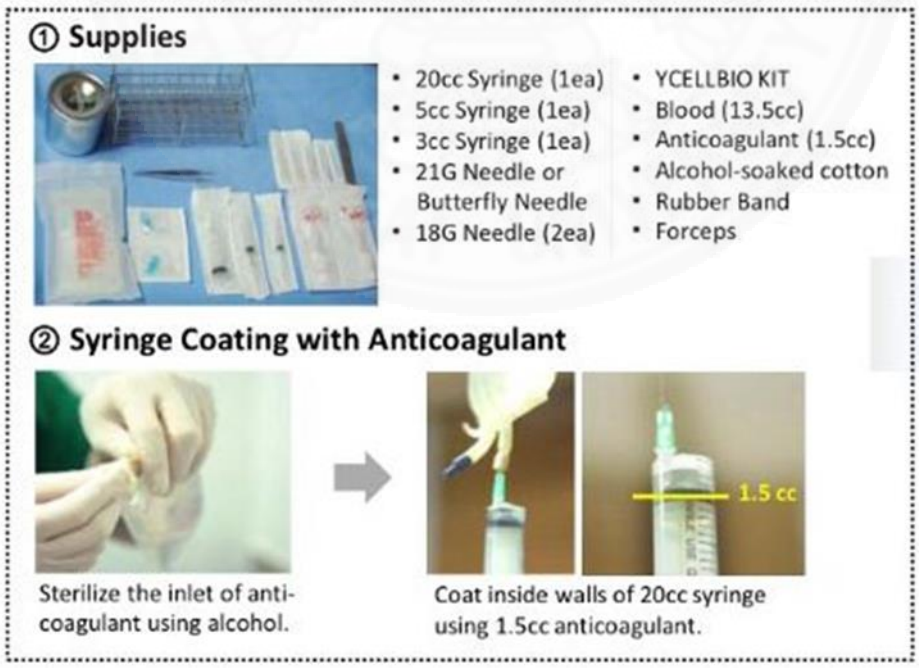


Figure 3.1 PRP preparation

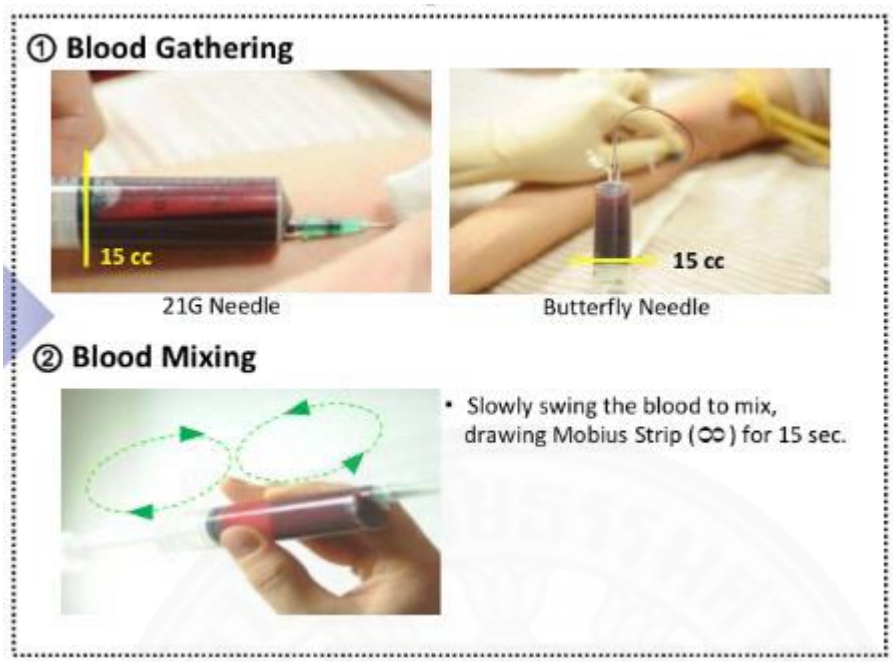


Figure 3.2 Blood gathering

3.1.2.1 Blood gathering

Draw 1.5 ml anticoagulant and 13.5 ml blood in 20 ml syringe using a butterfly cannula. Shake it slowly to mix, for 15 sec.

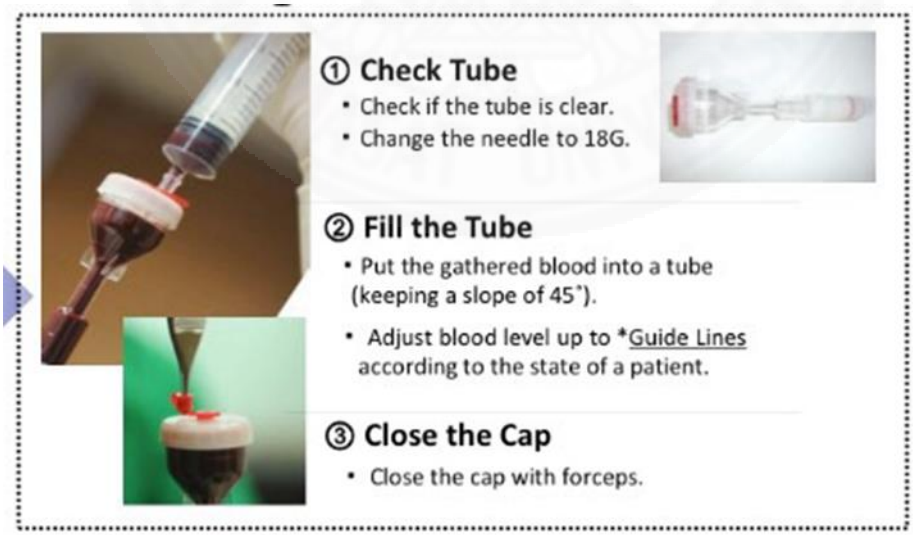


Figure 3.3 Blood insertion

Figure 3.4 RBC adjustment

3.1.2.2 Centrifugation

The 1st centrifugation will be at 3400-3600 rpm for 4 minutes at room temperature. After the centrifugation, If PRP line is unclear after 1st centrifugation, adjust RBC level as Figure 3.5 According to the RBC position adjusted, conduct the 2nd centrifugation at same rate.

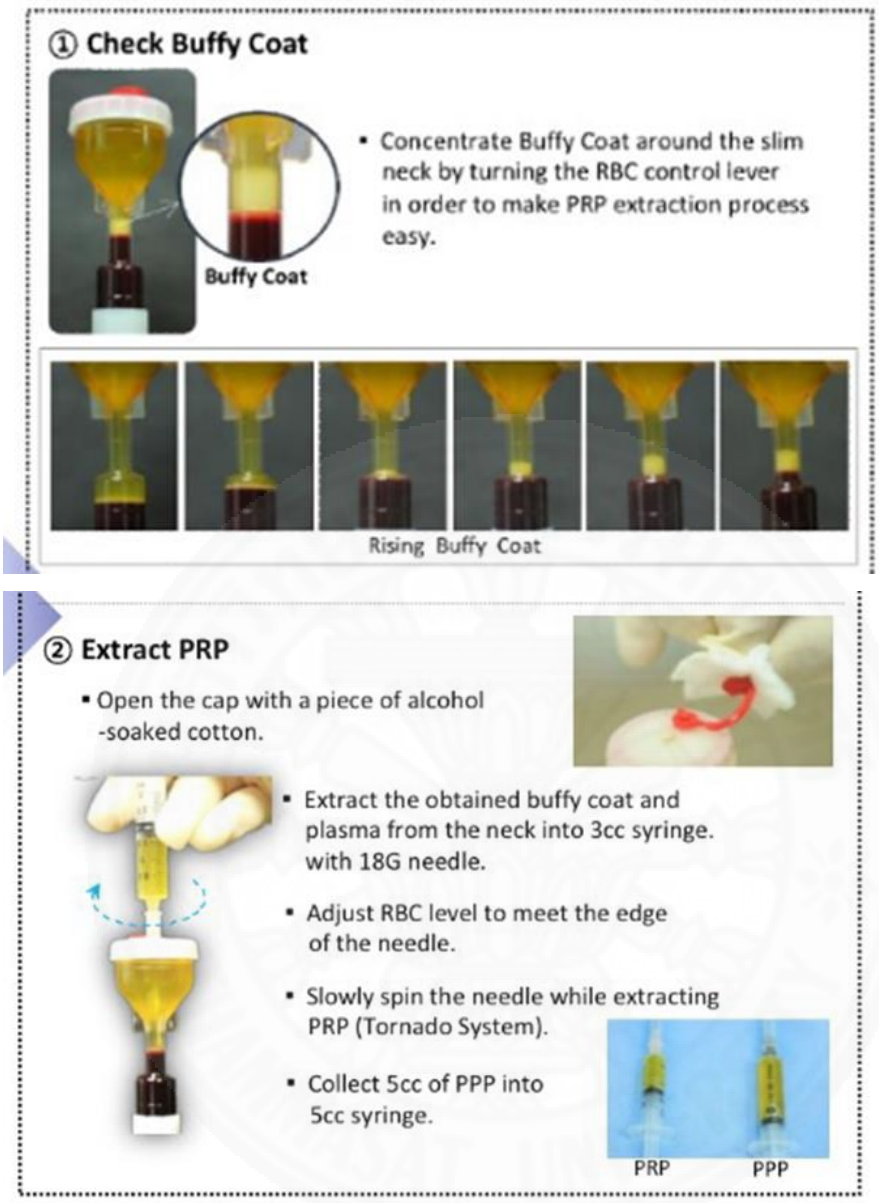


Figure 3.5 PRP extraction

3.1.2.3 Extraction

Control the level of RBC by the knob to meet the edge of needle and extract the obtained buffy coat and plasma from the neck by 3ml syringe and 18G needle. And then spin the needle slowly(Tornado Technique).

