THE EFFICACY OF POLY-D, L-LACTIC ACID (PDLLA) BIOSTIMULATOR INJECTION ON FACIAL REJUVENATION MARKERS IMPROVEMENT OF THAI MIDDLE AGE

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DOCTOR OF PHILOSOPHY
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THIS DISSERTATION IS A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ANTI-AGING AND REGENERATIVE MEDICINE

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Chaichana Srituravanich

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ABSTRACT

Background: The quality of skin, especially its elasticity, hydration, and texture, diminishes with age, contributing to visible signs of aging. Poly-D, L-lactic acid (PDLLA) has emerged as a biostimulator that can potentially improve facial skin quality through collagen stimulation.

Aim: This study aims to assess the efficacy of PDLLA in improving facial rejuvenation markers in early and late middle age groups.

Method: A quasi-experimental study was conducted on 30 participants divided into two age groups (early middle age group: 30-45 years and late middle age group: 45-60 years). Each participant received subdermal PDLLA injections over six months, with skin quality measurements taken at baseline, 2, 4, and 6 months using various instruments including sebum level, elasticity, skin hydration, transepidermal water, spot, pore, wrinkle, and texture. The present study also assesses for GAIS score, patients' satisfaction score and treatment-related side effect.

Results: Significant improvements in skin elasticity, wrinkles, pores, transepidermal water loss (TEWL), and skin hydration were observed starting from two months after treatment and monitored for six months, with more pronounced effects in the older age group. No severe adverse effects were reported.

Conclusion: PDLLA is an effective and safe biostimulator for enhancing facial skin quality of Thai middle age group.

Keywords: Poly-D, L-lactic Acid, Facial Rejuvenation, Middle Age, Sebum, Elasticity, TEWL, Hydration

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ABBREVIATIONS AND SYMBOLS

AU	Arbitrary Units
CaHA	Calcium Hydroxyapatite
CMC	Carboxymethyl Cellulose
GAIS	Global Aesthetic Improvement Scale
Mean diff	Mean Difference
PCL	Polycaprolactone
PLA	Poly Lactic Acid
PLLA	Poly-L-Lactic Acid
PDLLA	Poly-D,L-Lactic Acid
PDO	Polydioxanone
SD	Standard Deviation
TEWL	Transepidermal Water Loss
$\bar{x}$	Mean

CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Aging constitutes an inescapable and intricate biological phenomenon that exerts its influence on all living organisms, manifesting through a multitude of physiological and anatomical transformations. In the case of humans, one of the most conspicuous and notable indications of aging becomes evident within the integumentary system, specifically in the skin. Functioning as the body's largest organ, the skin assumes the pivotal role of shielding against environmental factors and actively contributing to the maintenance of overall health. With the progression of age, individuals undergo a series of structural and functional modifications in their skin, culminating in the emergence of diverse dermatological concerns, including but not limited to the appearance of wrinkles, sagging, irregularities in pigmentation, and a decrease in elasticity. (Lorenc & Lee, 2016). In the 20th and 21st centuries, the extension of life expectancy underscores the necessity for strategies promoting healthy aging. Preventing physical and mental ailments associated with old age has become imperative. Given its perceptible nature, the health and appearance of the skin possess the capacity to impact self-esteem, mental well-being, social interactions, and the overall quality of life. Over the years, a myriad of skincare products, treatments, and interventions have been developed to address the effects of aging skin, ranging from topical creams and minimally invasive procedures to surgical interventions. Among these approaches, the use of biostimulators has emerged as a promising avenue for rejuvenating aging skin, especially PLA.

PLA (Poly Lactic Acid, Polylactide) is an aliphatic thermoplastic polymer that originates entirely from renewable sources, such as corn and potato starch (Xiao et al., 2012). One remarkable feature of PLA is its biodegradability, which allows medical substances containing PLA to break down naturally within the body after fulfilling their intended functions. Unlike non-degradable polymers that necessitate surgical removal

to prevent chronic issues associated with foreign objects left in the body, biodegradable PLA eliminates this requirement, offering a significant advantage in medical applications (Farah et al., 2016). Furthermore, PLA exhibits pronounced hydrophobic properties and acts as a biostimulator, meaning it has the capability to trigger an inflammatory response within the tissues of living organisms (Xiao et al., 2012). Lactic acid exists as a chiral molecule, with two enantiomers known as L and D-lactic acid. Through the process of polymerization, PLA gives rise to four distinct PLA (Poly Lactic Acid) substances: poly-D-lactic acid (PDLA), poly-L-lactic acid (PLLA), poly-D, L-lactic acid (PDLLA), and meso-PLA (Pretula et al., 2016). It is noteworthy that a diverse array of biostimulators has emerged with promising experimental researches. Among these biostimulators, noteworthy examples include polycaprolactone (PCL) (Angelo-Khattar, 2022), Calcium Hydroxyapatite (CaHA) (Corduff et al., 2021), and Polydioxanone (PDO) (Kwon et al., 2019).

Among these biostimulators, poly-D, L-lactic acid (PDLLA) stands out as a collagen stimulant, offering distinctive and highly effective methods for addressing tissue defects, leading to a natural and long-lasting enhancement. PDLLA is presently recognized as one of the most enduring among all approved and certified biodegradable products. PDLLA is employed for addressing both deep and superficial facial wrinkles, and it is available in the form of lyophilized powder lumps contained within vials. While certain dermal fillers, such as hyaluronic acid, provide immediate volume upon injection, PDLLA take a biostimulatory approach. PDLLA is recognized as one of the products that stimulates collagen production, confirmed by experiments in animals and laboratory tubes (Kwon et al., 2019; Lin et al., 2019). PDLLA activates resident fibroblasts to generate autologous collagen gradually, resulting in a more natural and enduring enhancement. The mechanism by which PDLLA triggers this neocollagenesis involves initiating a foreign body reaction to the injected material, followed by a cellular inflammatory response that fosters the development of vascularized connective tissue (Lin et al., 2019). Subsequently, PDLLA undergoes hydrolysis into lactate, which is then converted to pyruvate and ultimately oxidized into carbon dioxide (Herrmann et al., 2018). Although the initial inflammatory response typically subsides within six months, the production of extracellular matrix continues, leading to a gradual increase in dermal thickness that can persist for at least

two years (No et al., 2015). PDLLA is considered safe, supported by research demonstrating its complete biodegradability. A study by Achtnich A and colleagues, using PDLLA screws in knee surgery, found no remaining PDLLA screws in MRI scans after 22 months, with no signs of inflammation and normal surrounding tissues and bones (Achtnich et al., 2014). Additionally, a study by No YA and colleagues, tracking outcomes after using PDLLA for cheek augmentation in 58 patients for two years, revealed improved skin quality and no severe side effects, only minor and self-resolving issues such as bruising (No et al., 2015). Furthermore, PDLLA has shown promising results in improving skin quality in studies using Poly-l-Lactic Acid (PLLA), a structurally similar substance, injected on the face by Bohnert K and colleagues. Parameters such as elasticity, hydration, transepidermal water loss, pigmentation, and clinical score assessments by both physicians and patients improved (Bohnert et al., 2019).

In conclusion, PDLLA emerges as an effective and safe collagen biostimulator, supported by evidence from in-vivo, in-vitro, and clinical trials. There is evidence suggesting improvements in patients' skin quality following PDLLA injections due to its sharing of enantiomer with PLLA. However, it's important to note that the evidence for PDLLA is currently scarce and indirect. Therefore, the primary aim of this study was to examine the overall impact on skin quality from repeated subdermal PDLLA injections on midface area at two different time points (1st injection at 0 months, and 2nd injection at 2 months). This evaluation encompassed both subjective assessments (utilizing the Global Aesthetic Improvement Scale by the investigators and Satisfactory score by the patients) and objective measurements obtained from the Visia CR system, which assesses spots, pores, wrinkles, and texture. Additionally, we employed other devices to measure sebum levels, elasticity, skin capacitance, transepidermal water loss, dermal thickness and monitored for side effects. These assessments were conducted at specific time points (0 months, 2 months, 4 months, and 6 months). To the best of our knowledge, this study represents the first investigation into the subjective and objective effectiveness by comparing before and after the injection of PDLLA in Thailand.

1.2 Research Questions

Does PDLLA have efficacy in facial rejuvenation in Thai middle age group?

1.3 Objectives

1.3.1 Primary Objective

To compare the efficacy of PDLLA on facial rejuvenation on month 0, 2, 4, and 6 of Thai middle age group

1.3.2 Secondary Objectives

1.3.2.1 To compare the effect of PDLLA on facial rejuvenation markers including sebum level, elasticity, skin capacitance, TEWL, spot, pore, wrinkle, and texture on month 0, 2, 4, and 6

1.3.2.2 To measure Global Aesthetic Improvement Scale after receiving PDLLA treatment for 2 months, 4 months, and 6 months

1.3.2.3 To assess the satisfaction score for PDLLA among participants at the end of the study

1.3.2.4 To determine the incidence of side effects from PDLLA during the entire study

1.4 Hypothesis

1.4.1 PDLLA has efficacy in facial rejuvenation:

1.4.1.1 PDLLA can increase elasticity, as measured by Cutometer® MPA580, when compared between 0 months, 2 months, 4 months, and 6 months

1.4.1.2 PDLLA can improve skin quality by the reduction of spots, pores, wrinkles, and texture using the Visia CR system, when compared between 0 months, 2 months, 4 months, and 6 months

1.4.1.3 PDLLA can reduce sebum levels, as measured by Sebumeter® SM815, when compared between 0 months, 2 months, 4 months, and 6 months

1.4.1.4 PDLLA can increase skin capacitance, as measured by

Corneometer® CM825, when compared between 0 months, 2 months, 4 months, and 6 months

1.4.1.5 PDLLA can reduce transepidermal water loss, as measured by Tewameter® TM300, when compared between 0 months, 2 months, 4 months, and 6 months

1.4.2 PDLLA can increase Global Aesthetic Improvement Scale when compared between 2 months, 4 months, and 6 months

1.4.3 There is no satisfaction score less than 3 among the participants after the study

1.4.4 There is no report of serious side effects during the 6-month study.

1.5 Variables of Research

1.5.1 Independent Variable

Poly-D,L-Lactic Acid (PDLLA)

1.5.2 Dependent Variables

1.5.2.1 Subjective Data for Facial Rejuvenation:

1. Global Aesthetic Improvement Scale (GAIS score) by the investigator
2. Satisfaction score by the participants

1.5.2.2 Objective Data for Facial Rejuvenation:

1. Skin Quality as measured by Visia CR system (Spots, Pores, Wrinkles, and Texture)
2. Sebum Level as measured by Sebumeter® SM815
3. Elasticity as measured by Cutometer® MPA580
4. Skin Capacitance as measured by Corneometer® CM825
5. Transepidermal Water Loss (TEWL) as measured by Tewameter® TM300

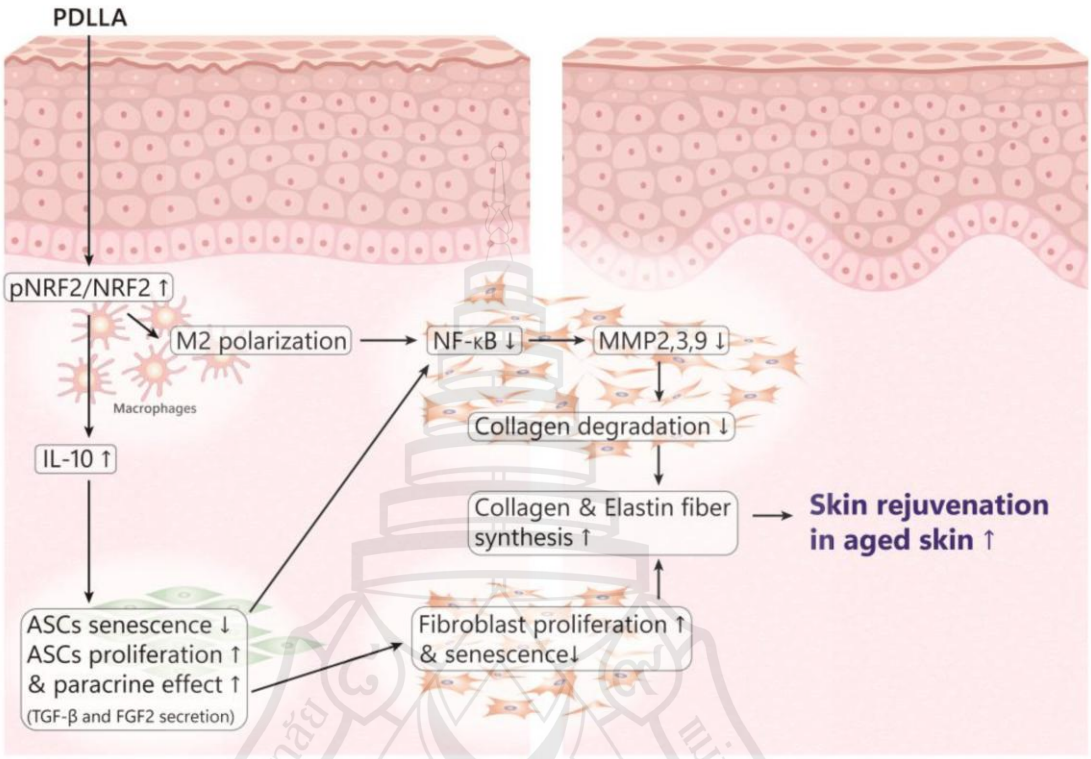
1.5.2.3 Side effect using the side effect record form

1.6 Scope of Research

We recruited 30 volunteers who came to visit and work at Mae Fah Luang University Hospital (Bangkok) as our study population. The included volunteers were either male or female aged between 30 to 60 years categorized to late middle age group (45-60 years, n = 15) and early middle age group (30-45 years, n = 15). We exclude those with a poor medical condition to ensure better cooperation and those with conditions that can interfere with the outcomes, such as active skin disease, acne scar, and those with pregnancy or on breast feeding.

After the recruitment, the participant will be scheduled for a total of 4 visits (0 months, 2 months, 4 months, and 6 months). They will be injected 2 sessions (2 months apart at 0 months and 2 months) of PDLLA on their midface area using 23G blunt canular on subdermal plane. During the 4 visits, the participants will be assessed using various parameters, including the subjective data for facial rejuvenation (Global Aesthetic Improvement Scale ,Satisfaction score), objective data for facial rejuvenation as measured by Visia CR system (Spots, Pores, Wrinkles, and Texture), Sebum Level as measured by Sebumeter® SM815, Elasticity as measured by Cutometer® MPA580, Skin Capacitance as measured by Corneometer® CM825, and Transepidermal Water Loss (TEWL) as measured by Tewameter® TM300. They will also be assessed for the Side effect using the side effect record form over the entire study duration.

1.7 Conceptual Framework



Source Lee et al. (2024)

Figure 1.1 Conceptual Framework of Mechanism of Action of poly-D,L-lactic acid (PDLLA) on Facial Rejuvenation

1.8 Operational Definitions

1.8.1 Poly Lactic Acid (PLA)

Poly lactic acid (PLA) is a biodegradable and biocompatible thermoplastic polymer derived from renewable resources, such as corn or potato starch. In the context of this manuscript, PLA refers to the specific polymer used for injectable cosmetic procedures. It is intended for subdermal use and is administered through injection to address various aesthetic concerns, including facial volume loss, the reduction of wrinkles, and the improvement of skin quality. PLA is expected to stimulate collagen production within the skin, resulting in an improvement in skin quality and the

reduction of visible signs of aging. There are several types of PLA. However, we only focus on:

Poly-D,L-Lactic Acid (PDLLA): Poly-D,L-Lactic Acid (PDLLA) is a biodegradable thermoplastic polymer composed of lactic acid molecules. In the context of this manuscript, PDLLA refers to a specific variant of poly lactic acid (PLA) known as AestheFill, developed by REGEN, received its initial approval for use in South Korea in 2014. The products are provided in the form of lyophilized powders contained within vials. Prior to injection, these powders must be reconstituted with sterile water. It is commonly used in aesthetic and medical procedures. When injected subdermally, it is intended to stimulate collagen production and improve skin quality (Lin et al., 2019).