3.1.2.4 Collection

1ml of collected PRP will be measured TGF-β1 by ELISA technique and platelet level.

Table 3.1 Properties of YCELLBIO Kit

	Blood Sample Volume	PRP Volume	Platelet count(μl)	Platelet enrichment
YCELLBIO KIT	13.5 – 15 ml	1 -2 ml	1,200,000 - 2,000,000	7-9%

Figure 3.6 YCELLBIO Kit Key Features

3.2 Research design

3.2.1 Research grouping

Ten female patients with bilateral mixed-type melasma were enrolled in a split-faced prospective trial. Ten patients were injected by PRP every 2 weeks for 4 times to one side of the face randomly every 2 weeks for 4 times. Patients were instructed that skin care during the study could consist only of a specific moisturizer and cleanser with a sun protection factor 50 sunscreen to be applied to both sides of the face in the morning.

3.2.2 Patients preparation

Study participants will be gotten informed consent prior to initiation of any study-related standard conditions. The study was performed in compliance with informed consent regulations.

Subjects will be asked to remove all make up and clean their faces with facial cleansers and water before treatment.

Front and 4 side view photos were taken with a digital camera (Panasonic Lumix GF8) before measured by mexameter and Antera equipment. The set of photos was taken in each visit pre-treatment and follow-up day using same place, camera and positioning.

Pretreatment melanin and erythema index are measured by mexameter and pretreatment photographs are taken and pretreatment skin quality are measured by Antera 3D® every sessions.

Preoperative topical analgesic cream (2.5% lidocaine and 2.5% prilocaine, EMLA ®) was applied and occluded at lesions 45 to 60 minutes before treatment and subsequently washed off to obtain completely dry skin surface.

All subjects were treated with PRP biweekly for a total number of four treatments. Entire melasma areas are injected with 30-gauge, 1 ml syringe needle under sterile condition. Injections of about 0.1 ml are injected intradermally on the lesions at 1 cm intervals.

No postoperative analgesic treatment was required beyond the application of ice compresses. No prophylactic antibiotics or antiviral were given in any patient.

Patients are instructed to apply broad-spectrum sunscreen with SPF 50, avoid sun exposure, and avoid the use of any topical preparation on the lesions for the period of the study. After ending of the study, control side will be treated by topical whitening treatment.

3.3 Outcome measurements

Table 3.2 Outcome measurements

Assessment	Treatment(wk)				F/U(wk)
Treatment	0	2	4	6	10
Photography	*	*	*	*	*
Antera 3D®	*	*	*	*	*
Mexameter	*	*	*	*	*
mMASI score	*	*	*	*	*
TGF-β1 levels	*	*	*	*	
Side effect		*	*	*	*
Quality of Life	*				*

Patients satisfaction		*	*	*	*
Pain score	*	*	*	*	
Physicians'global assessment		*	*	*	*

3.3.1 Objective assessment

3.3.1.1 Mexameter

Mexameter is based on absorption and reflection principles. The probe emits 3 specific light wavelengths. A receiver measures the light reflected by the skin. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated. The melanin is measured by specific wavelengths chosen to correspond to different absorption rates by the pigment. For the erythema measurement specific wavelengths are also used, corresponding to the spectral absorption peak of haemoglobin and to avoid other color influences (e.g. bilirubin). In this study, the darkest point of melasma will be measured.

3.3.1.2 Antera

Figure 3.7 Antera 3D® Skin Analysis Equipment

The Antera 3D® skin analysis equiment uses a hand-held, portable camera and software with complex algorithms to convert light reflected from the skin's surface into digital images that display topography, hemoglobin, and melanin. To measure skin quality, various light-emitting diodes and polarizers from camera illuminates a 60 mm square area and capture reflected light independent of surrounding lighting conditions. A matching tool incorporated into the Antera 3D interface allows accurate before-and-after camparisons in several ways such as skin color, texture, volumes, wrinkles, redness and pigmentation. In this study, we measure wrinkles, texture, melanin and haemoglobin.

3.3.1.3 TGF-beta 1 (details at Appendix A)

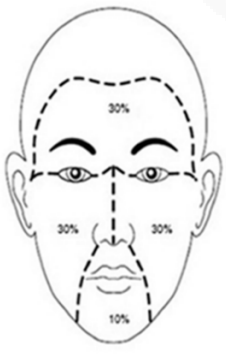
Measured TGF-β1 level from 1ml of PRP part by ELISA technique and then storaged at -80° celsius refrigerator.

3.3.1.4 Side effects

Side effects were assessed at all visits using objective such as erythema, scaling/peeling, edema, eczema, or irritation. Patients will be asked to report any adverse effects such as pain (be evaluated by visual analog scale: VAS), erythema, burning, itching, pigmentation change (hyperpigmentation, and hypopigmentation), purpura, textural alteration. The side effect will be checked on the 2nd week onward.

3.3.2 Subjective assessment

3.3.2.1 mMASI score



Modified MASI SCORING

Forehead	30%
Left malar	30%
Right malar	30%
Chin	10%

Area	Darkness
0 = no involvement	0 = absent
1 = <10%	1 = slight
2 = 10–29%	2 = mild
3 = 30–49%	3 = marked
4 = 50–69%	4 = severe
5 = 70–89%	
6 = 90–100%	

Figure 3.8 Modified MASI scores

Modified Melasma area and severity index (MASI) is a scoring system that has been devised to accurately quantify the severity of melasma at baseline

and any changes during therapy (2). The score of involvement area is divided into seven grades from 0 which means no involment to 6 which means almost cover all area. The score of darkness is divided into five grades from 0 to 4 which mean absent and severe respectively. Each forehead, right malar and left malar area is accounted for 30% of the whole face. While chin area is accounted for 10%. The score will be calculated by the equation below.

MASI = 0.3A(D)forehead + 0.3A(D)right malar
+ 0.3A(D)left malar + 0.1A(D)chin

The maximum score for MASI is 24, with 0 as minimum.

3.3.2.2 Patients satisfaction

0-100% score

from 0% = no change to 100% = completely resolved

3.3.2.3 WHO QOL

See details at Appendix D

3.3.2.4 Pain score

0 = no pain

1-3 = mild pain (annoying, interfering little with activities of daily living)

4-6 = moderate pain (interferes significantly with activities of daily living)

7-10 = severe pain (disabling; unable to perform activities of daily living)

3.4 Data analysis

All measured values will be expressed in the form of means and standard deviations (SD).

A Paired t test will be used to compared the change in mMASI and mexameter between the baseline and final follow-up visits

All statistical analyses will be performed out using SPSS

Statistically significant in all cases will be considered at the p-value < 0.05

3.5 Ethics approval

Project no. MTU-EC-OO-2-192/59

CHAPTER 4
RESULTS

4.1 Demographic data

Ten female patients with mixed-type melasma were enrolled in the study.

All of them completed the protocol. The average age was 46.2 years (range 33-58 years), with skin types III (20%), IV (80%). The details of the demographic data were revealed in table 4.1

Table 4.1 Demographic data

Subject No.	Age	Menstruation	Duration (Yr)
1	40	Yes	5-10
2	50	Yes	>10
3	58	No	5-10
4	33	Yes	<5
5	45	Yes	<5
6	53	No	>10
7	58	No	>10
8	55	No	>10
9	52	No	5-10
10	49	No	5-10

4.2 Objective assessment

All enrolled subjects were evaluated for melanin index and erythema index by mexameter and were examined for melanin, haemoglobin, wrinkles and textures levels by Antera 3D.

4.2.1 Mexameter

4.2.1.1 Melanin index

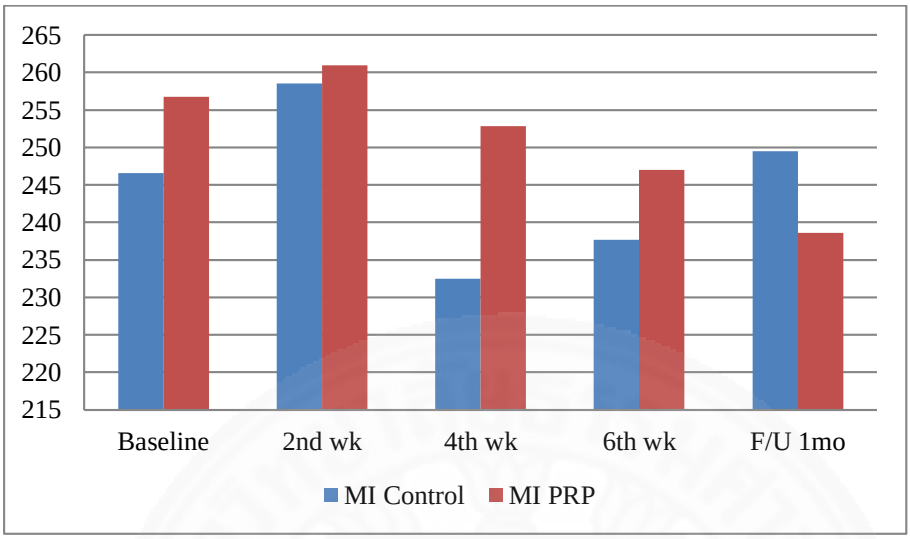


Figure 4.1 Melanin index results

Table 4.2 Melanin index results

Variables		PRP	Control	p-value[(b)]
	n	Mean ± SE.	Mean ± SE.	
Melanin Index				
Baseline	10	256.73 ± 17.68	246.57 ± 22.88	0.447
Week 2	10	260.97 ± 20.07	258.5 ± 23.02	0.846
Week 4	10	252.8 ± 17.53	232.5 ± 13.96	0.067
Week 6	10	246.97 ± 18.63	237.7 ±22.37	0.546
Week 10	10	238.63 ± 16.4	249.47±21.36	0.513
p-value[(w)]				
Week 2		0.679	0.073	
Week 4		0.823	0.421	
Week 6		0.652	0.576	
Week 10		0.334	0.841	

Values presented as mean and Standard error (SE.). P-value corresponds to Paired t test.
(b) Comparisons between treatments. (w) Comparisons within treatments.

According to Figure 4.1 and Table 4.2 demonstates melanin index of the participants at each visit. Clinical visits were scheduled at the baseline, week 2, week 4, week 6 and week 10. The data of melanin index was collected at

Thailand Tobacco Monopoly Hospital and the statistical analysis was evaluated by Paired t test comparison within groups, and comparison between PRP group and control group.

Within group analysis of the melanin index in PRP side from baseline to the end of week 10 was from the mean score of 256.73 ± 17.68 to 238.63 ± 16.4 (P = 0.334).

The melanin index of control side was increased from 246.57 ± 22.88 to 249.47±21.36. The results showed non-statistical significant in every visit from week 2 to week 10.

Between group analysis PRP side and control side were not significantly different with regard to the changes in the mean score of melanin index at 10 weeks of the study (P = 0.447, 0.846, 0.067, 0.546, 0.513).

4.2.1.2 Erythema index

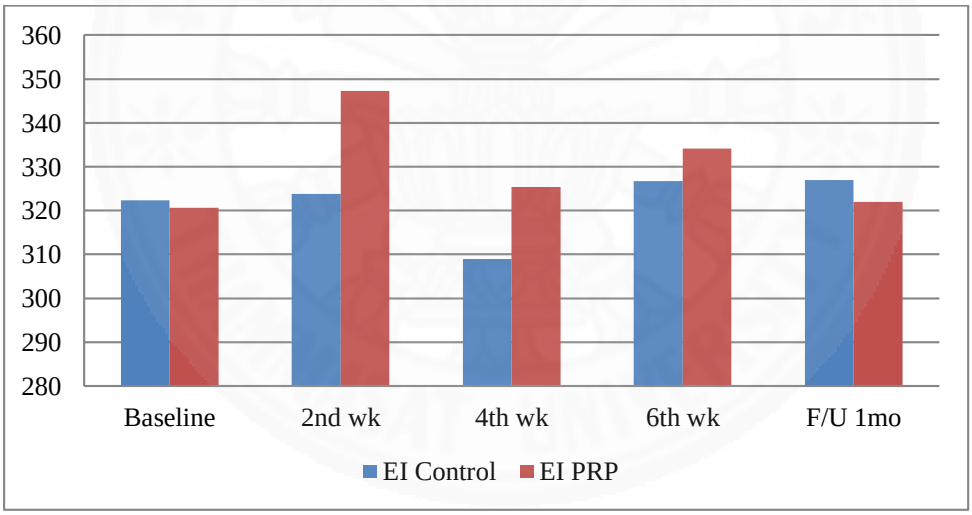


Figure 4.2 Erythema index results

Table 4.3 Erythema index results

Variables		PRP	Control	p-value[(b)]
	n	Mean ± SE.	Mean ± SE.	
Erythema Index				
Baseline	10	320.6 ± 13.75	322.3 ± 18.94	0.927
Week 2	10	347.23 ± 12.01	323.77 ± 19.29	0.171
Week 4	10	325.43 ± 15.82	309 ± 11.27	0.227
Week 6	10	334.13 ± 9.92	326.77 ± 13.69	0.446
Week 10	10	322.02 ± 13.27	327.0 ± 13.99	0.172
p-value[(w)]				
Week 2		0.200	0.926	
Week 4		0.823	0.524	
Week 6		0.406	0.874	
Week 10		0.938	0.802	

Values presented as mean and Standard error (SE.). P-value corresponds to Paired t test.
(b) Comparisons between treatments. (w) Comparisons within treatments.

According to Figure 4.2 and Table 4.3 demonstates erythema index of the participants at each visit. Clinical visits were scheduled at the baseline, week 2, week 4, week 6 and week 10. The data of erythema index was collected at Thailand Tobacco Monopoly Hospital and the statistical analysis was evaluated by Paired t test comparison within groups, and comparison between PRP group and control group.

Both PRP and control group at all visits did not show improvement in erythema index compared with baseline. Within group analysis of PRP group from baseline to the end of week 10 was from the mean score of 320.6 ± 13.75 to 322.02 ± 13.27 (P = 0.938). The mean score from control group was also increased from 322.3 ± 18.94 at baseline to 327.0 ± 13.99 at week 10 (P = 0.802).

Between group analysis PRP side and control side were not significant different with regard to the changes in the mean score of erythema index at 10 weeks of the study (P = 0.927, 0.171, 0.227, 0.446, 0.172).

4.2.2 Antera

4.2.2.1 Antera® melanin level

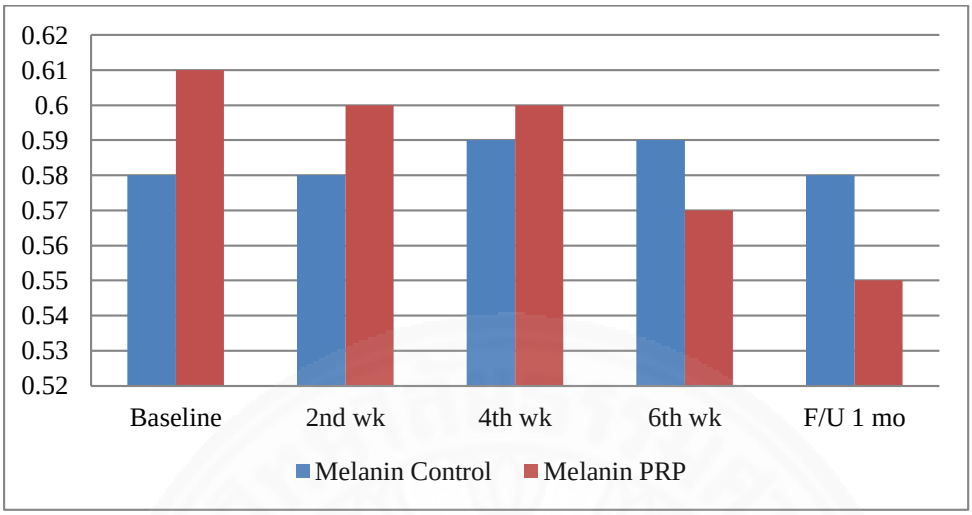


Figure 4.3 Antera® melanin level

Table 4.4 Antera® melanin level

Variables	PRP		Control	p-value[(b)]
	n	Mean ± SE.	Mean ± SE.	
Antera® melanin level				
Baseline	10	0.61 ± 0.02	0.58 ± 0.02	0.085
Week 2	10	0.60 ± 0.02	0.58± 0.02	0.139
Week 4	10	0.60 ± 0.02	0.59 ± 0.02	0.915
Week 6	10	0.57 ± 0.03	0.59 ± 0.02	0.309
Week 10	10	0.55 ± 0.06	0.58 ± 0.02	0.592
p-value[(w)]				
Week 2		0.206	0.692	
Week 4		0.538	0.197	
Week 6		0.038*	0.183	
Week 10		0.321	0.949	

Values presented as mean and Standard error (SE.). P-value corresponds to Paired t test.
(b) Comparisons between treatments. (w) Comparisons within treatments.

According to Figure 4.3 and Table 4.4 demonstates melanin levels of the participants at each visit evaluated from Antera 3D biometric instruction. Clinical visits were scheduled at the baseline, week 2, week 4, week 6 and week 10.

The statistical analysis was evaluated by Paired t test comparison within groups, and comparison between PRP group and control group.

The mean reduction of melanin level was statistically significant in PRP group at week 6. Within group analysis of PRP group from baseline to the end of week 6 was from the mean score of 0.61 ± 0.02 to 0.57 ± 0.03 (P = 0.038).

The mean score of control group at all visits did not show improvement in melanin levels compared with baseline. Within group analysis of control group from baseline to the end of week 10 was the same at 0.58 ± 0.02 (P = 0.949).

Between group analysis PRP side and control side were not significant differenct with regard to the changes in the mean score of melanin levels at 10 weeks of the study (P = 0.085, 0.139, 0.915, 0.309, 0.592).