1.8.2 Facial Rejuvenation Markers

Despite the increasing recognition of the significance of skin quality in human evolution, psychology, aesthetic treatments, clinical research, and practice, there remains a noticeable scarcity of literature and a lack of uniform descriptive terminology regarding skin quality parameters. Good skin quality is characterized by skin that exhibits a healthy, undamaged, and youthful appearance, often appearing younger than a person's chronological age (Goldie et al., 2021). One of the frameworks that identifies attributes contributing to skin quality in healthy skin was organized into three fundamental categories: visual, topographical, and mechanical (Humphrey et al., 2021). Visual attributes are discernible solely through sight, even after any topographic imperfections on the skin have been smoothed away; they are assessed by observing how light interacts with the skin. Topographical attributes are perceived both by touch and through topographic imagery. Mechanical attributes are associated with the skin’s movement and can be quantified through physical manipulation or deformation of the skin. In the context of this manuscript, the efficacy in facial rejuvenation will be evaluated through various markers, including:

1.8.2.1 Spots: It refers to skin lesions that exhibit hues of brown or red, encompassing conditions such as freckles, acne scars, hyperpigmentation, and vascular lesions. These spots are identifiable by their noticeable coloration, which contrasts with the surrounding skin tone. They can vary in size and typically exhibit a circular or rounded shape. In this study, we use the Visia® CR system (Canfield

Imaging Systems, Fairfield, NJ, USA). The VISIA-CR analysis provided results expressed as absolute scores in arbitrary units (a.u.).

1.8.2.2 Pores: They represent the circular surface openings of sweat gland ducts. These pores often appear darker than the surrounding skin due to shadowing, and they are recognized by their distinctively darker color and circular shape. The VISIA system differentiates pores from spots primarily based on their size; specifically, pores are characterized by a significantly smaller area compared to spots. In this study, we use the Visia® CR system (Canfield Imaging Systems, Fairfield, NJ, USA). The VISIA-CR analysis provided results expressed as absolute scores in arbitrary units (a.u.).

1.8.2.3 Wrinkles: They are defined as furrows, folds, or creases that develop in the skin, often becoming more pronounced with sun exposure and as skin elasticity decreases. The appearance of wrinkles can vary significantly from one image to another, largely influenced by the client's facial expressions. In this study, we use the Visia® CR system (Canfield Imaging Systems, Fairfield, NJ, USA). The VISIA-CR analysis provided results expressed as absolute scores in arbitrary units (a.u.).

1.8.2.4 Texture: They analysis primarily focuses on evaluating the smoothness of the skin. It assesses skin color and smoothness by detecting differences in color relative to the surrounding skin tone, and also identifies surface irregularities, such as peaks (highlighted in yellow) and valleys (highlighted in blue), which indicate variations in skin surface texture. In this study, we use the Visia® CR system (Canfield Imaging Systems, Fairfield, NJ, USA). The VISIA-CR analysis provided results expressed as absolute scores in arbitrary units (a.u.).

1.8.2.5 Sebum Levels: They refer to the quantity of sebum, which is an oily substance produced by the sebaceous glands within the skin. Sebum levels are typically assessed using the Sebumeter® SM 815 (manufactured by Courage & Khazaka GmbH, Cologne, Germany), a specialized device designed to measure the amount of sebum present on the skin's surface. The unit of measurement for sebum levels is expressed in “micrograms per square centimeter” ($\mu g/cm^2$). This measurement provides valuable insights into the skin’s oiliness or sebaceous activity, which can be important in dermatological and cosmetic assessments.

1.8.2.6 Elasticity: It pertains to the skin's ability to stretch and then return to its original state when subjected to mechanical deformation. Elasticity is typically assessed using the Cutometer® Dual MPA 580 (manufactured by Courage & Khazaka GmbH, Cologne, Germany), a specialized device designed to measure the mechanical properties of the skin. The unit of measurement for elasticity is expressed in "millimeters" (mm) or "percent" (%), depending on the specific parameter being evaluated. This measurement provides valuable information about the skin's resilience, firmness, and ability to recover its shape after being stretched or compressed, which are key factors in assessing skin health and aging.

1.8.2.7 Skin Capacitance: This term refers to the moisture content present within the skin, often referred to as skin hydration. Skin hydration plays a pivotal role in upholding skin health and its proper functioning. To quantitatively assess skin hydration, several non-invasive methods are available. One such tool is the Corneometer® CM 825, developed by Courage & Khazaka GmbH in Cologne, Germany. This device gauges the skin's water content by measuring its capacitance. Additionally, another technique called conductometry evaluates skin hydration by measuring its electrical conductivity. These methods yield numerical measurements of skin hydration, typically presented either as a percentage of the skin's water content or in arbitrary units (a.u.).

1.8.2.8 Transepidermal Water Loss: It involves the inherent mechanism of water evaporation occurring from the skin's outermost layer, scientifically referred to as the stratum corneum. This natural physiological process is under the regulation of the skin's protective barrier function, responsible for managing water loss and preserving skin moisture. Multiple factors, both intrinsic and extrinsic, including skin composition, temperature, humidity, and environmental conditions, can influence this process known as TEWL (Trans-Epidermal Water Loss) (Karen & Carol, 2004). In this study, we utilized the Tewameter® TM300 (manufactured by Courage & Khazaka GmbH, Cologne, Germany) to measure TEWL in $g/m^2/h$.

1.8.3 Side Effect

It refers to any unintended, adverse, or undesirable outcome or reaction resulting from the administration of PDLLA for the purpose of enhancing skin quality. These effects may include, but are not limited to, bruising, pain, skin irritation, redness, swelling, discomfort, lump, or any other condition that deviates from the desired improvement in skin quality. They will be evaluated for short-term adverse effects by a physician once in every visit, and also via phone calls or messages. Additionally, the researcher will conduct a causality assessment of any adverse events to determine whether they are related to the PDLLA injection. Subsequently, the researcher will gauge the relationship between the adverse events and the treatment, categorizing them into four levels (0-3) (Pande, 2018). For this study, only level 2, "Probable", and level 3, "Certain", symptoms will be considered as side effects resulting from PDLLA injection.

1.8.4 Global Aesthetic Improvement Scale (GAIS)

It is a standardized assessment tool commonly used in aesthetic and dermatological research to evaluate the overall improvement in a subject's appearance following a specific treatment or intervention. This scale typically ranges from "1" to "5", with each numerical value representing a different level of improvement (Carruthers & Carruthers, 2010): 1- Very Much Worse, 2- Worse, 3- No Change, 4- Improved, and 5- Very Much Improved. Evaluators assign a score based on their subjective perception of the subject's appearance using before-and-after photos of each participant, which were captured by the OLYMPUS PEN Lite E-PL5 and Visia CR system. A higher score indicates a more significant improvement, while a lower score suggests less improvement or potential worsening. The Global Aesthetic Improvement Scale provides a standardized way to assess the overall effectiveness and satisfaction of a given aesthetic or dermatological procedure.

1.8.5 Efficacy

The efficacy of Poly-D, L-Lactic Acid (PDLLA) biostimulator will be evaluated through facial rejuvenation markers:

1.8.5.1 Spots: The decrease in spots, as measured by Visia CR system, determines the efficacy of facial rejuvenation.

1.8.5.2 Pores: The decrease in pores, as measured by Visia CR system, determines the efficacy of facial rejuvenation.

1.8.5.3 Wrinkles: The decrease in wrinkles, as measured by Visia CR system, determines the efficacy of facial rejuvenation.

1.8.5.4 Texture: The decrease in texture, as measured by Visia CR system, determines the efficacy of facial rejuvenation.

1.8.5.5 Sebum Levels: The decrease in sebum levels, as measured by Sebumeter® SM815, determines the efficacy of facial rejuvenation.

1.8.5.6 Elasticity: The increase in elasticity, as measured by Cutometer® MPA580, determines the efficacy of facial rejuvenation.

1.8.5.7 Skin Capacitance: The increase in skin capacitance, as measured by Corneometer® CM825, determines the efficacy of facial rejuvenation.

1.8.5.8 Transepidermal Water Loss: The decrease in transepidermal water loss, as measured by Tewameter® TM300, determines the efficacy of facial rejuvenation.

CHAPTER 2

LITERATURE REVIEW

The researchers have categorized the related literature review topics as follows:

1. Skin Rejuvenation (Measurements and Treatments)
2. Biostimulator (CaHA, PLLA, PCL, PDO, and PDLLA)
3. Material and Equipment
4. Previous Studies Related to PDLLA and Skin Rejuvenation

2.1 Skin Rejuvenation

The quality of the skin plays a crucial role in human attractiveness, impacting the assessment of aesthetic treatment requirements and choices. It exerts a significant influence on the perception of attractiveness, aging, youth, and health. Even subtle alterations in skin texture and pigmented patterns can wield a considerable influence on the perceived appeal or attractiveness of the face, and this phenomenon considers true throughout diverse races and for both male and female faces.

Skin changes associated with aging are rooted in age-induced modifications occurring across all layers of tissue. Skeletal aging primarily results in diminished bony prominent, manifesting as a reduction in volume that contributes to a loss of support and imparts an aged appearance to the face (Shaw et al., 2011). This alteration in bony support also affects the positioning of true retaining ligaments. The aging process involving muscle mass and ligament fibers leads to the increase of areas of the face, emphasizing increased laxity and the perception of volume depletion (Gierloff et al., 2012). Age-related shifts in facial fat tissue contribute to both volume loss and sagging (Mendelson & Wong, 2013). Additionally, the skin's elasticity diminishes with age due to the loss of elastin and collagen (Krueger et al., 2011), while a rising in surface texture, possibly attributed to reduced hydration and oily content, plays a role in the development of skin rhytids, including wrinkles around the eyes (crow's feet) and lines on the forehead and glabella (Luebberding et al., 2014). The decline in viscoelastic

properties associated with aging becomes more evident after the age of 50, while inadequate skin capacitance is more prevalent in more youthful age groups, especially between the ages of 40 and 50. This underscores the necessity for a multidisciplinary and age-specific treatment plan aimed at improving quality of skin (Pearce & Grimmer, 1972).

Nevertheless, there are currently no internationally accepted criteria for assessing quality of skin (Cavallini et al., 2019). One framework that identifies attributes contributing to quality of skin which is organized into three fundamental categories: visual, topographical, and mechanical (Humphrey et al., 2021). Along with their measurements and treatments, it was concluded as follows:

2.1.1 Visual Attributes

Unequal pigmentation, often associated with differences in melanin levels, is a key visual characteristic determined by the melanin content of the skin, with darker skin having higher melanin levels (Jablonski, 2004). Photoaged skin might exhibit areas of hypo-hyperpigmentation, while melasma typically presents as hyperpigmented patches in a specific distribution, contributing to the appearance of mottling or blotchiness. Erythema (redness) is linked to underneath skin color, vascular burden, and visibility through the skin (Bielfeldt et al., 2018). Dullness and sallowness denote the absence of natural radiance and may be accompanied by a yellow undertone, representing visual indicators of compromised skin quality arising from various factors. On the contrary, skin's ability to reflect light, termed radiance or "glow," is considered both a visual and tactile quality, dependent on water levels and the presence of dead or dry skin that may obstruct light reflection. Oiliness/shine and dryness are also visual and tactile attributes (Mayrovitz et al., 2017). Oiliness, resulting from excess sebum production, can be influenced by intrinsic (hormonal) or extrinsic (oxidative stress) factors. Hydration is discernible through sight, touch, and biomechanics, with the moisture level impacting the skin's appearance, feel, and manipulability (Donofrio et al., 2016).

Measurement tools for pigmentation/discoloration include Mexameter (Courage + Khazaka, Cologne, Germany) (Clarys et al., 2000), Chroma Meter CR-400 (Konica Minolta, Ramsey, New Jersey, USA), LifeViz (Lee et al., 2016), and Skin Colorimeter CL 400 (Courage + Khazaka, Cologne, Germany) (Fossa Shirata et al.,

2019). Treatment options for pigmentation improvement encompass topicals/PSP-based cosmeceuticals, such as NEOCUTIS® (Dreher et al., 2013); lifestyle adjustments, such as sun protection (Krutmann et al., 2017); and laser therapies (Ungaksornpairote et al., 2020). Additionally, biostimulators (diluted) and HA microinjections are effective for undereye hyperpigmentation (Corduff, 2020). For erythema assessment, Mexameter, Chroma Meter, LifeViz, and standardized photos can be used (Fullerton et al., 1996). Treatment options include biostimulators (diluted) (Chao et al., 2018), and HA microinjections (Hertz-Kleptow et al., 2019). Laser treatments have demonstrated efficacy in enhancing skin tone evenness. Similarly, BoNT-A has been evidenced to improve skin tone uniformity (Guida et al., 2018). Skin glow, described by terms like radiance and luminosity, can be measured by Mexameter, Glossymeter (Courage + Khazaka, Cologne, Germany), or clinical scoring (Hertz-Kleptow et al., 2019). Treatment recommendations for improving glow involve HA (Hertz-Kleptow et al., 2019), biostimulators (Chao et al., 2018), MFU-V, BoNT-A, chemical peels (Rendon et al., 2010), and topicals/PSP-based cosmeceuticals (Dreher et al., 2013), with microinjections of HA showing promise for hydration and biostimulatory enhancement.

2.1.2 Topographical Attributes

Topographical characteristics are observable and assessed through specific methods. These features involve smoothness or roughness (texture), a crucial indicator of aging or photodamage, where excessively coarse skin may signal elastosis, while smooth skin is associated with a more youthful appearance. Fine or coarse lines and wrinkles contribute to topographical attributes, reflecting signs of aging. Enlarged pores are linked to skin topography and have been correlated with factors such as increased sebum production, aging, and male gender (Roh et al., 2006). Skin crepiness, indicative of the loss of underlying structural support, such as fat and/or muscle atrophy or the degradation of collagen and elastin fibers, results in thin skin hanging loosely in its place (Donofrio, 2000).

For assessing overall topographical attributes, VISIA (Canfield Scientific, Parsippany, New Jersey, USA) is a suitable option. It can assess pore, wrinkles, and textures (Merati et al., 2020). Typical choices for overall skin topography treatments include laser (Ungaksornpairote et al., 2020), platelet-rich plasma (PRP) (Cameli et

al., 2017), and chemical peels (Rendon et al., 2010). We can also aim for the targeted parameter. For pores, it generally includes hyperdiluted botulinum toxin type A (BoNT-A), diluted or hyperdiluted biostimulators (Chao et al., 2018), hyaluronic acid (HA) (Qian et al., 2018), and microfocused ultrasound with visualization (MFU-V). BoNT-A, often used in a hyperdiluted form, can be beneficial for tightening pores, especially in individuals with elevated sebaceous content, such as those with rosacea and acne (Sayed et al., 2020). Treatment for wrinkles and lines may involve the use of biostimulators, HA, BoNT-A, fractionated laser, carbon dioxide (CO2) laser, and diode laser (Archer and Carniol, 2019).

2.1.3 Mechanical Attributes

Elasticity, or the ability to recoil, is a mechanical attribute of the skin that diminishes when the stability of the network of elastic fibers in dermis is compromised (Ono, 2011). The firmness of the skin is connected to its pliability and has served as a significant measure of effectiveness in studies evaluating aesthetic treatments (Mayrovitz et al., 2017). The density and firmness of the skin — whether the skin is excessively thick or thin, tight or loose, these are also considered mechanical properties influenced by aging, both intrinsic or extrinsic. Variations in epidermal and dermal thickness, along with morphological changes, are often linked to sun exposure and the natural aging process. These factors contribute to the development of thicker or sagging skin, altering its appearance and texture over time (Choi et al., 2013).

Elasticity could be measured using a Cutometer (Courage + Khazaka, Cologne, Germany) (Ahn et al., 2007) and/or a snap test (Hussain et al., 2013). The Cutometer is a validated tool for assessing elasticity, while the snap test is a useful in-practice evaluation method. Biostimulators, including PDLLA, have been shown to have a collagen-stimulating effect, with both diluted and hyperdiluted forms. Microinjections of hyaluronic acid (HA) plus glycerol in dermal/subdermal plane are also employed to address skin elasticity (Hertz-Kleptow et al., 2019). Other treatment options include microfocused ultrasound with visualization (Kerscher et al., 2019), fractional laser (Kołodziejczak & Rotsztejn, 2017), deep peelings (Fabbrocini et al., 2009), and topicals such as retinoids (Korolkova et al., 2019).