4.2.2.2 Antera® haemoglobin level

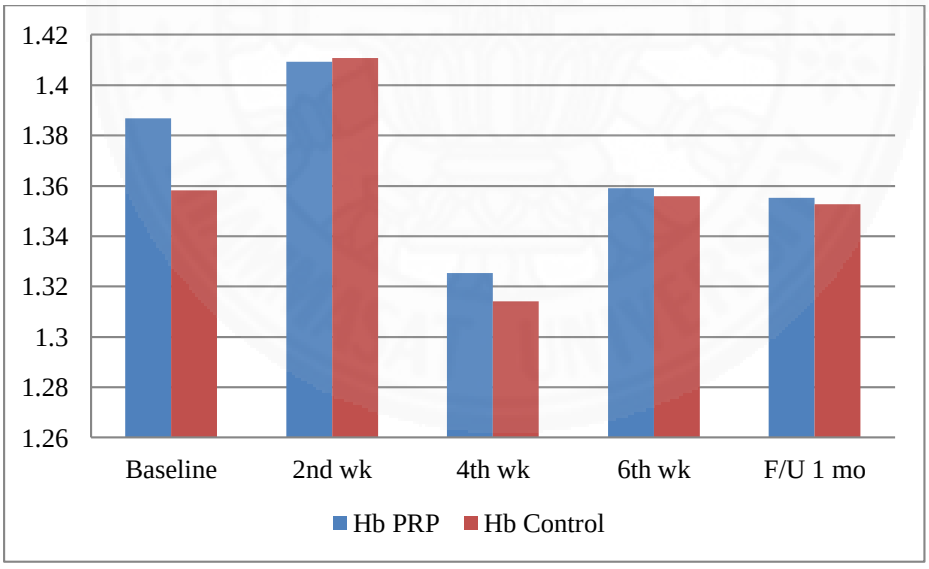


Figure 4.4 Antera® haemoglobin level

Table 4.5 Antera® haemoglobin level

Variables	PRP		Control	p-value[(b)]
	n	Mean ± SE.	Mean ± SE.	
Antera® haemoglobin level				
Baseline	10	1.39 ± 0.04	1.39 ± 0.03	0.929
Week 2	10	1.41 ± 0.05	1.41 ± 0.04	0.943
Week 4	10	1.31 ± 0.03	1.33 ± 0.02	0.632
Week 6	10	1.36 ± 0.03	1.36 ± 0.03	0.819
Week 10	10	1.35 ± 0.03	1.36 ± 0.02	0.907
p-value*(w)*				
Week 2		0.284	0.431	
Week 4		0.087	0.100	
Week 6		0.164	0.134	
Week 10		0.233	0.287	

Values presented as mean and Standard error (SE.). P-value corresponds to Paired t test.
(b) Comparisons between treatments. (w) Comparisons within treatments.

According to Figure 4.4 and Table 4.5 demonstates haemoglobin levels of the participants at each visit evaluated from Antera 3D biometric instruction. Clinical visits were scheduled at the baseline, week 2, week 4, week 6 and week 10. The statistical analysis was evaluated by Paired t test comparison within groups, and comparison between PRP group and control group.

Both PRP and control group at all visits did not show improvement in haemoglobin levels compared with baseline. Within group analysis of PRP group from baseline to the end of week 10 was from the mean score of 1.39 ± 0.04 to 1.35 ± 0.03 (P = 0.233). The mean score of control group from baseline to week 10 was from the mean score of 1.39 ± 0.03 to 1.36 ± 0.02 (P = 0.287).

Between group analysis PRP side and control side were not significantly different with regard to the changes in the mean score of haemoglobin levels at 10 weeks of the study (P = 0.929, 0.943, 0.632, 0.819, 0.907).

4.2.2.3 Antera® wrinkles level

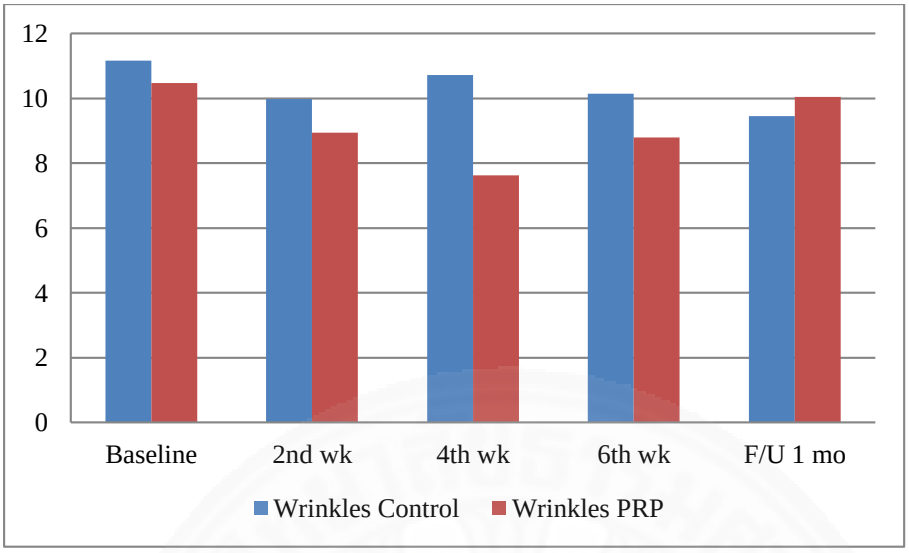


Figure 4.5 Antera® wrinkles level

Table 4.6 Antera® wrinkles level

Variables	PRP		Control	p-value[(b)]
	n	Mean ± SE.	Mean ± SE.	
Antera® wrinkles level				
Baseline	10	10.47 ± 1.26	11.17 ± 1.45	0.579
Week 2	10	8.94 ± 0.9	9.99 ± 1.43	0.233
Week 4	10	7.63 ± 1.06	10.73 ± 1.55	0.018*
Week 6	10	8.8 ± 0.87	10.15 ± 1.34	0.144
Week 10	10	10.05 ± 1.58	9.46 ± 0.71	0.678
p-value[(w)]				
Week 2		0.136	0.117	
Week 4		0.040*	0.569	
Week 6		0.121	0.186	
Week 10		0.709	0.166	

Values presented as mean and Standard error (SE.). P-value corresponds to Paired t test.
(b) Comparisons between treatments. (w) Comparisons within treatments.

According to Figure 4.5 and Table 4.6 demonstates wrinkes levels of the participants at each visit evaluated from Antera 3D biometric instruction. Clinical visits were scheduled at the baseline, week 2, week 4, week 6 and week 10.

The statistical analysis was evaluated by Paired t test comparison within groups, and comparison between PRP group and control group.

The mean reduction of wrinkles level was statistically significant in PRP group at week 4. Within group analysis of PRP group from baseline to the end of week 4 was from the mean score of 10.47 ± 1.26 to 7.63 ± 1.06 (P = 0.040).

The mean score of control group at all visits did not show improvement in wrinkles levels compared with baseline. Within group analysis of control group from baseline to the end of week 10 was from the mean score of 11.17 ± 1.45 to to 9.46 ± 0.71 (P = 0.166).

Between group analysis att week 4, the Antera3D® wrinkles levels in the PRP group was significantly lower than the control group (P = 0.018).

4.2.2.4 Antera® textures level

At 4th week, the Antera3D® textures level in the PRP group was significantly lower than baseline.

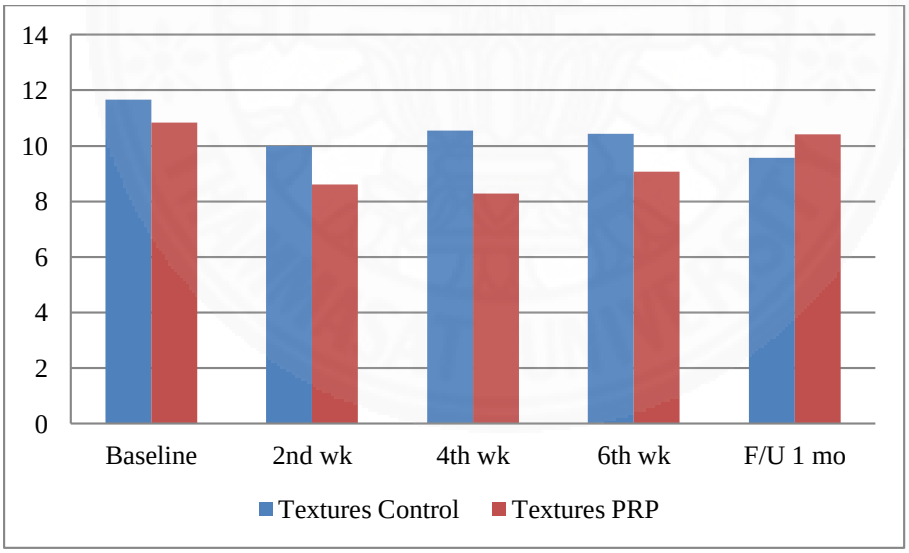


Figure 4.6 Antera® textures level

Table 4.7 Antera® textures level

Variables	PRP		Control	p-value[(b)]
	n	Mean ± SE.	Mean ± SE.	
Antera® textures level				
Baseline	10	10.83 ± 1.53	11.67 ± 1.58	0.525
Week 2	10	8.6 ± 0.87	10 ± 1.58	0.197
Week 4	10	8.28 ± 0.78	10.55 ± 1.52	0.061
Week 6	10	9.07 ± 0.97	10.44 ± 1.6	0.146
Week 10	10	10.41 ± 1.75	9.57 ± 0.84	0.608
p-value[(w)]				
Week 2		0.073	0.046*	
Week 4		0.041*	0.150	
Week 6		0.148	0.074	
Week 10		0.756	0.106	

Values presented as mean and Standard error (SE.). P-value corresponds to Paired t test.
(b) Comparisons between treatments. (w) Comparisons within treatments

According to Figure 4.6 and Table 4.7 demonstates textures levels of the participants at each visit evaluated from Antera 3D biometric instruction. Clinical visits were scheduled at the baseline, week 2, week 4, week 6 and week 10. The statistical analysis was evaluated by Paired t test comparison within groups, and comparison between PRP group and control group.

The mean reduction of textures level was statistically significant in PRP group at week 4. Within group analysis of PRP group from baseline to the end of week 4 was from the mean score of 11.67 ± 1.58 to 8.28 ± 0.78 (P = 0.041).

The mean reduction of textures level was statistically significant in control group at week 2. Within group analysis of control group from baseline to the end of week 4 was from the mean score of 10.83 ± 1.53 to 10 ± 1.58 (P = 0.046).

Between group analysis PRP side and control side were not significantly different with regard to the changes in the mean score of textures levels at 10 weeks of the study (P = 0.525, 0.197, 0.061, 0.146, 0.608).

4.2.3 TGF-beta1

Figure 4.7 TGF-beta1 plate

Table 4.8 Raw data of TGF-beta1

450 -570 nm	standard		subject1		subject2		subject3		subject4		subject5	
	sample1	sample2	sample1	sample2	sample1	sample2	sample1	sample2	sample1	sample2	sample1	sample2
A	1.32733	1.507741	0.019279	0.02072	0.015227	0.016501	0.012247	0.012026	0.017013	0.01596	0.02104	0.016208
B	0.351457	0.715879	0.021798	0.018771	0.012378	0.016802	0.013542	0.014001	0.015175	0.014804	0.017919	0.024587
C	0.513776	0.517252	0.018129	0.016648	0.015859	0.017807	0.017339	0.015281	0.017641	0.013482	0.018943	0.014721
D	0.272163	0.266817	0.019912	0.018212	0.013745	0.014545	0.014694	0.013629	0.087969	0.099728	0.014751	0.015874
450 -570 nm	standard		subject6		subject7		subject8		subject9		subject10	
	sample1	sample2	sample1	sample2	sample1	sample2	sample1	sample2	sample1	sample2	sample1	sample2
E	0.165125	0.084417	0.017878	0.017098	0.017478	0.018535	0.015856	0.019767	0.02184	0.017149	0.015424	0.017821
F	0.102035	0.110971	0.021833	0.01892	0.032695	0.025603	0.015122	0.015672	0.016118	0.016602	0.012768	0.01625
G	0.071913	0.054363	0.020001	0.01664	0.014233	0.017987	0.015676	0.016145	0.013196	0.014181	0.014284	0.014511
H	0.034882	0.020424	0.029813	0.024632	0.015371	0.016963	0.014329	0.014273	0.016543	0.021711	0.015368	0.016224

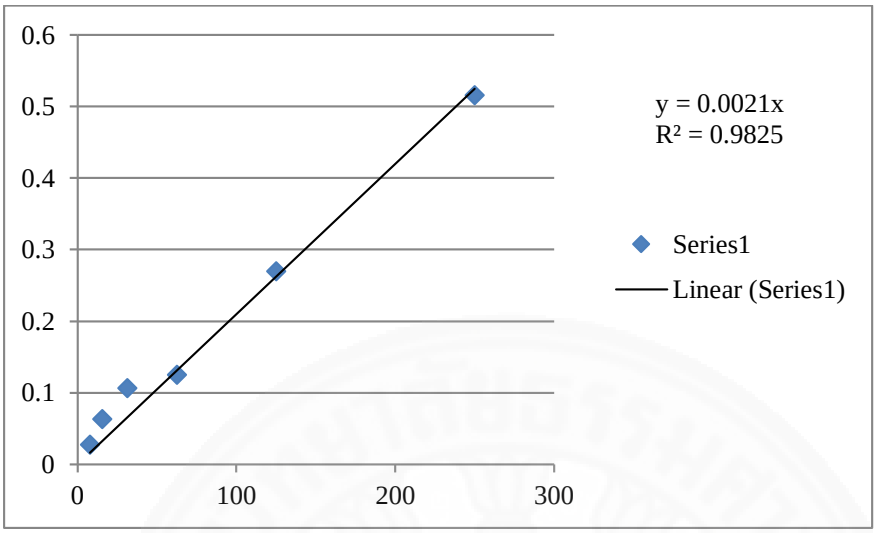


Figure 4.8 The equation of TGF-beta1 levels

Table 4.9 TGF-beta1 levels from the participants at the baseline, week 2, week 4 and week 6 (Positive value is equal or more than 7.8125 pg/mL).

TGF-beta1	Standard	Subject1		Subject2		Subject3	
(pg/mL)		OD	TGF-beta1	OD	TGF-beta1	OD	TGF-beta1
1000	1.4175358	0.0199998	-1.950125	0.0158637	-4.018175	0.0121366	-5.8817
500	0.5336684	0.0202844	-1.8078	0.0145901	-4.654975	0.0137717	-5.064175
250	0.5155144	0.0173885	-3.255775	0.016833	-3.5335	0.0163101	-3.794975
125	0.2694902	0.0190618	-2.4191	0.0141449	-4.877575	0.014161	-4.8695
62.5	0.1247708						
31.25	0.1065031						
15.625	0.0631379						
7.8125	0.027653						

TGF-beta1	Standard	Subject4		Subject5		Subject6	
(pg/mL)		OD	TGF-beta1	OD	TGF-beta1	OD	TGF-beta1
1000	1.4175358	0.0164864	-3.7068	0.0186243	-2.63785	0.0174879	-3.20605
500	0.5336684	0.0149894	-4.455325	0.0212527	-1.32365	0.0203765	-1.76175
250	0.5155144	0.0155619	-4.16905	0.0168321	-3.533975	0.0183207	-2.78965
125	0.2694902	0.0938482	34.9741	0.0153124	-4.2938	0.0272223	1.661125
62.5	0.1247708						
31.25	0.1065031						
15.625	0.0631379						
7.8125	0.027653						

TGF-beta1	Standard	Subject7		Subject8		Subject9		Subject10	
(pg/mL)		OD	TGF-beta1	OD	TGF-beta1	OD	TGF-beta1	OD	TGF-beta1
1000	1.4175358	0.0180064	-2.946825	0.0178116	-3.044225	0.0194948	-2.202625	0.0166225	-3.638775
500	0.5336684	0.0291492	2.624575	0.0153966	-4.2517	0.0163599	-3.770075	0.0145088	-4.695625
250	0.5155144	0.0161099	-3.895075	0.0159106	-3.9947	0.0136887	-5.105675	0.0143977	-4.75115
125	0.2694902	0.0161673	-3.866375	0.0143012	-4.799425	0.0191268	-2.3866	0.0157962	-4.051925
62.5	0.1247708								
31.25	0.1065031								
15.625	0.0631379								
7.8125	0.027653								

According to Figure 4.7, 4.8 and Table 4.8, 4.9 demonstates TGF-beta1 levels of the participants at each visit evaluated from Human TGF-β1 Immunoassay Procedure. Clinical visits were scheduled at the baseline, week 2, week 4, week 6 and week 10. Positive value is equal or more than 7.8125 pg/mL. Only one sample has been detected TGF-beta 1 level. (34.9741 pg/mL).

4.2.4 Side effects

At baseline, in PRP group had 3 mild pain during injection and 1 mild bruises after injection compared with control group which had 3 mild pain during injection.

At week 2, in PRP group had and 3 mild pain during injection compared with control group which had 4 mild pain during injection.

At week 4, in PRP group had 2 mild pain during injection compared with control group which had 4 mild pain during injection.

At week 6, in PRP group had 2 mild pain during injection compared with control group which had 3 mild pain during injection.

No irritation or edema was observed in both groups at all visits.