Tautness (Tightness) can be measured using a Cutometer (Ohshima et al., 2013). Additional assessment methods include a pinch test (Hussain et al., 2013) and a

comparison of before and after photos (McBean & Katz, 2009). Options for improving tautness include MFU-V (Kerscher et al., 2019), biostimulators (Yutskovskaya & Kogan, 2017), and HA (25 mg, intradermal and subdermal) (Hertz-Kleptow et al., 2019). MFU-V is known to enhance skin tightness, is safe for all types of skin, and does not cause hyperpigmentation including post-inflammatory hyperpigmentation (PIH) (Fabi et al., 2013).

Hydration levels can be assessed with a Corneometer (Courage + Khazaka, Cologne, Germany) (Hashimoto-Kumasaka et al., 1993). Lifestyle adjustments, such as avoiding excessive bathing, limiting sun exposure, and regular use of lotion or moisturizers, can contribute to improved skin hydration (Iizaka, 2017). Other treatment options include HA (Hertz-Kleptow et al., 2019) and topicals/PSP-based cosmeceuticals (Crowther, 2016).

2.2 Biostimulator

The utilization of fillers for soft-tissue augmentation has experienced a significant surge in popularity in recent years, providing substantial aesthetic enhancements in facial design and skin quality, surpassing outcomes previously achievable only through surgical procedures. According to the latest study from the American Academy of Plastic Surgeons (ASPS), filler procedures reached 3.4 million in 2020, ranking second among the 5-most aesthetic minimally invasive procedures, following injections of the neuromodulator.

Soft-tissue fillers are currently categorized into 3 types: (1) Permanent non-biodegradable fillers, such as polyacrylamide, polymethyl methacrylate, and silicone, alongside (2) biodegradable options like hyaluronic acid products and (3) collagen-stimulating polymers (Biostimulators), are commonly used in aesthetic treatments. Permanent materials face limited approval globally due to potential long-term and often permanent complications can occur, including delayed persistent granulomas that may develop years after implantation. Hyaluronans or hyaluronic acid (HA), despite requiring repeat injections for sustained aesthetic correction, stand out as the most commonly used soft-tissue filler materials worldwide. As natural compounds,

hyaluronans are subject to compromise by the natural hyaluronidase enzyme in the body, leading to a general longevity of 6–12 months. The longevity of each hyaluronic acid filler depends on factors such as the degree and type of cross-linking, along with the product's concentration and particle size. Although valuable in restoring facial volume, hyaluronic acid fillers exhibit limited collagen-stimulating effects (Lee et al., 2020).

In the early 2000s, the development of a new generation of collagen-stimulating fillers was driven by the quest for biodegradable substances that offer favorable safety profiles and longer-lasting effects, presenting biostimulators as follows:

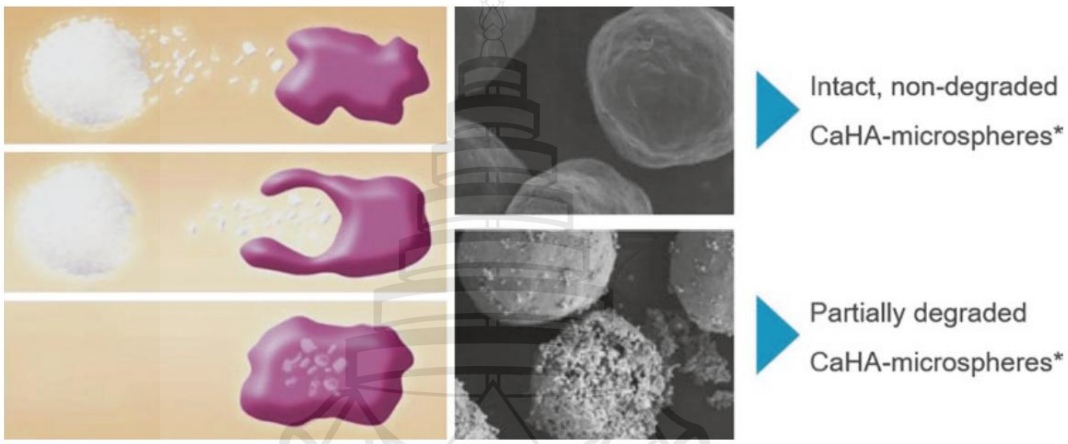
2.2.1 Calcium Hydroxyapatite (CaHA)

Calcium hydroxyapatite (CaHA) is a biostimulatory filler that is biodegradable, biocompatible, and capable of being resorbed by the body that incorporates calcium hydroxyapatite (CaHA) microspheres capable of inducing the natural production of collagen in the skin. Radiesse, a filler containing calcium hydroxyapatite by Merz Aesthetics, received FDA approval in 2001. This distinctive product serves as both a volume augmentation and collagen-stimulation agent as its main mechanisms of action (Kadouch, 2017). Initial replacement is facilitated by the presence of the carboxymethylcellulose or CMC gel, accompanying the particles. However, after around 9–12 months, the CaHA substances undergo metabolism into calcium and phosphate, eventually terminated through the urination (Loghem et al., 2015). A CaHA filler with high viscoelasticity, applied either undiluted or with minimal dilution, delivers immediate correction, followed by progressive tissue formation driven by elastin synthesis, neocollagenesis, angiogenesis, and the proliferation of dermal cells (Silvers et al., 2006). It is well-suited for both supraperiosteal plane and subdermal injection, resulting in a sustained aesthetic enhancement for at least 18 months, characterized by firmed and tight skin with increased density (Yutskovskaya & Kogan, 2017). When Radiesse is hyperdiluted (e.g., 1.5 mL of product mixed with ≥1.5 mL of diluent), it shows minimal or no immediate volumizing effects due to the dispersion of the carboxymethylcellulose gel. Instead, it generates long-term effect to the skin through the CaHA microspheres, enabling its injection at a more superficial level for

rejuvenation of the skin and the distribution of adjacent injected areas (Yutskovskaya & Kogan, 2017).

Since 2004, research has shown that the long-lasting effectiveness of Calcium Hydroxylapatite (CaHA) stems from a regulated inflammatory response that initiates a primarily fibroblastic reaction, ultimately replacing the aqueous gel with a dense collagen deposit (Coleman et al., 2008). Studies have shown that the majority of collagen deposited is type 1, which is linked to enhanced mechanical properties of the skin, rather than type 3 collagen, which is often associated with fibrotic processes (Berlin et al., 2008). Moreover, CaHA was found to induce more type 1 collagen and elastin production, leading to increased fibroblast proliferation compared to hyaluronic acid (Yutskovskaya et al., 2014). CaHA has been observed to not only promote fibroblast proliferation but also enhance their contractile function (Couderot et al., 2016). A separate study utilizing a 1:1 dilution revealed a peak in type 1 collagen production, observed both with the hyperdiluted product alone and when combined with microfocused ultrasound with visualization (MFU-V) (Casabona & Pereira, 2017). Immunohistochemical analysis revealed the effectiveness of Radiesse in increasing type 1 collagen and elastin production even at large dilutions (1:2 to 1:6), with results persisting 7 months after injection (Yutskovskaya & Kogan, 2017). Magnetic resonance imaging conducted 2.5 years post-injection revealed persistent soft tissue enlargement, even after the full absorption of CaHA particles. Clinical studies further highlighted a 50% increase in skin thickness within three months of treatment, which was sustained in 91% of participants throughout an 18-month observation period (Pavicic, 2015). It was reported improved laxity and dermal thickness in various body regions as early as 5 weeks after the procedure (Wasylkowski, 2015). In 2015, Amselem reported improved arm laxity following two treatment sessions conducted one month apart (Amselem, 2015). In conclusion, consensus meetings held in 2016 endorsed the application of hyperdiluted CaHA for dermal rejuvenation across larger areas in combination with other procedures for the face and body.

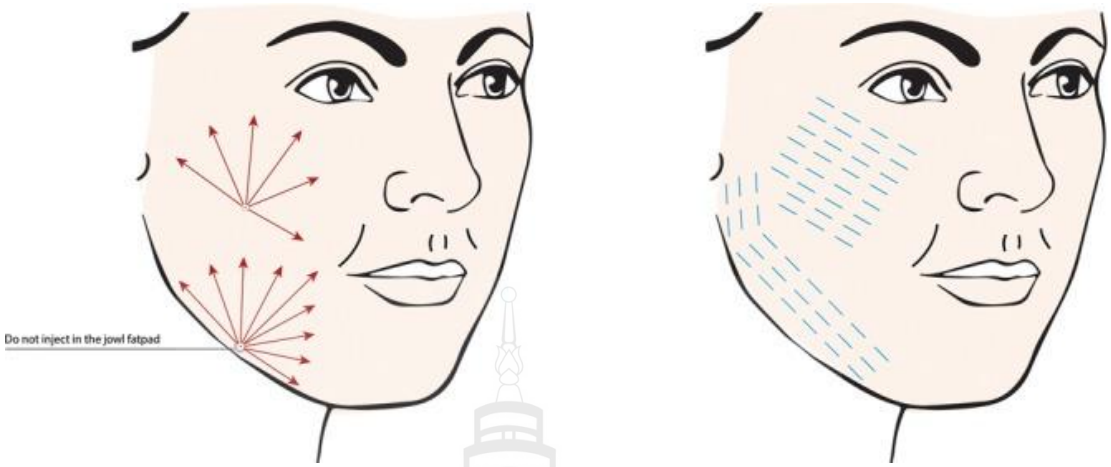
Mechanism of Action: Radiesse (calcium hydroxyl apatite filler, Merz aesthetics) is composed of microspheres of Calcium Hydroxylapatite (CaHA) suspended in an aqueous gel carrier. When injected, it provides immediate volume and correction while simultaneously triggering the body to produce its own natural collagen. Over time, the gel is absorbed, and the CaHA microspheres are metabolized by the body, ultimately leaving behind your own natural collagen (Figure 2.1).



Source Palermo and Anzai (2020)

Figure 2.1 Mechanism of Action of Calcium hydroxyapatite (CaHA)

Current practice and consensus statements for facial treatments provide expert-guided recommendations for the application of Calcium Hydroxylapatite (CaHA). The product is administered via retroinjection using cannulas, applying techniques such as fanning or the “asterisks” method, utilizing 2–4 entry points per side of the face. When needles are used, the short linear threading technique is the preferred method (Figure 2.2). For facial treatment, a dilution of 1:1 (1.5 mL of diluent) is recommended, with typically one syringe used per session.

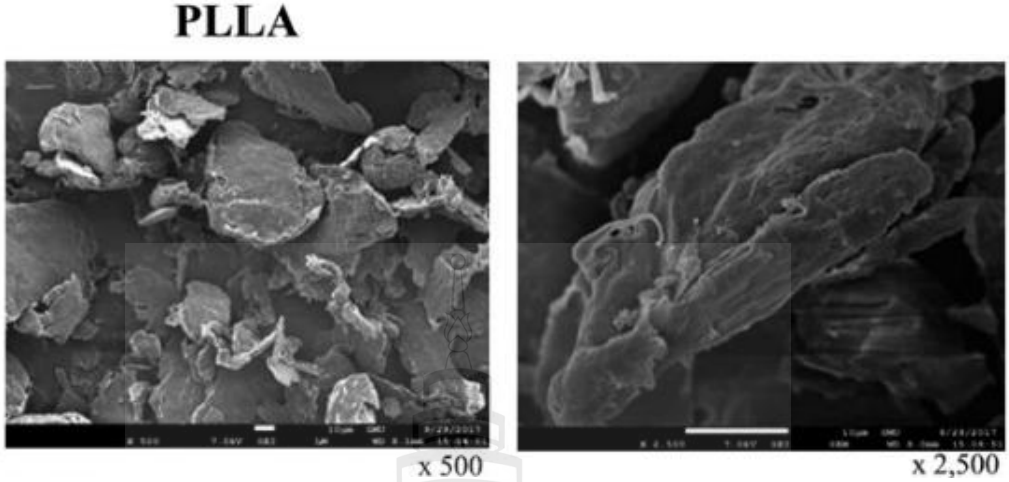


Source de Almeida et al. (2019)

Figure 2.2 Techniques for Calcium Hydroxyapatite (CaHA) of the Face

2.2.2 Poly-L-Lactic Acid (PLLA)

PLLA, a synthetic polymer belonging to the alpha-hydroxy acid family, is both biocompatible and biodegradable (Simamora & Chern, 2006). Over the past three decades, PLLA has demonstrated satisfactory and safe results in various medical applications (Alam & Tung, 2018). Sculptra (poly-l-lactic acid, Galderma, USA) received FDA approval in 2004. Sculptra® is provided in a sterile glass vial containing lyophilized powder, which consists of non-pyrogenic mannitol, sodium carboxymethylcellulose, and PLLA crystalline microparticles. These microparticles are irregularly shaped and range in size from 40 μm to 63 μm in diameter, a characteristic commonly seen in injectable dermal fillers (Alam & Tung, 2018) (Figure 2.3). The PLLA microparticles initiate a mild, localized inflammatory response, which recruits immune cells such as monocytes, macrophages, and fibroblasts. This immune reaction promotes the gradual degradation of the particles and stimulates the synthesis of type I collagen, ultimately leading to enhanced skin thickness and improved tissue structure over time (Kwon et al., 2019). Neocollagenesis initiates around 2 to 10 days post-application and persists for 8-24 months until complete product degradation and cessation of the subclinical inflammatory response (Lacombe, 2009).



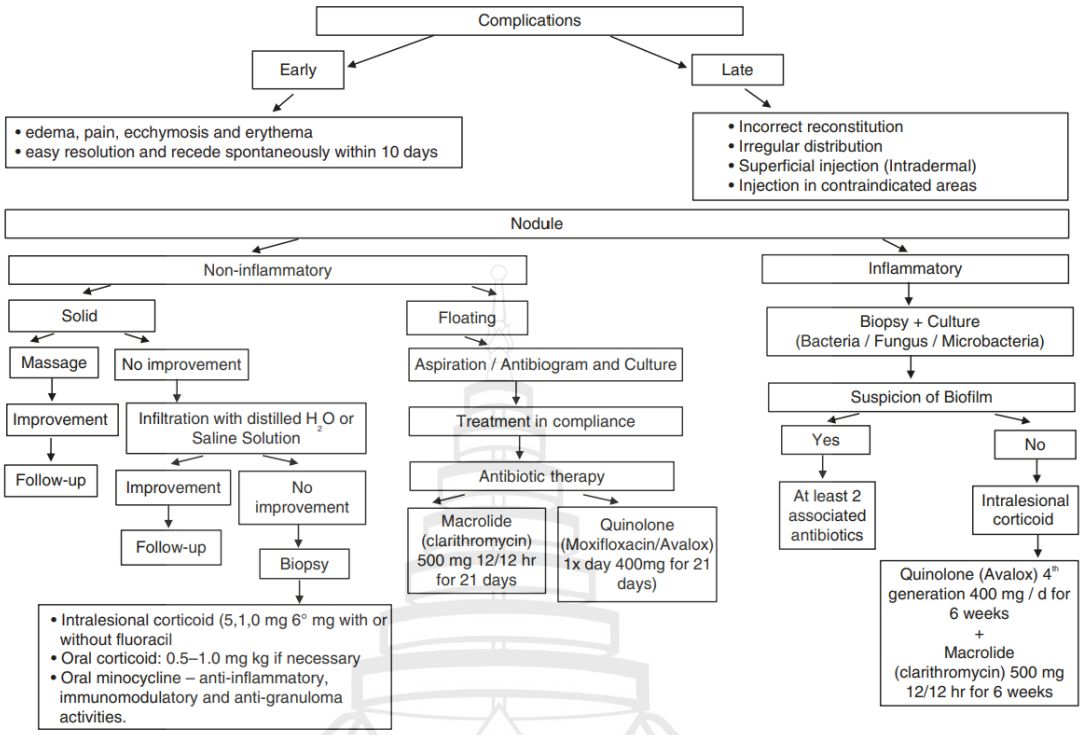
Source Kwon et al. (2019)

Figure 2.3 Particle Morphology of Poly-L-Lactic Acid (PLLA)

The Sculptra® treatment, administered over multiple sessions, has demonstrated efficacy and long-lasting results in enhancing facial contour and addressing sagging (Lee & Lorenc, 2016). It is especially effective in improving the appearance of sagging skin and restoring volume to areas that have lost fat or structure, including wrinkles, furrows, skin depressions, atrophic scars, and alterations from conditions like lipoatrophy or changes in bone structure (Alessio et al., 2014). This implies an enhancement in skin quality and firmness, contributing to an overall facial rejuvenation (Bohnert et al., 2019).