4.3 Subjective assessment

4.3.1 mMASI score

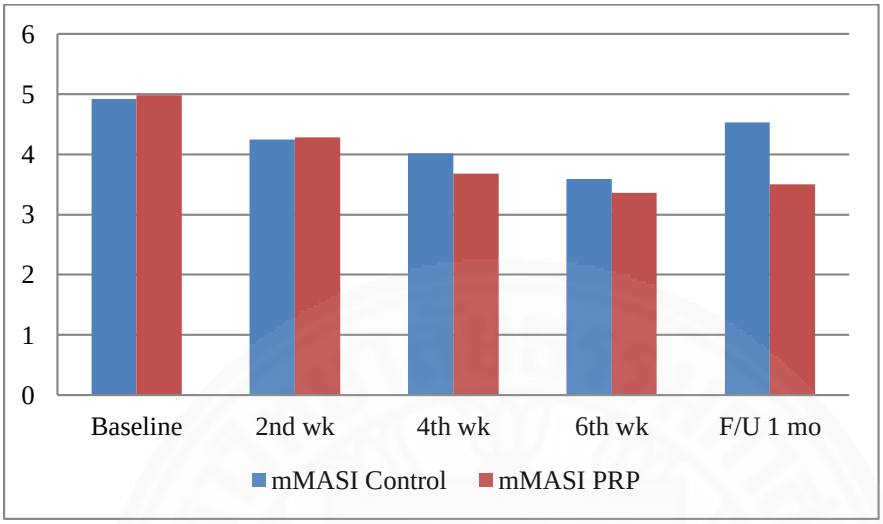


Figure 4.9 mMASI score results

Table 4.10 mMASI score results

Variables		PRP	Control	p-value[(b)]
	n	Mean ± SE.	Mean ± SE.	
mMASI score				
Baseline	10	4.98 ± 0.86	4.92 ± 0.96	0.903
Week 2	10	4.28 ± 0.77	4.25 ± 0.76	0.951
Week 4	10	3.68 ± 0.71	4.02 ± 0.81	0.416
Week 6	10	3.36 ± 0.63	3.59 ± 0.73	0.639
Week 10	10	3.5 ± 0.67	4.53 ± 0.96	0.042*
p-value[(w)]				
Week 2		0.014*	0.022*	
Week 4		0.002*	0.012*	
Week 6		<0.001*	0.014*	
Week 10		0.005*	0.566	

Values presented as mean and Standard error (SE.). P-value corresponds to Paired t test.
(b) Comparisons between treatments. (w) Comparisons within treatments.

According to Figure 4.9 and Table 4.10 demonstates mMASI score of the participants at each visit evaluated by two binded dermatologists. Clinical visits

were scheduled at the baseline, week 2, week 4, week 6 and week 10. The statistical analysis was evaluated by Paired t test comparison within groups, and comparison between PRP group and control group.

The mean reduction of mMASI score was statistically significant in PRP group at week 2, 4, 6 and 10. Within group analysis of PRP group from baseline to the end of week 2, 4, 6 and 10 was from the mean score of 4.92 ± 0.96 to 4.28 ± 0.77 (P = 0.014*), 3.68 ± 0.71 (P = 0.002*), 3.36 ± 0.63 (P = <0.001*) and 3.5 ± 0.67 (P = 0.005*) respectively.

The mean reduction of mMASI score was statistically significant in control group at week 2, 4 and 6. Within group analysis of control group from baseline to the end of week 2, 4 and 6 was from the mean score of 4.98 ± 0.86 to 4.25 ± 0.76 (P = 0.022*), 4.02 ± 0.81 (P = 0.012*) and 3.59 ± 0.73 (P = 0.014) respectively.

Between group analysis PRP side and control side were significantly different with regard to the changes in the mean score of mMASI score at week 10 of the study (P = 0.042).

4.3.2 Patients satisfaction

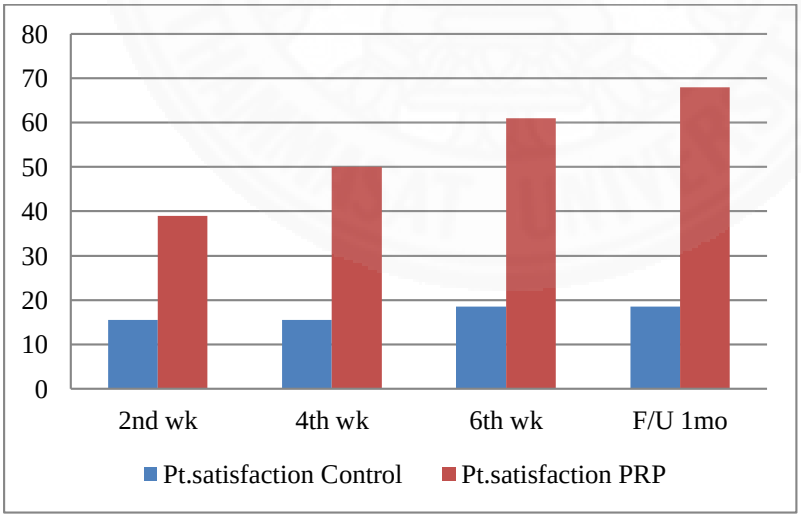


Figure 4.10 Patients satisfaction

Table 4.11 Patients satisfaction

Variables	PRP		Control	p-value[(b)]
	n	Mean ± SE.	Mean ± SE.	
Pt.satisfaction				
Week 2	10	39 ± 4.14	15.5 ± 2.17	0.001*
Week 4	10	50 ± 4.94	15.5 ± 2.17	<0.001*
Week 6	10	61 ± 4.07	18.5 ± 2.36	<0.001*
Week 10	10	68 ± 2.91	18.5 ± 2.36	<0.001*
p-value[(w)]				
Week 4		0.013*	N/A	
Week 6		<0.001*	0.081	
Week 10		<0.001*	0.081	

Values presented as mean and Standard error (SE.). P-value corresponds to Paired t test.
(b) Comparisons between treatments. (w) Comparisons within treatments.

According to Figure 4.10 and Table 4.11 demonstates patients satisfaction of the participants at each visit. Clinical visits were scheduled at the baseline, week 2, week 4, week 6 and week 10. The statistical analysis was evaluated by Paired t test comparison within groups, and comparison between PRP group and control group.

The patients satisfactionof PRP group was statistically significant increase at every visits. Within group analysis of PRP group from week 2 to week 4,6,10 was from the mean score of 39 ± 4.14 to 50 ± 4.94, 61 ± 4.07, 68 ± 2.91 respectively (P = <0.001*). The mean score of control group from baseline to week 10 was from the mean score of 15.5 ± 2.17 to 18.5 ± 2.36 (P = 0.081)

Between group analysis PRP side and control side were significantly different with regard to the changes in the mean score of the patients satisfaction at 10 weeks of the study (P = <0.001*).

4.3.3 WHO QOL (See details of the questionnaire at appendix D)

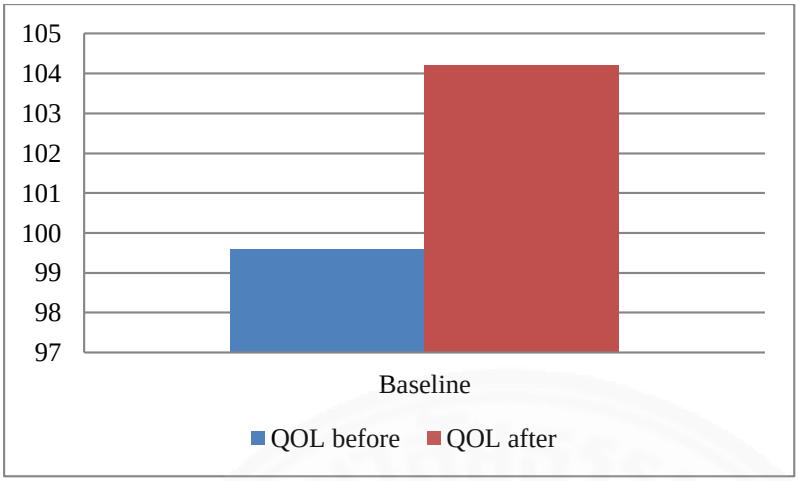


Figure 4.11 QOL of subjects.

Table 4.12 QOL of subjects.

	Before	After	Difference	p-value
Quality of life	99.6 ± 4.03	104.2 ± 3.8	4.6 ± 3	0.160

Values presented as mean and Standard error (SE.). P-value corresponds to Paired t test.

According to Figure 4.11 and Table 4.12 demonstates QOL score of the participants before and after the experiment. The statistical analysis was evaluated by Paired t test. QOL score before the experiment was increased from 99.6 ± 4.03 to 104.2 ± 3.8 after the experiment (P = 0.160).

4.3.4 Pain score

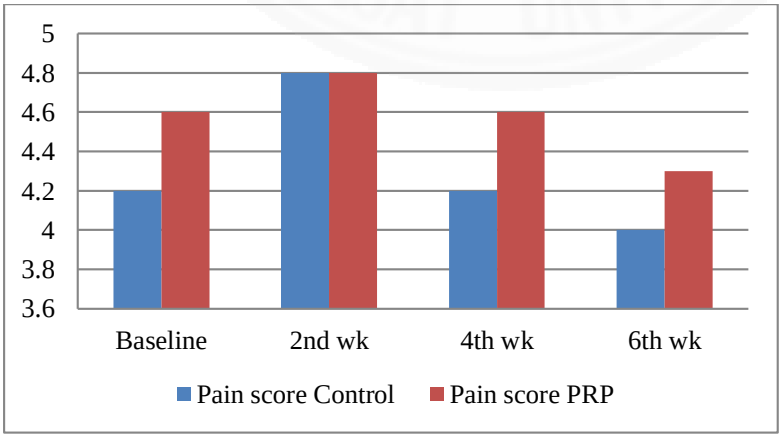


Figure 4.12 Pain score

Table 4.13 Pain score

Variables		PRP	Control	p-value[(b)]
	n	Mean ± SE.	Mean ± SE.	
Pain score				
Baseline	10	4.6 ± 0.69	4.2 ± 0.76	0.399
Week 2	10	4.8 ± 0.68	4.8 ± 0.59	1.000
Week 4	10	4.6 ± 0.75	4.2 ± 0.61	0.168
Week 6	10	4.3 ± 0.78	4 ± 0.56	0.343
p-value[(w)]				
Week 2		0.758	0.239	
Week 4		1.000	1.000	
Week 6		0.656	0.591	

Values presented as mean and Standard error (SE.). P-value corresponds to Paired t test.
(b) Comparisons between treatments. (w) Comparisons within treatments.