The precise technique for preparing and administering injectable PLLA is a crucial aspect for optimizing results. This approach also emphasizes ensuring the correct reconstitution and hydration of the product, targeted application to specific regions under local anesthesia, and adhering to post-procedure care guidelines. For increased dilution, the recommended mixture is 10 ml per vial, comprising 8 ml of distilled water and 2 ml of lidocaine without vasoconstrictor, ensuring proper formulation for optimal results. While the conventional recommendation suggests product reconstitution 24 to 72 hours before application, recent studies, including one incorporated in this systematic review, propose immediate reconstitution (Bravo & Carvalho, 2021). A prospective study involving 26 patients using Sculptra® for collagen biostimulation in the patients' face revealed that it was safe to immediately

reconstitute the product, with a no adverse effects reported (Bravo & Carvalho, 2021). This technique offers the benefit of reduced clinical time and minimized product loss. However, it is essential to conduct well-structured randomized clinical trials to confirm these results. PLLA administration necessitates supraperiosteal injection in regions with bone support or subcutaneous tissue in the absence of bone structure (Vleggaar et al., 2014). For supraperiosteal and subcutaneous applications, the most suitable techniques are depot application and fan-retroinjection, respectively (Lorenc, 2012). In areas of bone resorption, PLLA should be injected supraperiosteally in small boluses. This technique involves the use of 1- or 3-ml syringes and 24G 3/4 or 25G needles, with 0.1–0.3 ml administered at each injection point. The needle is inserted into the skin at a 90-degree angle, and aspiration should be performed before injection. In regions experiencing volume loss, PLLA is best administered using a 21G or 22G cannula. The procedure involves first inserting a larger gauge needle into the subcutaneous plane and then retroinjecting with a fan pattern, typically around 0.2 ml/cm2 per injection.The incorporated articles support these principles (Brandt, 2011; Bravo & Carvalho, 2021; Brown, 2011; Chen, 2015; Fabi & Goldman, 2021; Masveyraud, 2011; Narins, 2010; Palm, 2010). Intradermal injections are discouraged due to their potential to increase the risk of developing papules or nodules, which can lead to complications in the treated area (Lorenc, 2012). The guideline to manage complication from PLLA injection is in figure 2.4. Massage of the treated area is crucial for achieving optimal results, as it aids in uniform product distribution and enhances the overall outcome. Immediate post-procedure massage by the physician is recommended, and the patient should continue self-massage for at least 7 days, twice daily, for 5 minutes each session at home.



Source Albuquerque et al. (2020)

Figure 2.4 Algorithm to Treat Complications of Poly-L-Lactic Acid (PLLA)

The effects of Sculptra® treatment last until the patient is satisfied with the outcome. While the quantity of sessions is different, generally, satisfactory results can be confirmed after 3 to 5 sessions (Vleggaar et al., 2014). The “treat, wait and evaluate” approach is considered a prudent strategy for determining the number of sessions. Typically, a follow-up session is recommended after 4 to 6 weeks, allowing time for the full effects of the previous treatment to become visible before making any adjustments (Xiong et al., 2020). Follow-up treatments are typically scheduled annually after the initial series to help maintain the achieved improvements with fewer application sessions (Vleggaar et al., 2014). The studies included in the review showed significant variation in the number of sessions and the intervals between them (ranging from 1 to 12 sessions and intervals of 14 to 121 days), which limits the overall conclusions of the review (Brandt, 2011; Bravo & Carvalho, 2021; Brown, 2011; Chen, 2015; Fabi & Goldman, 2021; Masveyraud, 2011; Narins, 2010; Palm, 2010).

2.2.3 Polycaprolactone (PCL)

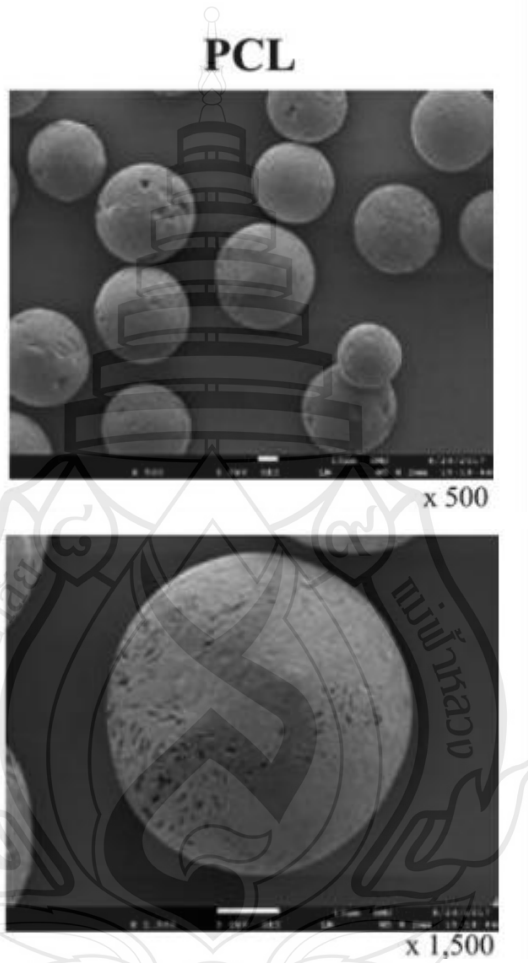
Polycaprolactone (PCL) is a well-established material with a considerable history in the medical field. It is a synthetically developed polymer that is biocompatible, biodegradable, and bioresorbable, belonging to the same chemical family as poly-L-lactic acid (PLLA) and polyglycolic acid (PGA). PCL stands as a biocompatible, biodegradable, and bioresorbable polymer, classified within the poly-α-hydroxy acid group, sharing this chemical category with polylactic and polyglycolic acids. Originating from the early 1930s, PCL emerged through the ring-opening polymerization of the cyclic monomer Ɛ-caprolactone (Labet & Thielemans, 2009). Comprising a chain with a repeated single-unit sequence of Ɛ-caprolactone $(C_6H_{10}O_2)_n$, the length (n) of the PCL chain, or its corresponding molecular weight, dictates its degradation timeline through ester-bond hydrolysis and its overall persistence.

Recognized for its non-toxic, non-allergic, and bioresorbable nature (Yusup et al., 2004), PCL finds diverse applications, including controlled-release drug delivery systems, tissue engineering (utilized in 3D scaffolds/matrices), absorbable sutures, and more. The versatility of PCL stems from its superior viscoelastic properties compared to other polymers, enabling practitioners to manipulate its structure with ease (Casalini, 2017). In the field of aesthetics, PCL serves as a collagen stimulator, primarily found in the form of threads and dermal injectables containing PCL microspheres. These products have demonstrated efficacy in stimulating collagen production. PCL-based fillers are formulated according to the polymer's chain length (molecular weight), with PCL-1 (Ellansé-S) and PCL-2 (Ellansé-M) being the primary types used for soft tissue augmentation. Both CMC and PCL have been granted GRAS (Generally Recognized as Safe) status by the FDA, signifying their safety in medical applications. Furthermore, PCL has a well-established track record as a resorbable material, notably as a key component in Monocryl™ (Ethicon, Inc.; Somerville, New Jersey, USA), which has been a standard suturing material in surgeries for years (Bezwada et al., 1995). PCL is also employed in a range of biomedical applications, including sutures, wound dressings, and tissue engineering scaffolds, due to its biocompatibility and slow degradation rate. Its use in drug delivery systems, like the Capronor™ contraceptive capsule, highlights its versatility in providing controlled and sustained release of therapeutic agents (Ma et al. 2006). Recently, Gouri® (PCL

filler, Edencolor, Seoul, Republic of Korea) is a new type of fully solubilized PCL filler. Utilizing the Collagen Enabled Solubilized Active and Biodegradable Polymer Technology (CESABP technology), a patented innovation by DEXLEVO, GOURI stands out as a filler devoid of micro-particles; it is entirely solubilized. This unique characteristic ensures GOURI's effortless spread to surrounding areas. With only 10 injection points, GOURI effectively stimulates collagen synthesis across the entire face. Post-injection, this dermal filler creates an expansive 3D matrix, contributing to its prolonged duration without succumbing to phagocytosis. Additionally, GOURI minimizes side effects associated with micro-particles, including vascular compression, necrosis, granuloma formation, and skin discoloration.

In 2009, Ellansé, a polycaprolactone-based soft tissue filler by Sinclair Pharma (London, UK), received CE marking as a Class III medical device, leading to its growing popularity in Europe and across various global markets. Ellansé comprises PCL microspheres with a diameter ranging from 25 to 50 μm, constituting 30% of the volume (Figure 2.5). These microspheres are suspended in a carrier gel composed of phosphate-buffered saline, glycerin, and carboxymethyl cellulose (CMC), which constitutes the remaining 70% of the volume (Nicolau, 2007). The gel is absorbed within a span of 4 to 5 weeks, facilitating the individual encapsulation of each microsphere. This method effectively prevents the formation of clusters and minimizes the likelihood of nodule development (Nicolau, 2007). The high viscosity of the CMC gel, around 1000 Pa, along with its significantly higher G' compared to hyaluronic acid fillers, enhances its stability within the tissue. This results in minimal risk of product migration, allowing for accurate placement and prolonged effect in the treated area (Stocks et al., 2011). PCL microspheres are synthesized by esterifying hydroxy-caproic acid molecules, resulting in a chemical structure composed exclusively of carbon, oxygen, and hydrogen. These microspheres are gradually absorbed through hydrolysis, maintaining their spherical shape and volume as they break down from within. This process produces only carbon dioxide and water, leaving no lasting by-products. The encapsulation process observed with any filler results in the continuous generation of protective, fibrotic type III collagen, influenced by alterations in the particles' shape and volume, while preserving the initial inflammatory response. Ellansé stands out as the sole filler with demonstrated higher production of collagen

type I compared to collagen type III. This distinction leads to a biological rejuvenation of the skin rather than just the thickening characteristic of scar tissue (Nicolau & Marijnissen-Hofsté, 2013). Given the known degradation rate of PCL, a series of four products has been designed for concerning the duration of the microspheres' presence in the tissues, ranging from 1 to 4 years (Pitt, 1990; Sun et al., 2006).



Source Kwon et al. (2019)

Figure 2.5 Particle Morphology of Polycaprolactone (PCL)

Injections can be applied to the subdermal layer for more superficial volumizing effects or deeper into the supra-periosteal area to target significant volume loss and enhance the production of type I collagen. Due to its ease of injection, it can be utilized with either needles or cannulas, ranging from 27G to 25G, based on the required duration, without necessitating specialized needles, syringes, or cannulas (Nicolau, 2020).

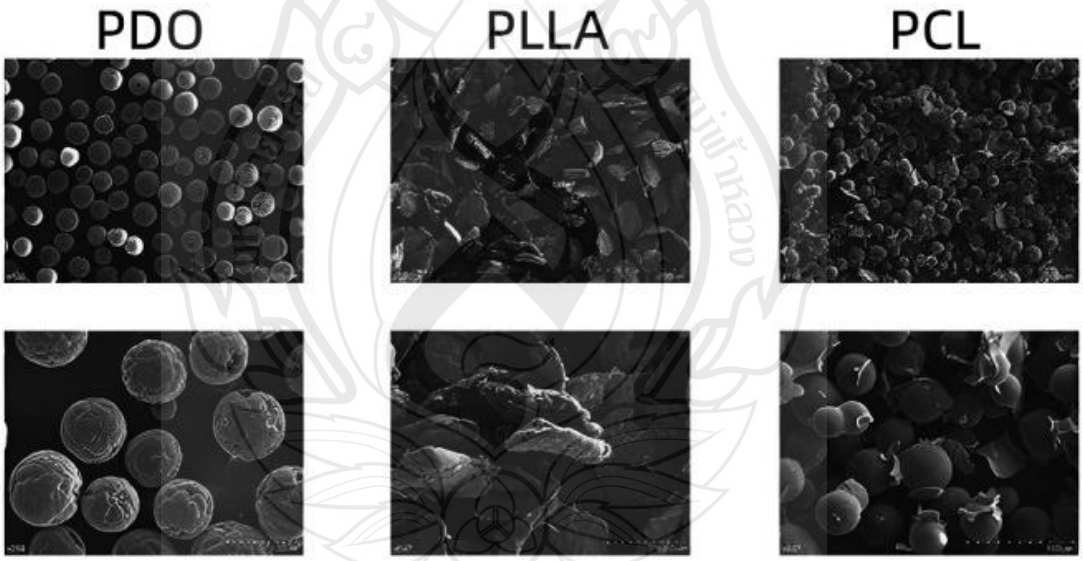
A prospective randomized study investigated the longevity of aesthetic effects for both PCL-1 and PCL-2, involving forty subjects treated for nasolabial fold correction. Additionally, the treatment outcomes were assessed through both subjective patient feedback and investigator evaluation, revealing high satisfaction rates. At the 12-month follow-up, significant improvements were reported in wrinkle severity and overall aesthetic appearance, with sustained results of 90% for WSRS and 91.3% for GAIS. These findings suggest long-term efficacy and patient satisfaction with the treatment. At 24 months, patient satisfaction remained high, with 81.5% for PCL-2 and 72.4% for PCL-1, indicating sustained positive outcomes for both formulations (Moers Carpi & Sherwood, 2013). The PCL-based filler has also undergone studies in other areas such as the forehead and hands. Another study demonstrated its efficacy in forehead augmentation in 58 Asian subjects up to 24 months post-injection (Bae et al., 2016). Further analysis from the pilot study indicated that the PCL filler effectively improved skin texture and elasticity, leading to a more youthful appearance of the hands (Figueredo, 2013).

2.2.4 Polydioxanone (PDO)

Polydioxanone (PDO) is a biodegradable polymer with ester-linked structures. The ester bond, characterized by its polar nature and lesser stability, is more reactive and susceptible to breakdown through hydrolysis, leading to the formation of (2-Hydroxyethoxy) acetic acid (Neirotti et al., 2008). Traditionally, it is a synthetic polymer primarily employed as absorbable suture material, known for its use in areas subjected to prolonged tension with minimal side effects. It has gained extensive use as absorbable thread-lifting material to stimulate collagen production (Teoh et al., 2016; Kim et al., 2017). Absorbable PDO threads for skin lifting have been in development since 2011. PDO exhibits a long half-life and induces fewer tissue reactions compared to catgut and polyglycolic acid (Patsalos et al., 2003).

Currently, the efficacy and safety of polydioxanone (PDO) implants have been investigated, demonstrating their suitability as a collagen inducer in animal studies (Martins et al., 2020). An animal study aimed at understanding the histological changes and absorption mechanisms of PDO threads and poly-caprolactone (PCL) found that PDO threads stimulate fibroblast proliferation through the TGF-β signaling system. The study proposes that TGF-β signal transduction triggers fibroblast

proliferation, promoting collagen formation and tissue remodeling. It suggests that a larger surface area between the thread and the tissue results in a heightened tissue response, leading to increased inflammatory cells, myofibroblasts, and fibroblasts. This makes the tissue remodeling and rearrangement effect highly variable and dependent on the thread's shape (Ha et al., 2022). As a powdered biostimulator, PDO compounds exhibit qualities compatible with those of PLLA and PCL in terms of the inflammatory response and the ability to form collagen. However, it has been demonstrated that this material offers superior biodegradability and a significant reduction in skin surface roughness when applied to animals undergoing photoaging (Kwon et al., 2019). In the in vitro test, the particles of the PDO filler exhibited a consistent size with a spherical shape and an uneven surface. On the other hand, PLLA microspheres were characterized by a rough and non-uniform size, appearing flat with a pointed shape. In contrast, PCL microspheres were smooth and consistently sized spherical particles as in Figure 2.6 (Zhou et al., 2023).



Source Zhou et al. (2023)

Figure 2.6 Particle Morphology of Biostimulators

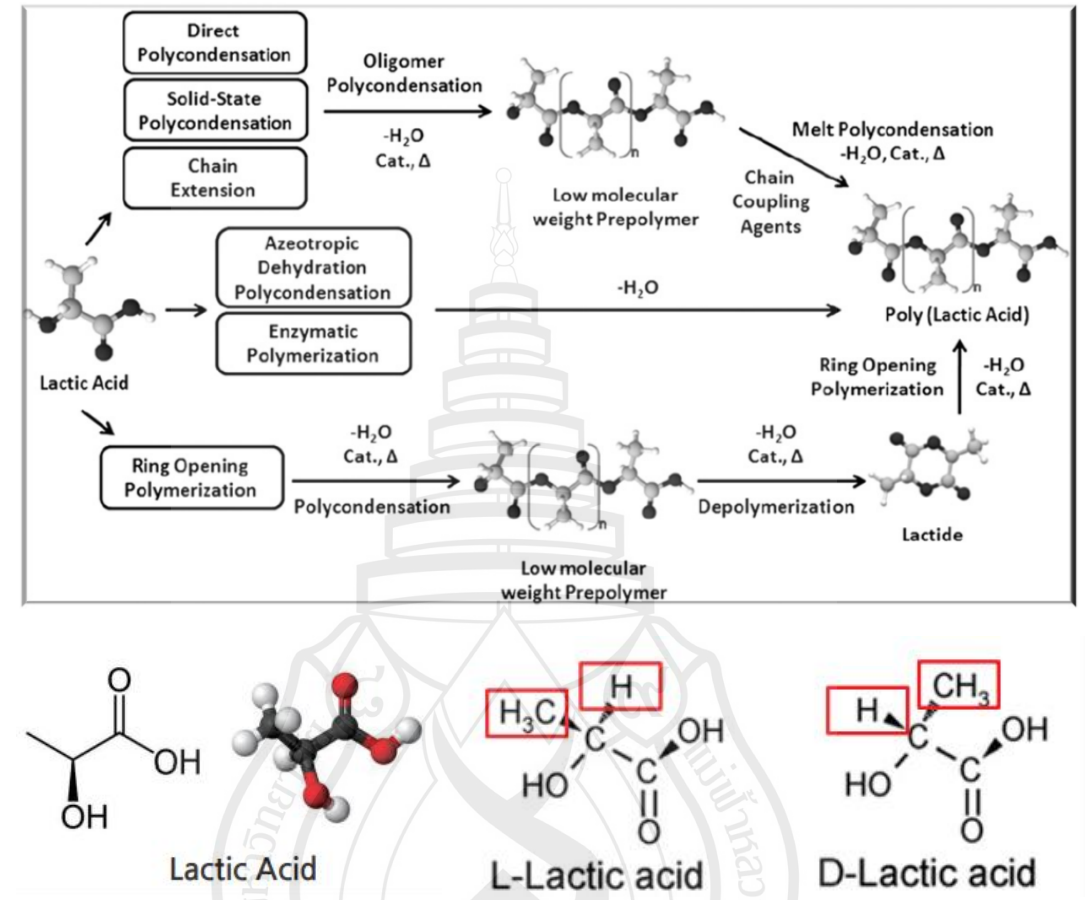
PDO filler (Ultracol®, Ultra V Co., Ltd., Seoul, Korea) obtained Korea Food and Drug Administration approval (KFDA), European CE certification, Thai Food and Drug Administration approval (TFDA), and USA Food and Drug Administration approval (USFDA) in 2022. Through a unique technology, over 1400 PDO mono

threads are transformed into micro particles and combined with carboxymethylcellulose (CMC). Carboxymethylcellulose is a highly tolerable and medically safe carrier substance, utilized in the food industry and pharmaceuticals, serving as a binding agent to prevent unintended clumping. UltraCol 200 mg comprises 170 mg Polydioxanone and 30 mg Carboxymethylcellulose (CMC). In terms of collagen stimulation, ULTRACOL 200mg, 1 vial surface area, is equivalent to 1,400 pieces of PDO Mono thread (29G 38/50mm). The ingredient ensures biodegradability and safety, decomposing safely over approximately six months to minimize the likelihood of side effects. Retention in the body for over a year may increase the risk of granuloma/nodule formation (Lee & Kim, 2015). Recommended injection is with a 21G – 25G cannula depending on the indication.

2.2.5 Polydioxanone (PDO)

Poly-D,L-lactic acid (PDLLA) is a synthetic polymer known for its biocompatibility and biodegradability. It falls under the category of Poly lactic acid (PLA). Poly lactic acid (PLA) was first synthesized from α-hydroxy acids by French chemists in 1954. This polymer is utilized safely in resorbable suture materials, as well as in plates and screws for orthopedic, neurological, and craniofacial surgery. PLA gives rise to chiral molecules, including poly-L type (PLLA), poly-D type, poly-D, L-lactic acid (PDLLA), and meso-PLA. Chiral molecules possess non-identical mirror images, making them distinct and a characteristic feature of natural compounds. Polylactic Acid (PLA), often referred to as “bioplastics”, has a chemical formula of $C_3H_6O_3$, derived from plant fermentation sources like corn starch or sugar cane, making it a cost-effective production material. PLA finds diverse applications, including in plastic films, bottles, and biodegradable medical devices. Biodegradable PLA in medical substances can break down in the body after fulfilling its function, eliminating the need for surgical removal and preventing issues associated with non-degradable polymers that may leave foreign objects in the body. Bio PLA Lactic Acid, a recent application of PLA, utilizes its monomer, poly Lactic acid, which consists of two optical isomers, L-lactic acid and D-lactic acid. These isomers, or enantiomers, have the same molecular formula but differ in their arrangement of atoms, exemplifying chirality. A monomer, defined as a low-molecular-weight molecule,

contributes to forming a polymer by linking numerous monomers together (Farah et al., 2016).

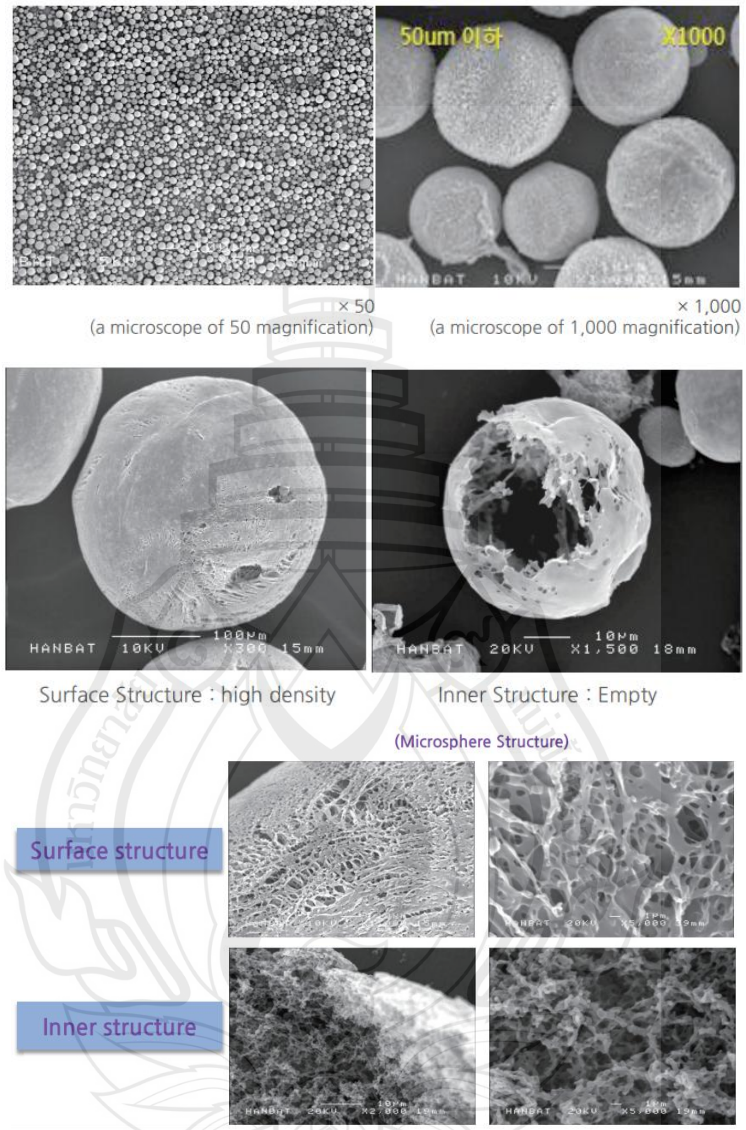


Source Farah et al. (2016)

Figure 2.7 Molecular, Atomic Structure, and Synthesis of Poly Lactic Acid (PLA)

PDLLA filler known as AestheFill (REGEN Biotech, Inc., Seoul, Korea) represents a recently developed injectable Poly Lactic Acid (PLA) product designed for a range of soft tissue augmentation procedures. This product received its initial approval from the Korean Food and Drug Administration in 2014 (Lin et al., 2019). This product was also approved by TFDA number 66-2-1-2-0004634. It also got the U.S. FDA- approved medical material for filler devices with patent BPM Technology. The PDLLA microsphere size around 30-70 micrometers (40-53μm) as in figure2.8. The particle surface features numerous densely distributed pores, strategically designed to attract fibroblasts into the microsphere. Its spongiform inner structures are distinctive, providing a residence for fibroblasts and facilitating the activation of

collagen formation (Kwon et al., 2019). The products are provided in the form of freeze-dried powders contained within vials. It consists of 200 mg (154 mg of PDLLA, 46 mg of Carboxymethyl Cellulose or CMC) in a vial.



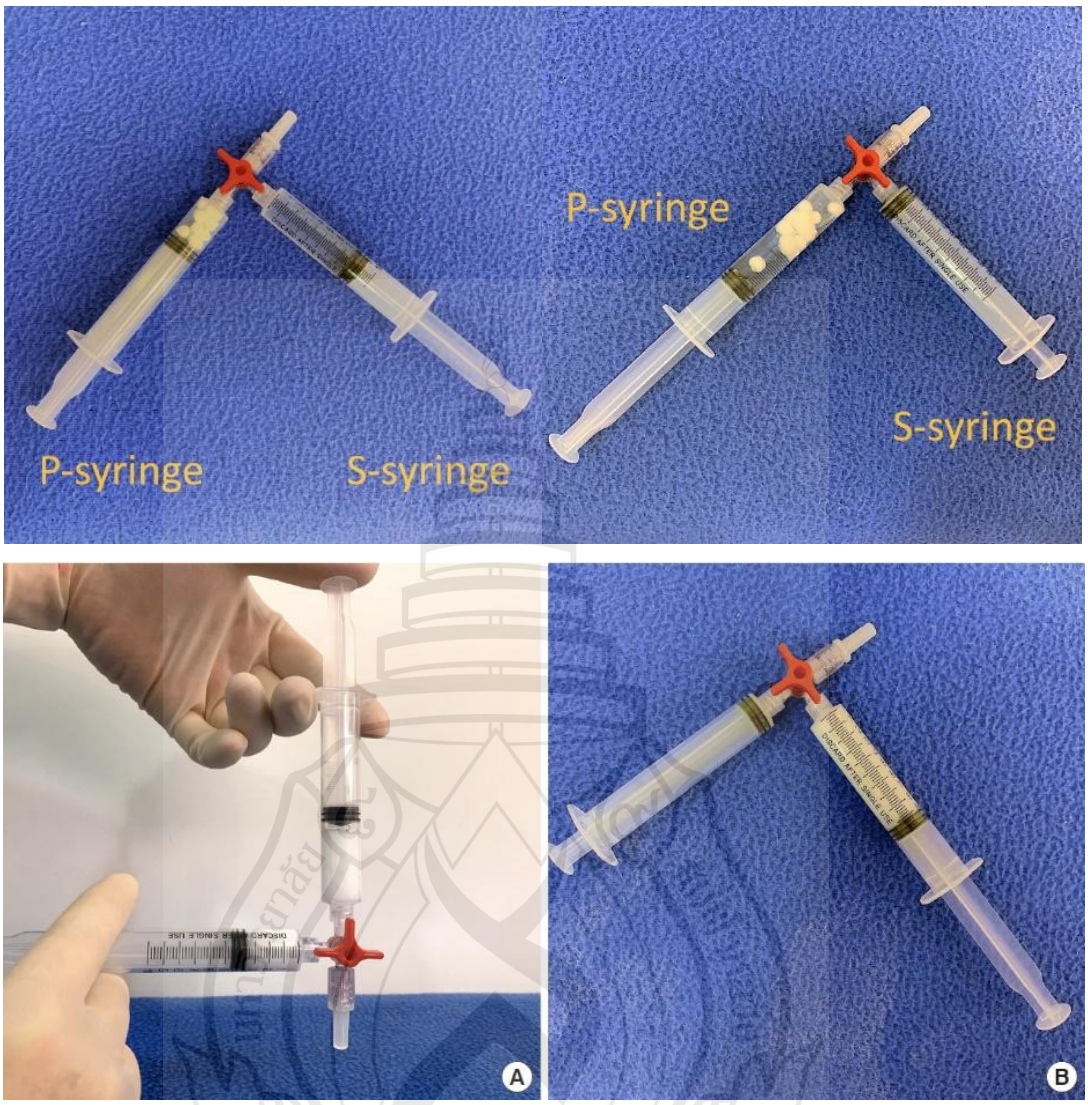
Source Kwon et al. (2019)

Figure 2.8 Particle Morphology of Poly Lactic Acid (PLA)

Implantation of biomaterials invariably triggers an inflammatory reaction, commonly referred to as a Foreign Body Reaction (FBR). This reaction is characterized by short-term acute or chronic inflammation, typically lasting less than two weeks and confined to the implant site. When the particle size exceeds 20 microns, macrophages are unable to phagocytose, resulting in encapsulation and the formation

of fibrous avascular capsules. Collagen production usually initiates within 4-8 weeks. The mechanism of Aesthefill involves two processes. Firstly, there is an immediate effect that enhances wrinkles and adds volume immediately after injection. However, this effect diminishes within a few days as water is absorbed. Around one week later, some volume loss occurs due to the absorption of water into the body. Secondly, there is a tissue response validated by animal models (Lin et al., 2019). In the second week, inflammatory cells are observed around microspheres, while by the fourth week, there is a tendency for a decrease in inflammatory cells. At the second week, the formation of the actin component begins, and by the fourth week, type I collagen starts to form as part of the extracellular matrix. One month later, wrinkles are corrected as lost volume is restored through collagen stimulation. PLA undergoes degradation in the body through hydrolysis, resulting in the formation of lactic acid and glycogen. Subsequently, PLA is excreted from the body in the form of water and CO_2 (Lin et al., 2019).

After the addition of 8 mL of sterile water to the PDLLA vial, the mixture was thoroughly agitated. Prior to injection, 2 mL of 2% lidocaine without adrenaline was incorporated into the solution, ensuring a total volume of 10 mL for the PDLLA, prepared using the back-and-forth method for optimal consistency (Chen et al., 2020) (Figure 2.9). After mixing the suspension and lidocaine, carefully inspect the solution to ensure it is completely homogeneous, with no remaining lumps or clumps of poly-D,L-lactic acid. Once confirmed, proceed to prepare for the injection. The final product should be a smooth, evenly mixed solution that is ready for dermal injection. During the injection, use a fine needle or cannula to carefully introduce the suspension into the treatment areas. Ensure that the injection is performed slowly and evenly to avoid any undue pressure or trauma to the skin. Maintain the appropriate depth according to the area being treated—typically subdermal or deeper, depending on the desired effect. Continue to observe the injection site for any immediate reactions, and apply gentle pressure if necessary to reduce any potential swelling. Use a 18 G sterile needle to withdraw the suitable amount of suspended solution and advise using a 25 G to 26 G sterile cannula needle for the procedure. Inject the solution into the subcutaneous layer to amplify the lifting effect and increase dermal tissue thickness. Administer 1cc per injection site during the treatment (Chen et al., 2020).