According to Figure 4.12 and Table 4.13 demonstates pain score of the participants at each visit. Clinical visits were scheduled at the baseline, week 2, week 4, week 6. The statistical analysis was evaluated by Paired t test comparison within groups, and comparison between PRP group and control group.

Withing group analysis both PRP and control group at all visits did not show any difference in pain scores compared with baseline. Pain score of PRP group from baseline to the end of week 10 was from the mean score of 4.6 ± 0.69 to 4.3 ± 0.78 (P = 0.656). The mean score of control group from baseline to week 10 was from the mean score of 4.2 ± 0.76 to 4 ± 0.56 (P = 0.591).

Between group analysis PRP side and control side were not significantly different with regard to the changes in the mean score of pain scores at every visits. (P = 0.399, 1.000, 0.168, 0.343).

CHAPTER 5
DISCUSSIONS AND RECOMMENDATIONS

5.1 Discussion

Melasma is an acquired hyperpigmented skin disorder commonly found in Thailand. Despite several treatments for melasma, the treatment results are variable success with some complications such as irritation, PIH and rebound hyperpigmentation.

In recent times, PRP is becoming to get attention in aesthetic medicine (26). PRP is blood plasma that has been enriched with platelets. As a concentrated source of autologous platelets, PRP contains several different growth factors and other cytokines that can stimulate various effects of soft tissue. Interestedly, we aimed to assess the effectiveness of PRP injection in melasma treatment. Hence, PRP could be a promising treatment with less side effects and rebound hyperpigmentation compared to other melasma treatments.

In 2014, Mutlu Çayırlı *et al.* studied the effects of PRP injection in one patient by injecting whole face every two weeks for three times. At the end of the treatment, more than 80% reduction in epidermal hyperpigmentation was observed with no recurrence in six months (19).

A case reported by Yew CH *et al.* showed that the reduction of mean mMASI score in two cases (33.5% and 20%) after administered ID PRP with QS-Nd:YAG montly for two sessions and applied topical alpha arbutin every day (18).

To our knowledge, our trial is the only controlled study applying PRP injection in split face of melasma patients.

In our study, ten volunteers with melasma were randomly treated with ID PRP injection at one side of the face and ID normal saline injection at another side as control group every two weeks for four times and then follow up one month after the last treatment.

The study was conducted during 10 weeks to evaluate melasma patients in both the objective assessment inclucing mexameter and Antera 3D camera analysis and

the subjective assessment including mMASI score, Patients satisfaction. Moreover, we also measured TGF-beta1 levels at every treatment visits.

The most notable finding of this study was that the mean mMASI score was significantly reduced from 4.92 ± 0.96 to 3.5 ± 0.67, showing 28.9 % improvement after four sessions of treatment in PRP group, whereas in control group, the score reduced from 4.98 ± 0.86 to 4.53 ± 0.96 at week 10. The decrease in the mMASI score between these two groups was statistically significant (P = 0.042).

In addition, the mean melanin level of PRP group was significantly reduced from 0.61 ± 0.02 at baseline to 0.57 ± 0.03 at week 6 (P = 0.038).

Even though between group analysis of both melanin index and Antera melanin levels were not significantly different, PRP group had a trend toward lower the levels than control group.

Accordingly, the patients' satisfaction in the PRP group was significantly improved when compared with the control group from week 2 to week 10.

These results showed that PRP could not improve the melasma lesions significantly. The different result from earlier case report could be explained by the younger subject of previous study, the different anticoagulant and method of PRP preparation.

Both treatment regimens did not show different improvement from baseline in term of both erythema index and Antera 3D haemoglobin levels. Meanwhile, the values of PRP group have a lower trend, that of control group had a higher trend. This might suggesting that PRP be less concern in the mechanism of redness.

In case of wrinkles, PRP group showed significantly lower in value as compared to that of control at week 4 (P = 0.018). There are many studies shown the rejuvenation effect of PRP by stimulating fibroblast and collagen proliferation, enhancing hyaluronic acid synthesis. The main growth factors with extracellular matrix production properties in PRP are EGF, PDGF, TGF-beta1 and FGF (45).

Correlatively, texture PRP group demonstrated a significant reduction at week 4 comparing with baseline (P = 0.041). However, texture level of control group also showed a significant reduction at week 2. All sessions of both groups were not significantly different. The texture improvement of both groups could be the effect of mechanical injury inducing collagen production.

Growth factos'levels of each subject was considered to be a part of treatment success. Evanson J et al. demonstrated that both age and gender are the main factors for various GFs. All GFs that they analyzed (EGF,HGF,IGF-1,PDGF-AB,PDGF-BB,TFGβ-1) had higher levels in younger than 25 females.. Five of GFs (EGF,IGF-1,PDGP-AB,PDGF-BB and TGFβ-1) achieved significance of people ≤ 25 years old (47).

In case of TGF-beta1 level in our study, the positive result was found only in one sample from the youngest subject (age 33), while the samples from other subjects were negative. Our previous hypothesis believed that TGF-beta1 is relate to the improvement of melasma. In this study the results showed no statistically significant change and the levels of TGF-beta1 was lower than expected. So we could not conclude whether TGF-beta1 level correlate with melasma improvement.

Another possible reason can help explaining was that beside TGF-beta1, there were several other growth factors performing in the improvement of pigmentation. Previous knowledge demonstrated that TGF-beta1 is not the only factors, but EGF is also play an important role as melanogenesis inhibition.

It has been reported that PRP injection cause adverse effects, such as mild pain and bruises. All side effects were mild and resolved withing a few days. Furthermore, no significant difference was observed between the PRP and the normal saline groups using pain score.

In this study, we have demonstrated that the treatment of melasma with PRP injection obtains a non-significant improvement in the QOL (P = 0.160).

The limitations of the study design include its small sample size and the relatively short follow-up period of 1 month.

In conclusion, PRP injection can significantly improve melasma within 10 weeks of treatment with regard to the changes in the mMASI score and the patients the patients' satisfaction. Interestingly, there were not statistically significant difference between PRP and control in the melanin index, erythema index, Antera 3D levels (melanin, haemoglonbin and texture) during 10 weeks of the study. Therefore, the improvement obtained on the NSS side is largely due to the daily use of SPF 50 sunscreen.

5.2 Recommendations

5.2.1 PRP injection can demonstrate the improvement of melasma in the subjective assessments.Though, both regimens did not reveal significant difference objective results, there are some trends in more reducing pigmentation from PRP than control.

5.2.2 With non-significant difference of treatment results, intradermal PRP could be an adjuvant therapy for melasma. However, larger and longer randomized, double-blinded, placebo-controlled trials are recommended for long-term efficacy and safety.

5.2.3 PRP would permit its use in other indications such as skin rejuvenation.

5.2.4 Young and healthy patients will get more effectiveness of PRP than old and unhealthy ones.

5.2.5 Further studies in other growth factor expression according to melanogenesis reduction which will benefit in providing further information on hyperpigmentation improvement.

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APPENDICES

APPENDIX A
Human TGF-β1 Immunoassay Procedure

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-53 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-53 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-53 (diluted 1:4). May contain a precipitate. Mix well before and during use.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

TGF-β1 Standard - Refer to the vial label for reconstitution volume. Reconstitute the TGF-β1 Standard with Calibrator Diluent RD5-53 (diluted 1:4). Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-53 (diluted 1:4) into each tube. Use the standard stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted TGF-β1 Standard serves as the high standard (2000 pg/mL). Calibrator Diluent RD5-53 (diluted 1:4) serves as the zero standard (0 pg/mL). Discard any unused reconstituted TGF-β1 Standard after use.

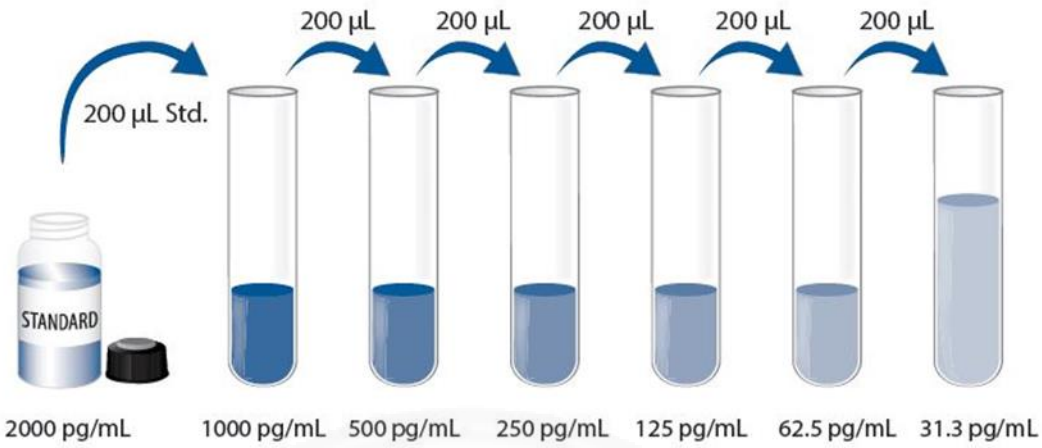

Figure 6.1 Calibrator Diluent RD5-53 preparation

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, standard dilutions, and activated samples as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Add 50 µL of Assay Diluent RD1-73 (*for serum/plasma samples*) to each well.