Source Chen et al. (2020)

Figure 2.9 Back-and-Forth Method

2.3 Material and Equipment

2.3.1 Material and Equipment for Intervention

Participants in the study were administered the biostimulators product (PLA). We used subdermal biostimulator poly-D, L-lactic acid (PDLLA), known as AestheFill by REGEN Biotech, Seoul, Korea, will be administered. This product received its initial approval from the Korean Food and Drug Administration in 2014 (Lin et al., 2019). This product was also approved by TFDA number 66-2-1-2-0004634. The products are

provided in the form of freeze-dried powders contained within vials. It consists of 200 mg (154 mg of PDLLA, 46 mg of Carboxymethyl Cellulose or CMC) in a vial. Once the 8 mL of sterile water was added to the PDLLA vial, the mixture was allowed to rehydrate for a short time. Just before the injection procedure, 2 mL of 2% lidocaine without adrenaline was added to the vial. This combination resulted in a total volume of 10 mL of reconstituted PDLLA, ensuring proper consistency and reducing discomfort during the injection process. The injections were administered using a fanning technique into the subdermal plane, following a grid pattern, and utilizing a 23-G needle with a length of 5 cm, entering at an angle of 30 to 40 degrees. Each subject received a total of up to 2.5 mL of PDLLA on each side.

2.3.2 Measurement Tools for Skin Biophysical Properties

2.3.2.1 VISIA® CR system (Canfield Imaging Systems, Fairfield, NJ, USA) for spots, pores, wrinkles, and texture of midface area: It is a sophisticated imaging tool used in dermatology, cosmetic medicine, and skin research. To utilize the system effectively, one must ensure it is well-set up and calibrated. Patient preparation is vital; explaining the procedure, obtaining consent, and having the patient remove makeup and lotions are necessary steps. Proper positioning of the patient is crucial for consistent imaging, and the face should align with the camera. The system captures a series of high-resolution images using various lighting and filters to evaluate different skin attributes, such as spots, pores, wrinkles, and texture. The software then analyzes these images, generating a comprehensive report on the patient's skin condition. This report forms the basis for treatment recommendations, which are discussed with the patient during a consultation. Follow-up appointments are scheduled to monitor treatment progress. The VISIA® CR system plays a pivotal role in objectively assessing skin conditions, enabling tailored treatment plans and informed decisions for both healthcare providers and patients. Proper adherence to manufacturer guidelines and training is essential for accurate and consistent results. The device is highly reliable, with an average of 0.74 to 0.945 (Henseler, 2022).

2.3.2.2 Sebumeter® SM815 (Courage & Khazaka, Germany) for sebum levels of the midface area: It is a widely used instrument in dermatology and skincare research for assessing sebum levels in the skin. To effectively utilize this device, the following steps should be taken: The measurement area should be properly cleansed

and allowed to acclimatize to the room conditions. The instrument probe is then gently applied to the skin's surface, where it absorbs sebum for a specified duration. The sebum level is determined by measuring the electrical capacitance of the probe, which is proportional to the amount of sebum absorbed. This provides an objective assessment of the skin's sebum content, aiding in the development of personalized skincare regimens and the evaluation of skincare product effectiveness. It is essential to follow the manufacturer's guidelines and undergo proper training to ensure accurate and consistent results when using the Sebumeter® SM815. The device is reliable, with a range of 0.513 to 0.987 (Richter et al., 2016).

2.3.2.3 Cutometer® MPA580 (Courage & Khazaka, Germany) for elasticity of midface area: It is a specialized device commonly employed in dermatological and cosmetic research to measure skin elasticity. To ensure its accurate usage, the following steps are typically followed: The device is placed in direct contact with the skin, and a controlled suction is applied, gently deforming the skin's surface. The probe measures the skin's mechanical response, specifically its ability to return to its original position when the suction is released. This data is then analyzed to assess various skin parameters, including elasticity and firmness. The Cutometer® MPA580 is a valuable tool for quantifying skin properties, which is essential for evaluating the effectiveness of skincare treatments and cosmetic procedures. As with any scientific instrument, adhering to the manufacturer's instructions and obtaining proper training is crucial to achieve consistent and reliable results. The device is highly reliable, with a range of 0.86 to 0.96 (Klimitz et al., 2023).

2.3.2.4 Corneometer® CM 825 (Courage & Khazaka, Germany) for skin capacitance of midface area: It is a widely used device in dermatology and skincare research for measuring skin hydration levels. To effectively utilize this instrument, the following steps are typically followed: The measurement area on the skin is cleaned and allowed to equilibrate with room conditions. The Corneometer probe is then gently applied to the skin's surface, where it measures the skin's capacitance, which is directly related to its hydration level. This provides an objective assessment of the skin's moisture content, aiding in the development of tailored skincare routines and the evaluation of the efficacy of skincare products. Proper adherence to the manufacturer's guidelines and adequate training is essential to ensure accurate and

consistent results when using the Corneometer® CM 825. The device is highly reliable, with a range of 0.985 to 0.984 (Anthonissen et al., 2015).

2.3.2.5 Tewameter® TM300 (Courage & Khazaka, Germany) for transepidermal water loss (TEWL) of midface area: It is a widely utilized instrument in the field of dermatology and skincare research for measuring transepidermal water loss (TEWL). To effectively employ this device, a few key steps are generally followed: The measurement area on the skin is properly cleansed and acclimated to the room's conditions. The Tewameter probe is then gently placed in direct contact with the skin's surface. The instrument quantifies the rate at which water evaporates from the skin's outermost layer, known as the stratum corneum, which is regulated by the skin's barrier function and affects skin hydration. This data is valuable for assessing skin health and its ability to retain moisture. As with any scientific instrument, it is crucial to adhere to the manufacturer's guidelines and undergo adequate training to ensure accurate and consistent results when using the Tewameter® TM300. The device is highly reliable, with a range of 0.85 to 0.94 (Gardien et al., 2014).

2.4 Previous Studies Related to PDLLA and Facial Rejuvenation

Hyun et al. (2015) conducted a multicenter, randomized, evaluator-blinded study to investigate the effects of hyaluronic acid (HA) fillers versus poly-L-lactic acid (PLA) fillers on nasolabial folds. The study aimed to compare the effectiveness and safety of a novel injectable PLA filler with a well-established biphasic HA filler in treating moderate to severe nasolabial folds. Subjects underwent randomization for injections with PLA or HA in both nasolabial folds, and efficacy was evaluated by assessing the change in Wrinkle Severity Rating Scale (WSRS) relative to baseline. At week 24, the poly-L-lactic acid (PLA) treatment showed a mean improvement in the Wrinkle Severity Rating Scale (WSRS) of 2.09 ± 0.68, compared to 1.54 ± 0.65 for the hyaluronic acid (HA) treatment. Both treatments were well tolerated, with predominantly mild and transient adverse reactions. In conclusion, PLA demonstrated

noninferior efficacy to HA, with a significant improvement in treating moderate to severe nasolabial folds, maintained for six months after the procedure.

The cases of three patients who underwent facial injectable PDLLA administration, as detailed in the study by Lin & Lin (2020), are discussed. In the first case, a 30-year-old female received PDLLA injections throughout the entire face over five sessions, with 2–4 vials per session and intervals of more than one month. Three months post-injection, a positive outcome was observed across all treated areas, yielding a more harmonious appearance. The second case involved a 54-year-old male receiving PDLLA injections in the temporal fossa, malar, sub-malar, and cheeks. Two sessions, with four vials per session spaced two months apart, were administered. Postoperative views four months after the second session revealed favorable effects in the treated regions. In the third case, a 60-year-old female underwent PDLLA injections in the temporal fossa and cheeks during a single session, using two vials. Six months post-injection, positive results were evident. In conclusion, PDLLA stands out for its ability to stimulate collagen production, offering long-term aesthetic benefits by gradually improving skin texture and volume. It is well-tolerated, with fewer adverse reactions and a lower incidence of complications. Its rapid reconstitution process and minimal water requirement make it convenient for use, ensuring quicker preparation times compared to other fillers. Overall, PDLLA provides a highly effective and safe option for facial rejuvenation, combining biocompatibility, sustainability, and efficacy in a single injectable treatment.

Another interesting study by Bohnert et al. (2019) is to assess the effectiveness of multiple PLLA injections on skin quality. In this randomized, controlled, double-blind, multicenter clinical trial study, 40 healthy women underwent three treatments at 4-week intervals, receiving either PLLA (treatment group) or normal saline (placebo group) injections on each side of the face. Follow-up assessments occurred at 6, 9, and 12 months post the final treatment, employing various evaluation methods, including biophysical measuring instruments (e.g., skin elasticity and hydration), live ratings by investigators, patient questionnaires, and the assessment of standardized photographs by a blinded evaluator. The biophysical measurements were used to objectively quantify improvements in skin texture and elasticity. The live ratings and patient questionnaires provided subjective evaluations, while the standardized photographs

allowed for visual comparison across time points. This comprehensive approach ensured a thorough assessment of the skin quality improvements following the treatment. At the 12-month follow-up, the results showed a statistically significant improvement in skin elasticity and hydration in the PLLA-treated subjects. Both groups exhibited a reduction in transepidermal water loss. Furthermore, the PLLA-treated group demonstrated significant decreases in pigmentation, erythema, and pore size, while radiance and smoothness were significantly enhanced, according to the blinded investigator ratings. Notably, no serious side effect from the intervention were observed. In conclusion, multiple PLLA treatments have the potential to progressively improve skin quality over time.

While these studies provide valuable insights into the efficacy and safety of hyaluronic acid (HA) fillers, poly-L-lactic acid (PLA) fillers, and injectable poly-D, L-lactic acid (PDLLA) in addressing facial rejuvenation and improving skin quality, there are some limitations and gaps in knowledge. Firstly, the study by Hyun et al. (2015) primarily focused on comparing the effectiveness of PLA and HA fillers in treating nasolabial folds. However, the scope of the study did not delve into potential long-term effects, and the follow-up period was limited to 24 weeks. To establish a more comprehensive understanding of the longevity and sustained benefits of these fillers, longer-term follow-up studies with extended observation periods may be warranted. Secondly, the cases presented by Lin & Lin (2020) offer valuable clinical insights into the positive outcomes of facial injectable PDLLA administration. Nevertheless, the limited number of cases and the absence of a control group hinder the ability to draw definitive conclusions about the comparative effectiveness of PDLLA in different facial regions. Further research with larger sample sizes and controlled study designs could enhance the generalizability of these findings. Lastly, the study by Bohnert et al. (2019) contributes valuable data on the impact of repeated PLLA injections on skin quality. However, the study did not explore the potential variations in treatment response among different age groups or skin types. A more nuanced analysis considering these factors could provide a more tailored understanding of the effects of PLLA on diverse patient populations.

In summary, while these studies make noteworthy contributions to the field, there is a need for additional research with extended follow-up periods, larger sample sizes, controlled study designs, and a focus on diverse patient characteristics to address the identified limitations and further advance our knowledge of these interventions. Addressing these limitations will enhance the generalizability and reliability of the results and further our understanding of the effects of PDLLA injection on skin quality. There is evidence to show improvements in patients' skin quality suggesting the potential of changes in sebaceous glands and adipocytes following PDLLA injections. The evidence is scarce and indirect. Therefore, the primary aim of this study was to examine the overall impact on skin quality from repeated subdermal PDLLA injections on midface area at two different time points (0 months, and 2 months). This evaluation encompassed both subjective assessments (utilizing the Global Aesthetic Improvement Scale by the blinded investigators and Satisfactory score by the patients) and objective measurements obtained from the Visia CR system, which assesses spots, pores, wrinkles, and texture. Additionally, we employed other devices to measure sebum levels, elasticity, skin capacitance, transepidermal water loss, and monitored for side effects. These assessments were conducted at specific time points (0 months, 2 months, 4 months, and 6 months).

CHAPTER 3

METHODOLOGY

3.1 Study Design

This study employed a prospective quasi-experimental design with a time series approach, in which a dependent variable was measured at multiple time points in a single group before and after the researcher-administered treatment.

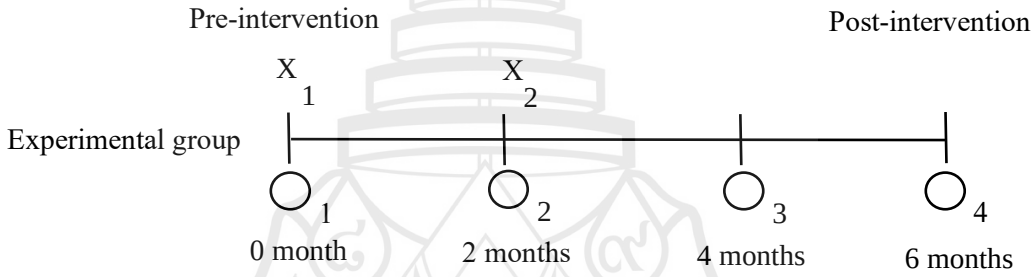


Figure 3.1 Study Design

O_1 Participants evaluated before the intervention at 0 month

X_1 Intervention: PDLLA 1st Injection

O_2 Participants evaluated at 2 months

X_2 Intervention: PDLLA 2nd Injection

O_3 Participants evaluated at 4 months

O_4 Participants evaluated after the intervention at 6 months

3.2 Study Area

Mae Fah Luang University Hospital Asoke, Bangkok

3.3 Population and Sample

3.3.1 Population

Any volunteers who came to visit or work at Mae Fah Luang University Hospital Asoke are our study population.

3.3.2 Sample and Sample Size Calculation

Thai middle age (both male and female) who came to visit or work at Mae Fah Luang University Hospital Asoke are our study population.

Based on the previous literature review by Bohnert et al. (2019) as a reference for the inputs of our sample size calculation. It was the study to compare the skin quality after PDLLA injection. The study protocol was completed by 40 patients. At month 12, the researcher got the mean difference of skin quality rating of 1.4±1.45. (p-value < 0.001). Therefore, this literature was used to calculate the sample size in the formula, as outlined by Chow et al. (2003):

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \sigma^2}{(\mu - \mu_0)^2}$$

Where :

n = sample size

α = 5%

Type I error; $Z_{1-\alpha/2}$ = 1.96 (Two-tailed test)

β = 10%

Type II error $Z_{1-\beta}$ = 1.28

μ_0; the mean of skin quality at baseline = 5.2±1.24

μ; the mean of skin quality at 12 months = 6.6±1.25

σ^2 ; Pooled variance

$$\sigma_p^2 = \frac{(n_0 - 1)S_0^2 + (n_1 - 1)S_1^2}{(n_0 - 1) + (n_1 - 1)}$$

$$= \frac{(40-1)1.24^2 + (40-1)1.25^2}{(40-1)+(40-1)}$$

$$= 1.55^2$$

We will get the sample size as the following calculation;

$$n = \frac{(Z_{1-\alpha/2}+Z_{1-\beta})^2\sigma_p^2}{(\mu_0-\mu_1)^2}$$

$$= \frac{(1.96 + 1.28)^2(1.55)^2}{(6.6 - 5.2)^2}$$

$$= 12.8676$$

In order to obtain reliable data and minimize the potential for dropouts (20%), we increased sample size to total of 15 people.

3.4 Selection Criteria

3.4.1 Inclusion Criteria

3.4.1.1 Male or Female Age 30-60 years

3.4.1.2 Able to provide informed consent, photograph record, and participate to all procedures

3.4.1.3 Committed to refraining from undergoing any treatments that could impact facial wrinkles (such as fillers, botulinum toxin, radiofrequency, laser, IPL, and ultrasound) or affect skin quality (including procedures like microdermabrasion, peels, acne treatments) throughout the entire study period.

3.4.2 Exclusion Criteria

3.4.2.1 Primary medical conditions such as chronic kidney disease (CKD), liver cirrhosis, cardiovascular disease (CVD), diabetes mellitus

3.4.2.2 History of allergic reaction to any biostimulators (PLLA, PDLLA, PCL, PDO, or CaHA)

3.4.2.3 Pregnancy and lactation

3.4.2.4 History of aesthetic facial procedure 6 months prior to the study

3.4.2.5 Active skin disease concomitant to area of interest

3.4.3 Withdrawal Criteria

3.4.3.1 Participants who chose to exit the program for any reason.

3.4.3.2 Participants who experienced significant treatment complications, illnesses, fatalities, or accidents.

3.4.3.3 Participants who were no longer accessible for follow-up.

3.5 Materials and Equipment

The materials and equipment in this study consisted of: 3.5.1 Material and equipment for intervention, 3.5.2 Measurement tools for skin quality, 3.5.3 Material and equipment for data collection.

3.5.1 Material and Equipment for Intervention

Participants in the study were administered the biostimulators product. We used subdermal biostimulator poly-D, L-lactic acid (PDLLA), known as AestheFill by REGEN Biotech, Seoul, Korea, will be administered. This product received its initial approval from the Korean Food and Drug Administration in 2014 (Lin et al., 2019). This product was also approved by TFDA number 66-2-1-2-0004634. The products were provided in the form of freeze-dried powders contained within vials. It consisted of 200 mg (154 mg of PDLLA, 46 mg of Carboxymethyl Cellulose or CMC) in a vial. The product was stored at room temperature below 30°C, and to ensure the stability of the substance, the entire vial must be used in one session. Prior to the injection, 8 mL of sterile water was introduced into the PDLLA vial. Just before the injections, an additional 2 mL of 2% lidocaine without adrenaline was added to achieve a final dilution of 10 mL of PDLLA using the back-and-forth technique. The local anesthesia was done at pre-hole (0.2 ml per site). Pre-hole point was the imaginary line between the mid-pre auricular line and lateral canthus line. The injections were administered using a fanning technique into the subdermal plane, following a same pattern (figure 3.1), and utilizing a 23-G needle with a length of 5 cm, entering at an angle of 30 to 40 degrees. Each line of injection used 0.5 ml per line (total of 5 lines). Each subject received up to 2.5 mL of PDLLA on each side (total of 5 ml per participant per session).

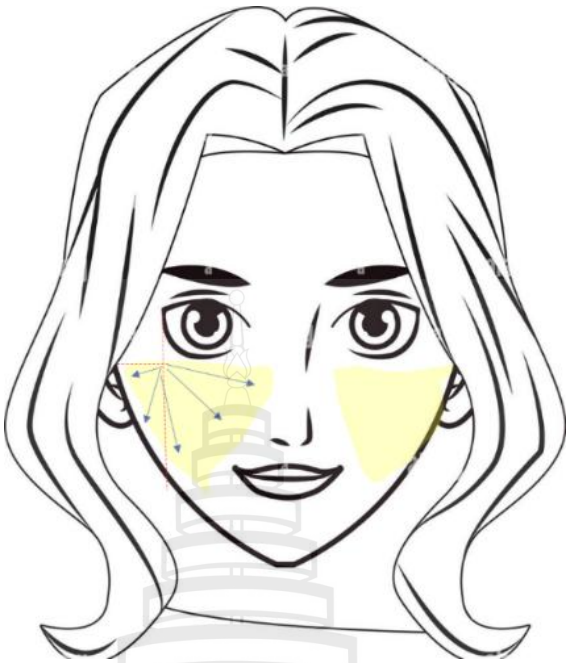
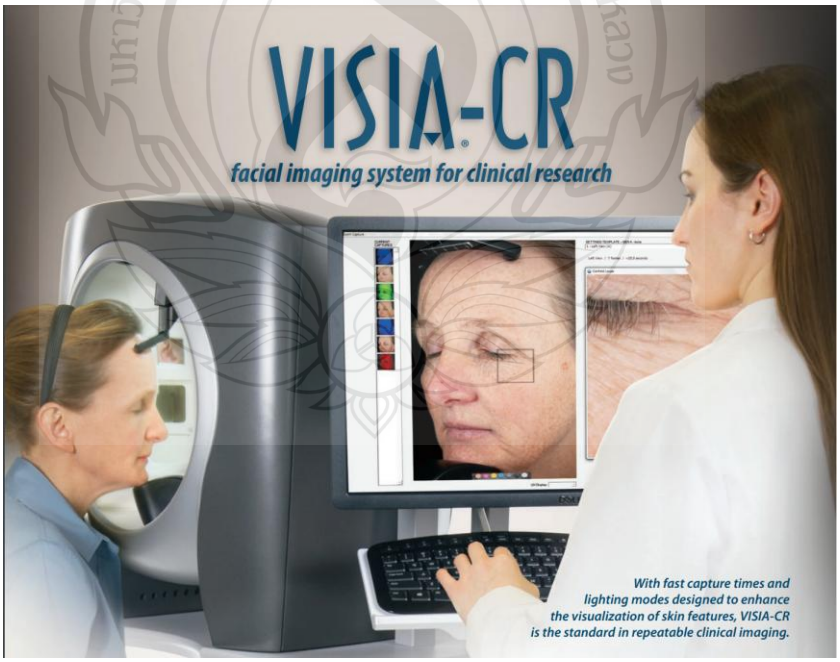


Figure 3.2 PDLLA Injection Technique

3.5.2 Measurement Tools for Facial Rejuvenation

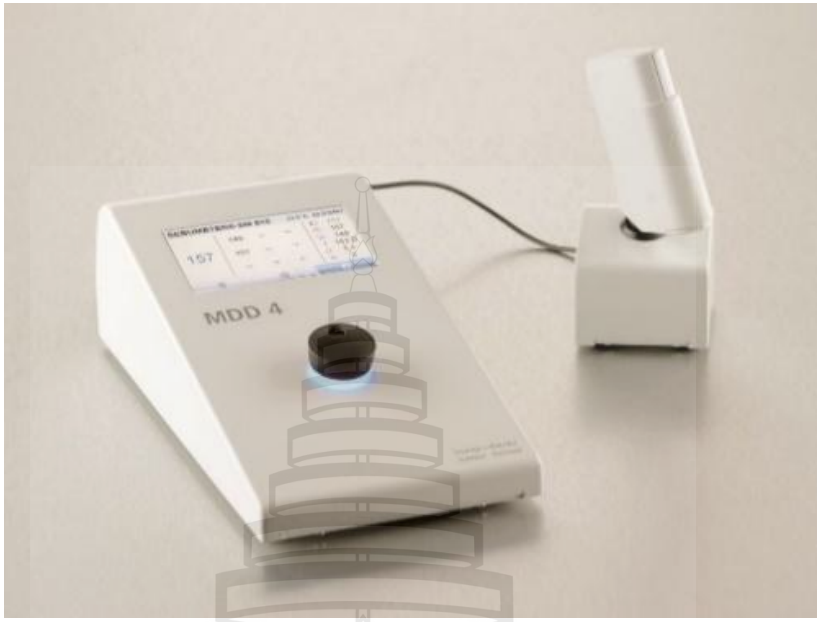
3.5.2.1 VISIA® CR system (Canfield Imaging Systems, Fairfield, NJ, USA) for spots, pores, wrinkles, and texture of midface area (TFDA number USA6205860)



Source Canfield Scientific, Inc. (2023)

Figure 3.3 VISIA® CR system (Canfield Imaging Systems, Fairfield, NJ, USA)

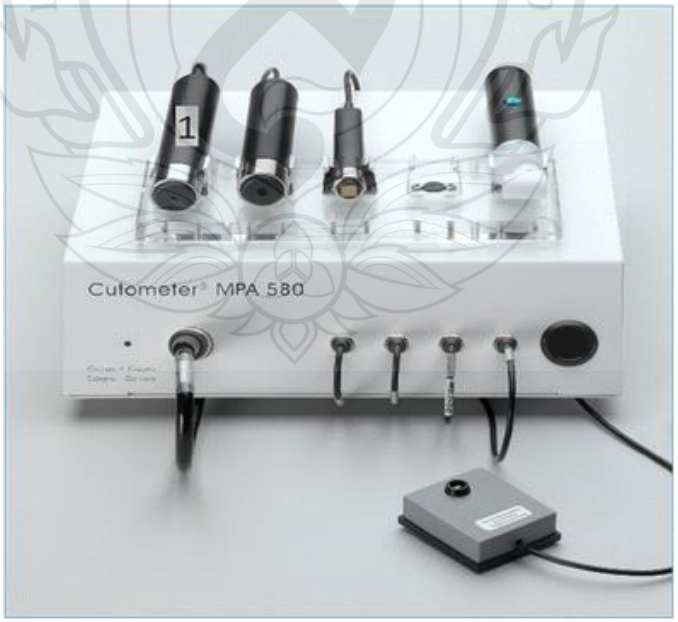
3.5.2.2 Sebumeter® SM815 (Courage & Khazaka, Germany) for sebum levels of the midface area



Source Courage + Khazaka electronic GmbH (2019)

Figure 3.4 Sebumeter® SM815 (Courage & Khazaka, Germany)

3.5.2.3 Cutometer® MPA580 (Courage & Khazaka, Germany) for elasticity of midface area



Source Courage + Khazaka electronic GmbH (2019)

Figure 3.5 Cutometer® MPA580 (Courage & Khazaka, Germany)

3.5.2.4 Corneometer® CM 825 (Courage & Khazaka, Germany) for skin capacitance of midface area

Source Courage + Khazaka electronic GmbH (2019)

Figure 3.6 Corneometer® CM 825 (Courage & Khazaka GmbH, Germany)

3.5.2.5 Tewameter® TM300 (Courage & Khazaka, Germany) for transepidermal water loss (TEWL) of midface area

Source Courage + Khazaka electronic GmbH (2019)

Figure 3.7 Tewameter® TM300 and its result (Courage & Khazaka, Germany)

3.5.2.6 Global Aesthetic Improvement Scale (GAIS)

To assess subjectively of participants' skin quality, the Global Aesthetic Improvement Scale (GAIS) was utilized. The evaluation process involved presenting before-and-after photos of each participant, which were captured by the OLYMPUS PEN Lite E-PL5 and Visia CR system. Following the 6-month study, the outcome was evaluated by a specialized doctor using grading scales presented in table 3.1.

Table 3.1 Global Aesthetic Improvement Scale (GAIS)

Score	Degree	Description
1	Extremely improved	Excellent corrective result
2	Very improved	Marked improvement of the appearance, but not completely optimal
3	Improved	Improvement of the appearance, better compared with the initial condition, but a further treatment is advised
4	No change	The appearance substantially remains the same compared with the original condition
5	Worse	The appearance has worsened compared with the original condition

3.5.3 Materials and Equipment for Data Collection

3.5.3.1 Informed consent form

3.5.3.2 Document describing data and research procedures

3.5.3.3 Qualification letter from ethical committee

3.5.3.4 Patient profile and data record form

3.5.3.5 Side effect record form

3.5.3.6 Treatment satisfactory questionnaires for the participants using the bipolar scale of 5-points Likert scale shown below (Boone HN & Boone DA, 2012):

1 = Extremely dissatisfied

2 = Dissatisfied

3 = Neutral

4 = Satisfied

5 = Extremely satisfied

3.6 Study Procedure

3.6.1 The process of selecting research participants followed predetermined inclusion and exclusion criteria. Participants in the study were males or females, aged 30–60 years, who received treatment or worked at Mae Fah Luang University Hospital Asoke, Bangkok.

3.6.2 Each participant was assigned a screening number for identification purposes.

3.6.3 The researcher thoroughly explained the study's objectives, procedures, potential risks, and benefits to the participants.

3.6.4 Informed consent forms were signed by the participants, and personal information was collected, including medical and disease history, allergies, personal history, occupation, and menstrual history.

3.6.5 The participants were subjectively assessed for skin quality using the Global Aesthetic Improvement Scale (GAIS) by investigators.

3.6.6 All the participants were undergone the test by the investigator for objective Skin Quality using Visia CR system (Spots, Pores, Wrinkles, and Texture), Sebum Level using Sebumeter® SM815, Elasticity using Cutometer® MPA580, Skin Capacitance as using Corneometer® CM825, and Transepidermal Water Loss (TEWL) using Tewameter® TM300.

3.6.7 All of the participants were received 2 sessions of PDLLA injection (2 months apart). The products were provided in the form of freeze-dried powders contained within vials. It consisted of 200 mg (154 mg of PDLLA, 46 mg of Carboxymethyl Cellulose or CMC) in a vial. Prior to the injection, 8 mL of sterile water was introduced into the PDLLA vial. Just before the injections, an additional 2 mL of 2% lidocaine without adrenaline was added to achieve a final dilution of 10 mL of PDLLA. The injections were administered using a fanning technique into the subdermal plane, following a same pattern (figure 3.1), and utilizing a 23-G needle with a length of 5 cm, entering at an angle of 30 to 40 degrees. Each subject received a total of up to

2.5 mL of PDLLA on each side (total of 5 ml in each participant per session). Any immediate side effect of the treatment such as bruising, pain, skin irritation, redness, swelling, discomfort, and lump were closely monitored.

3.6.8 After the 1st injection at 0 months, the participants were scheduled for the 2nd injection at 2 months, follow-up visits at 4 months, and 6 months. In these follow-visits, they were tested by the investigator for:

1. Global Aesthetic Improvement Scale (GAIS score)
2. Visia CR system (Spots, Pores, Wrinkles, and Texture)
3. Sebum Level as measured by Sebumeter® SM815
4. Elasticity as measured by Cutometer® MPA580
5. Skin Capacitance as measured by Corneometer® CM825
6. Transepidermal Water Loss (TEWL) as measured by Tewameter® TM300
7. Side effect using the side effect record form

3.6.9 After the 6-month study period, participants were provided with a questionnaire to assess their satisfaction score regarding the PDLLA injection.

3.6.10 All the data were recorded in a case record and SPSS data file based on the participant's identification number.

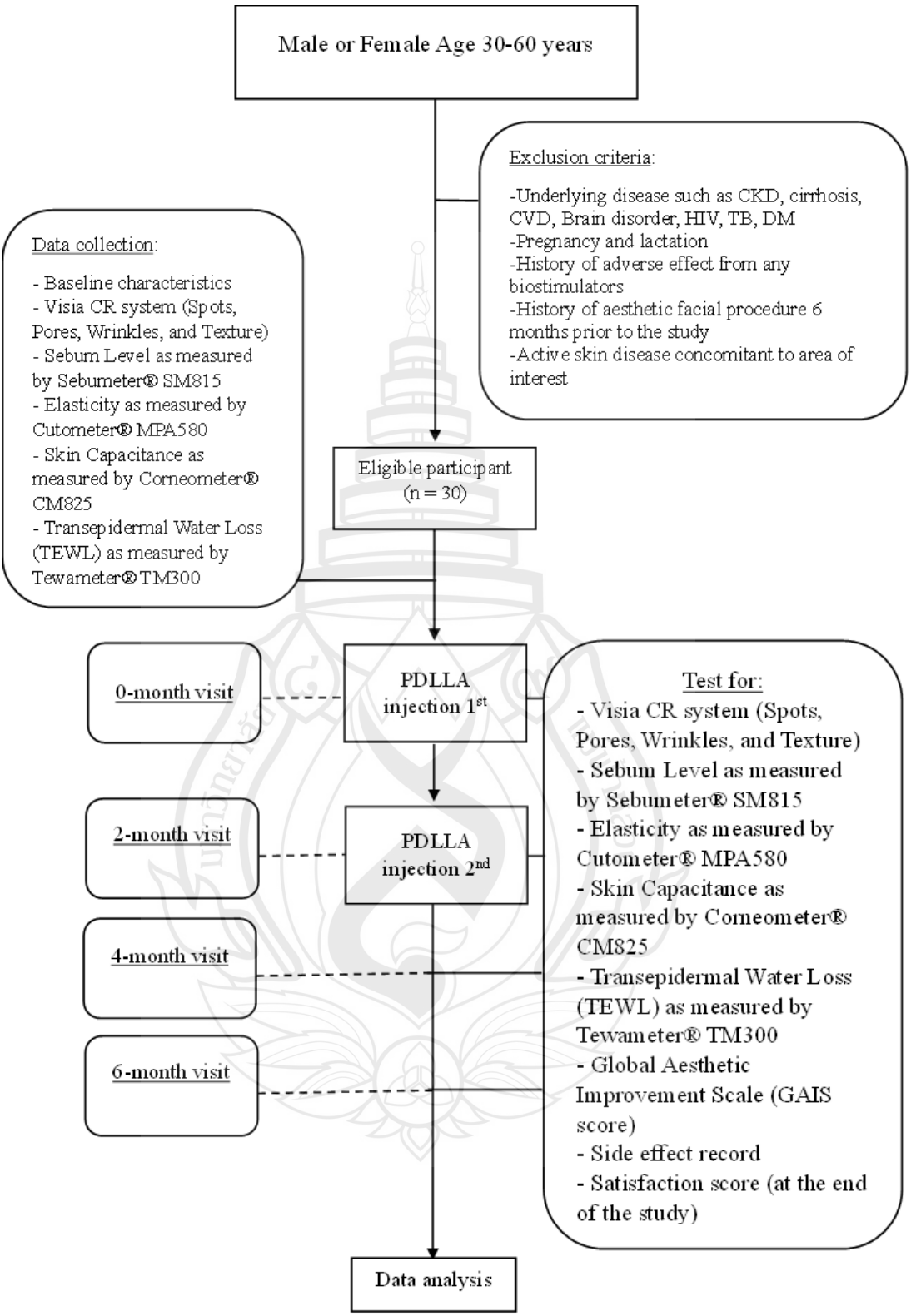


Figure 3.8 Flowchart of Data Collection and Study Procedure

3.7 Data Collection

3.7.1 Demographic characteristics of the research participants, including gender, weight, height, BMI, occupation, underlying diseases, family history, smoking history, personal history, history of drug and/or food allergies, family history, and contraceptive history in female participants.