4. Add 50 µL of Standard, control, or activated sample* per well. Tap the plate gently to mix. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.

5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 100 µL of TGF-β1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.

7. Repeat the aspiration/wash as in step 5.

8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**

9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

APPENDIX B
DIGITAL IMAGES OF SUBJECTS

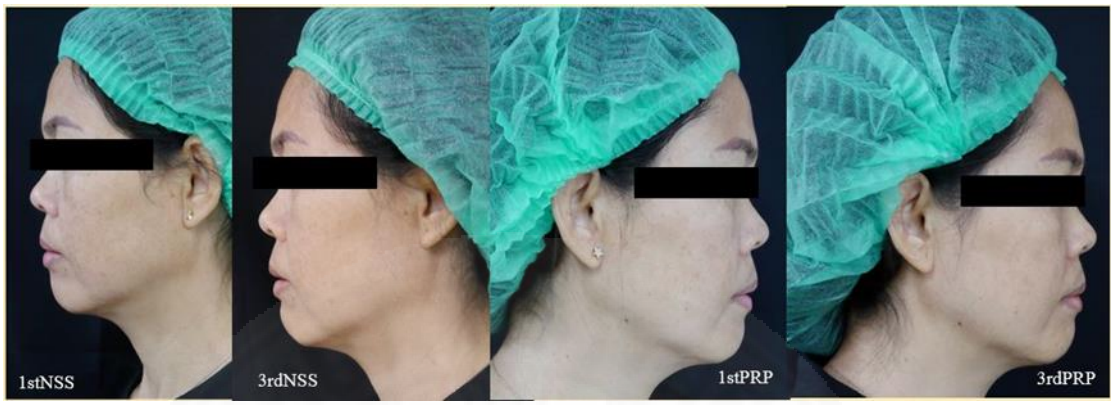

Figure 6.1 Photographs of subject 1 showing, (A) NSS-treated side at 2nd week , (B) NSS-treated side at 6th week, (C) PRP-treated side at 2nd week, (D) PRP-treated side after 6th of treatment

Figure 6.2 Photographs of subject 2 showing, (A) NSS-treated side at 2nd week , (B) NSS-treated side at 6th week, (C) PRP-treated side at 2nd week, (D) PRP-treated side after 6th of treatment

APPENDIX C

RECORD FORM

Record form

General information

1. Province
   Province [ ]
2. Sex Female
3. Age …… years Age [ ]
4. Smoking 1. Yes 2. No
   Smoking [ ]
5. Drinking 1. Yes 2. No
   Drinking [ ]
6. Underlying disease 1. Yes …….. 2. No
   Disease [ ]
7. Allergy
   7.1 Drugs 1. Yes …….. 2. No Drug [ ]
   7.2 Food 1. Yes …….. 2. No Food [ ]
8. Duration of lesion 1. < 5 yrs 2. 5-10 yrs 3. >10 yrs
   Durtion [ ]
9. Effect to daily activity 1. None 2. Mininal 3. Mild
   Activity [ ]
   4. Moderate 5. Severe

Physical examination

1. Color 1.Light brown 2. Brown 3. Dark brown
   Color [ ]
2. Site
3. Size

4. Outcome measurement

Assessment	Treatment(wk)				F/U(wk)
Treatment	0	2	4	6	10
Photography	*[ ]	*[ ]	*[ ]	*[ ]	*[ ]
Antera 3D®	*[ ]	*[ ]	*[ ]	*[ ]	*[ ]
Mexameter	*[ ]	*[ ]	*[ ]	*[ ]	*[ ]
mMASI score	*[ ]	*[ ]	*[ ]	*[ ]	*[ ]
TGF-β1 level	*[ ]	*[ ]	*[ ]	*[ ]	
Side effect		*[ ]	*[ ]	*[ ]	*[ ]
Patients satisfaction		*[ ]	*[ ]	*[ ]	*[ ]
Quality of life index	*[ ]				*[ ]
Pain score	*[ ]	*[ ]	*[ ]	*[ ]	

APPENDIX D
The World Health Organization Quality of Life (WHOQOL) Questionaire

ABOUT YOU

Before you begin we would like to ask you to answer a few general questions about yourself: by circling the correct answer or by filling in the space provided.

What is your **gender**? Male Female

What is you **date of birth**? _____ / _____ / _____
Day / Month / Year

What is the highest **education** you received?
None at all
Primary school
Secondary school
Tertiary

What is your **marital status**?
Single Separated
Married Divorced
Living as married Widowed

Are you currently **ill**? Yes No

If something is wrong with your health what do you think it is?
_____illness/ problem

Instructions

This assessment asks how you feel about your quality of life, health, or other areas of your life. **Please answer all the questions.** If you are unsure about which response to give to a question, **please choose the one** that appears most appropriate. This can often be your first response.

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life **in the last two weeks.** For example, thinking about the last two weeks, a question might ask:

		Not at all	Not much	Moderately	A great deal	Completely
	Do you get the kind of support from others that you need?	1	2	3	4	5

You should circle the number that best fits how much support you got from others over the last two weeks. So you would circle the number 4 if you got a great deal of support from others as follows.

		Not at all	Not much	Moderately	A great deal	Completely
	Do you get the kind of support from others that you need?	1	2	3	4	5

You would circle number 1 if you did not get any of the support that you needed from others in the last two weeks.

Please read each question, assess your feelings, and circle the number on the scale for each question that gives the best answer for you.

		Very poor	Poor	Neither poor nor good	Good	Very good
1(G1)	How would you rate your quality of life?	1	2	3	4	5

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
2 (G4)	How satisfied are you with your health?	1	2	3	4	5

The following questions ask about **how much** you have experienced certain things in the last two weeks.

		Not at all	A little	A moderate amount	Very much	An extreme amount
3 (F1.4)	To what extent do you feel that physical pain prevents you from doing what you need to do?	1	2	3	4	5
4(F11.3)	How much do you need any medical treatment to function in your daily life?	1	2	3	4	5
5(F4.1)	How much do you enjoy life?	1	2	3	4	5
6(F24.2)	To what extent do you feel your life to be meaningful?	1	2	3	4	5

		Not at all	A little	A moderate amount	Very much	Extremely
7(F5.3)	How well are you able to concentrate?	1	2	3	4	5
8 (F16.1)	How safe do you feel in your daily life?	1	2	3	4	5
9 (F22.1)	How healthy is your physical environment?	1	2	3	4	5

The following questions ask about **how completely** you experience or were able to do certain things in the last two weeks.

		Not at all	A little	Moderately	Mostly	Completely
10 (F2.1)	Do you have enough energy for everyday life?	1	2	3	4	5
11 (F7.1)	Are you able to accept your bodily appearance?	1	2	3	4	5
12 (F18.1)	Have you enough money to meet your needs?	1	2	3	4	5
13 (F20.1)	How available to you is the information that you need in your day-to-day life?	1	2	3	4	5
14 (F21.1)	To what extent do you have the opportunity for leisure activities?	1	2	3	4	5

		Very poor	Poor	Neither poor nor good	Good	Very good
15 (F9.1)	How well are you able to get around?	1	2	3	4	5

The following questions ask you to say how **good or satisfied** you have felt about various aspects of your life over the last two weeks.

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
16 (F3.3)	How satisfied are you with your sleep?	1	2	3	4	5
17 (F10.3)	How satisfied are you with your ability to perform your daily living activities?	1	2	3	4	5
18(F12.4)	How satisfied are you with your capacity for work?	1	2	3	4	5
19 (F6.3)	How satisfied are you with yourself?	1	2	3	4	5
20(F13.3)	How satisfied are you with your personal relationships?	1	2	3	4	5
21(F15.3)	How satisfied are you with your sex life?	1	2	3	4	5
22(F14.4)	How satisfied are you with the support you get from your friends?	1	2	3	4	5
23(F17.3)	How satisfied are you with the conditions of your living place?	1	2	3	4	5
24(F19.3)	How satisfied are you with your access to health services?	1	2	3	4	5
25(F23.3)	How satisfied are you with your transport?	1	2	3	4	5

The following question refers to **how often** you have felt or experienced certain things in the last two weeks.

		Never	Seldom	Quite often	Very often	Always
26 (F8.1)	How often do you have negative feelings such as blue mood, despair, anxiety, depression?	1	2	3	4	5

Did someone help you to fill out this form?.....

How long did it take to fill this form out?.....

Do you have any comments about the assessment?

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THANK YOU FOR YOUR HELP

BIOGRAPHY

Name	Arada Dannarongchai
Date of Birth	30 April 1988
Educational Attainment	The Degree of Doctor of Medicine, Faculty of Medicine Vajira Hospital, Mahidol University
Work Position	General Physician
	Master of Science (Dermatology) Chulabhorn International College of Medicine,Thammasat University Academic Year 2016
Work Experiences	2012-currently : Private clinics as general physician
Publication	None
Award	None