3.7.2 Data of the facial rejuvenation markers from the Visia CR system (Spots, Pores, Wrinkles, and Texture): By placing the participant on machine, it automatically captured photos from the participants and analyzing 4 parameters including Spots, Pores, Wrinkles, and Texture in arbitrary unit (a.u.).

3.7.3 Data of the facial rejuvenation markers including the Sebum Level as measured by Sebumeter® SM815, Elasticity as measured by Cutometer® MPA580, Skin Capacitance as measured by Corneometer® CM825, Transepidermal Water Loss (TEWL) as measured by Tewameter® TM300: For each measurement (except Visia CR), the investigators used the probe to touch the skin at the measurement site (Figure 3.9). The investigators conducted the measurement 5 times on each side at each follow-up, totaling 10 times per follow-up which were adapted from the protocol by Jung et al. (2019).

1^{st}	=	the imaginary line between the tragus line and mid-pupil line
2^{nd} ,3^{rd}	=	1 cm lateral to the 1^{st} point
4^{th} ,5^{th}	=	1 cm vertical to the 1^{st} point

To enhance measurement accuracy and ensure consistency in measuring the exact same point each time, the investigators compared the measurement site with the previous photograph using the OLYMPUS PEN Lite E-PL5 and Visia CR system.

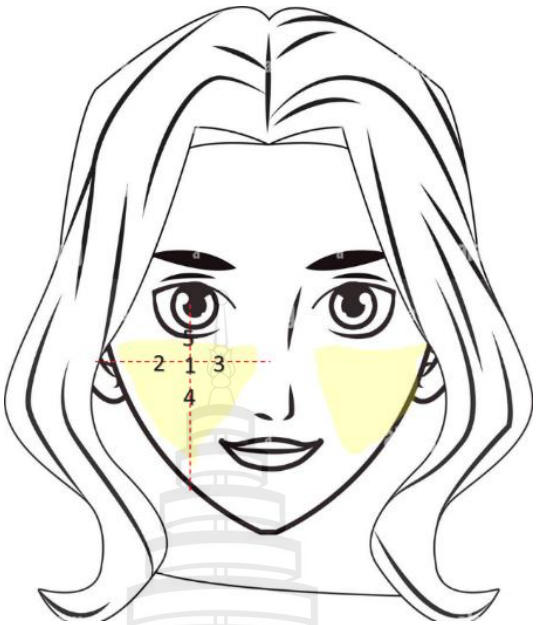


Figure 3.9 Measurement Site for the Data Collection

3.7.4 Data of Global Aesthetic Improvement Scale (GAIS): Each participant was assessed by two different investigators. The data were used as the mean of the two values. Investigators assigned a score based on their subjective perception of the subject's appearance using before-and-after photos of each participant, which were captured by the OLYMPUS PEN Lite E-PL5 and Visia CR system.

3.7.5 Information related to side effects occurring during treatment was recorded using a side effect record form. These effects included, but were not limited to, bruising, pain, skin irritation, redness, swelling, discomfort, lumps, or any other condition deviating from the desired improvement in skin quality. They were evaluated for short-term adverse effects by a physician once per session (0 months, 2 months, 4 months, and 6 months).

3.7.6 Data of participants' satisfactory score: After the 6-month study period, participants were provided with a satisfaction questionnaire to assess their satisfaction score regarding the PDLLA injection.

3.8 Statistical Analysis

The data of this study were analyzed by computer through package software (SPSS: Statistical Package for Social Sciences) version 28.0 for Windows. The p-value was set to < 0.05 for statistical significance.

3.8.1 Descriptive Statistics

3.8.1.1 Categorical data were reported as frequency and percentage. These included data on demographic features (gender, occupation, underlying diseases, family history, smoking history, and BMI).

3.8.1.2 For continuous data:

1. In the case of normal distribution, the mean and standard deviation (SD) were reported. This included data on age, weight, height, and BMI.

2. In the case of non-normal distribution, the median and inter-quartile range (IQR) were reported.

3.8.1.3 For side effects, we used descriptive statistics, which were reported as a percentage of the total events for each side effect, representing both the left and right sides of the face.

3.8.1.4 For satisfactory score, we used descriptive statistics, which were reported as frequency and percentage. We used the data collecting from the treatment satisfactory questionnaires for the participants using the scale of 5-points Likert scale shown below (Boone & Boone, 2012):

1 = Extremely dissatisfied
2 = Dissatisfied
3 = Neutral
4 = Satisfied
5 = Extremely satisfied

3.8.2 Inferential Statistics

For comparing continuous data, repeated measure ANOVA was used for normally distributed data. For data that were not normally distributed, the non-parametric Friedman test was used. The data that could be compared including the subjective Skin Quality using Global Aesthetic Improvement Scale (GAIS), objective

Skin Quality using Visia CR system (Spots, Pores, Wrinkles, and Texture), Sebum Level using Sebumeter® SM815, Elasticity using Cutometer® MPA580, Skin Capacitance as using Corneometer® CM825, and Transepidermal Water Loss (TEWL) using Tewameter® TM300. We analyzed data obtained from an average of 10 measurements per session (5 measurements on each side left & right during each follow-up).

3.9 Ethical Consideration

All protocol of this study has been approved from the Ethics Committee on Human Research at MFU (approval no. COA 97/2024 EC 24011-20). The study adhered to the Good Clinical Practice (GCP) guidelines outlined in the Declaration of Helsinki of 1975 and its 2000 amendment, which sets international standards for ethical and scientific quality in trials involving human subjects, including designing, conducting, recording, and reporting. The study's objectives, procedures, and protocol were communicated clearly and precisely to all participants, who were informed about the study in its entirety without individualized details.

The GCP guidelines encompassed various aspects such as protecting human rights, ensuring the safety and efficacy of newly developed compounds, defining the roles and responsibilities of clinical trial sponsors, clinical research investigators, and monitors, and setting standards for conducting clinical trials.

Prior to participation, all subjects were informed in detail and given the option to withdraw from the study at any time without affecting their relationship with the hospital. In order to preserve the rights of research volunteers, the researcher requested their consent to participate in the study and complete the questionnaires in a private location. The participants were allowed to answer the questionnaires at their own pace and without any pressure to reduce any potential discomfort. Moreover, participants were given the flexibility to take breaks during the study to address any issues or needs that may arise. The researcher ensured that data confidentiality was maintained throughout the study and that no identifying pictures of individuals were presented. All subject information was deleted one year after the study concluded.

CHAPTER 4

RESULTS

This chapter presented the findings from the study on the efficacy of Poly-D, L-Lactic Acid (PDLLA) and facial rejuvenation markers, focusing on two distinct age groups: early middle age and late middle age. The primary aim of this study was to examine the overall impact on skin quality from repeated subdermal PDLLA injections on midface area at two different time points (0 months, and 2 months). This evaluation encompassed both subjective assessments (utilizing the Global Aesthetic Improvement Scale by the blinded investigators and Satisfactory score by the patients) and objective measurements obtained from the Visia CR system, which assesses spots, pores, wrinkles, and texture. Additionally, we employed other devices to measure sebum levels, elasticity, skin capacitance, transepidermal water loss, and monitored for side effects. These assessments were conducted at specific time points (0 months, 2 months, 4 months, and 6 months). To the best of our knowledge, this study represents the first investigation into the subjective and objective effectiveness by comparing before and after the injection of PDLLA in Thailand.

The results are divided into two main sections. Part I reported on the efficacy of PDLLA in the early middle age group (30-45 years). Part II focused on the late middle age group (45-60 years). By comparing the outcomes between the two age groups, we could assess variations in PDLLA's effects based on age. Both groups were analyzed using the same subjective and objective measures to evaluate PDLLA's impact comprehensively. We presented into these categories including demographic data, facial rejuvenation markers, Global Aesthetic Improvement Scale, Satisfactory score, and side effects.

4.1 Part I: Early Middle Age Group (30-45 Years)

4.1.1 Demographic Data

We presented the baseline characteristics of the 15 participants in the early middle age group who participated in the study (Table 4.1). The average age of the participants was 36.33 years, with a standard deviation of 3.85 years. All participants were female, as no males were included in this group. Participants came from various occupational backgrounds, with the majority being housekeepers (26.7%), followed by therapists (20%), nurses (20%), office employees (13.3%), and business owners (6.7%). A small proportion (13.3%) of the participants were unemployed. In terms of skin type, the Fitzpatrick classification was used, showing that 60% of the participants had Type 3 skin, while 40% had Type 4 skin. No other skin types were represented in this group.

Regarding underlying health conditions, the vast majority of participants (86.7%) had no reported underlying diseases, while two participants (13.3%) had dyslipidemia (DLP). None of the participants had a history of food or drug allergies, and none reported taking current medications, including antibiotics or nonsteroidal anti-inflammatory drugs (NSAIDs). Additionally, none of the participants were current smokers, though two participants (13.3%) reported consuming alcohol. These baseline characteristics provided a comprehensive understanding of the participants' demographics and health status, ensuring homogeneity within the group and offering valuable context for interpreting the results of the study on PDLLA efficacy for facial rejuvenation in this age cohort. The absence of severe underlying conditions or other risk factors, such as smoking or medication use, suggested that the study results were less likely to be influenced by confounding factors related to participants' health.

The baseline characteristics in Table 4.1 provided a comprehensive overview of the 15 participants in the early middle age group, ensuring a well-rounded understanding of their demographics, health status, and lifestyle factors. This data established a foundation for evaluating the efficacy of PDLLA in a relatively homogeneous sample.

Table 4.1 Baseline characteristics of the 15 participants in early middle age group

Characteristic	Mean±SD
Age (year)	36.33±3.85
	Number of case
Sex	
Male	0
Female	15
Occupation	
Housekeeper	4
Therapist	3
Nurse	3
Office Employee	2
Business Owner	1
Unemployed	2
Fitzpatrick Skin Type	
Type 3	9
Type 4	6
Other	0
Underlying Disease	
None	13
DLP	2
History of Food/Drug Allergy	0
Current Medication (Antibiotic, NSAIDs)	0
Current Smoker	0
Alcohol drinking	2

4.1.2 Facial Rejuvenation Markers

4.1.2.1 Mean Value of Spot, Pore, Wrinkle, and Texture measured by the Visia® CR system

The mean values of facial rejuvenation markers (Spot, Pore, Wrinkle, and Texture) measured by the Visia® CR system in 15 participants from the early middle age group at four different time points: baseline, 2 months, 4 months, and 6 months (Table 4.2):

The Spot marker showed a relatively stable trend over time. At baseline, the mean Spot value was 30.36 ± 2.47, which remained consistent at 2 months (30.36 ± 2.70). By 4 months, there was a slight increase to 30.98 ± 2.63, followed by a small decrease to 30.68 ± 2.45 at 6 months, indicating minimal fluctuation in skin spot characteristics.

In contrast, the Pore marker demonstrated a significant reduction over time. At baseline, the mean Pore value was 14.51 ± 2.86, which slightly increased to 14.70 ± 2.63 at 2 months. However, a notable decrease was observed at 4 months (11.46 ± 2.48), with a further reduction at 6 months (10.78 ± 2.28), suggesting a gradual improvement in pore size after PDLLA treatment.

Wrinkle values followed a downward trend, indicating a positive response to treatment. The baseline mean was 31.69 ± 5.76, which slightly increased to 33.16 ± 6.36 at 2 months. However, a marked decrease was observed at 4 months (29.12 ± 4.70) and continued to decline at 6 months (24.08 ± 4.41), showing improvement in wrinkle reduction.

The Texture marker remained relatively stable throughout the study. At baseline, the mean value was 27.33 ± 9.31, with a slight decrease to 26.86 ± 9.43 at 2 months and a further decrease to 26.54 ± 9.13 at 4 months. By 6 months, the value slightly increased to 26.77 ± 9.82, indicating minor fluctuations in skin texture over time. Overall, the table reflects improvements in pores and wrinkles, with relatively stable results in skin spots and texture following PDLLA treatment over six months.

Table 4.2 presented data on facial rejuvenation markers measured at baseline, 2 months, 4 months, and 6 months. The markers included spot, pore, wrinkle, and texture. The table provided a detailed comparison of changes in these markers over time, allowing for the assessment of trends across the different time points.

Table 4.2 The mean of facial rejuvenation markers (Spot, Pore, Wrinkle, and Texture) measured by Visia® CR system in early middle age group at baseline, 2 months, 4 months, and 6 months (n = 15)

Markers	Baseline ($\bar{x}$±SD)	2 months ($\bar{x}$±SD)	4 months ($\bar{x}$±SD)	6 months ($\bar{x}$±SD)	p-value
Spot	30.36±2.47	30.36±2.70	30.98±2.63	30.68±2.45	1.000
Pore	14.51±2.86	14.70±2.63	11.46±2.48	10.78±2.28	<0.001
Wrinkle	31.69±5.76	33.16±6.36	29.12±4.70	24.08±4.41	<0.001
Texture	27.33±9.31	26.86±9.43	26.54±9.13	26.77±9.82	1.000

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), $\bar{x}$ for (Mean)

4.1.2.2 Mean Difference of Spots, Pores, Wrinkles, and Texture measured by the Visia® CR system

Table 4.3 presented the comparison of the mean differences in facial rejuvenation markers—spot, pore, wrinkle, and texture—measured by the Visia® CR system across multiple time points in the early middle age group (n = 15). The markers were analyzed between baseline, 2 months, 4 months, and 6 months using Repeated Measure ANOVA and post hoc tests with Bonferroni adjustments.

For the spot marker, the mean differences between the various time points were relatively small, with a mean difference of -0.01 ± 0.12 from baseline to 2 months, -0.62 ± 0.67 from baseline to 4 months, and -0.32 ± 0.66 from baseline to 6 months. The differences between the 2-month and 4-month periods, 2-month and 6-month periods, and 4-month and 6-month periods were -0.62 ± 0.69, -0.32 ± 0.68, and 0.30 ± 0.11, respectively. However, none of the p-values reached statistical significance, as they were all equal to 1.000 except for the comparison between the 4-month and 6-month periods, where the p-value was 0.082.

For the pore marker, the mean difference showed greater variation over time. From baseline to 2 months, the mean difference was -0.20 ± 0.38, while from baseline to 4 months and 6 months, the differences were 3.04 ± 0.51 and 3.728 ± 0.51, respectively. Comparisons between the 2-month and 4-month periods, 2-month and 6-

month periods, and 4-month and 6-month periods showed differences of 3.24 ± 0.27, 3.93 ± 0.27, and 0.69 ± 0.08, respectively. The p-values for these comparisons indicated statistically significant changes in the pore marker over time, with p-values of less than 0.001 in all but the baseline to 2-month comparison, which had a p-value of 1.000.

The wrinkle marker showed a notable progression in mean difference. From baseline to 2 months, the mean difference was -1.46 ± 1.51, while from baseline to 4 months and baseline to 6 months, the differences increased to 2.57 ± 1.13 and 7.62 ± 1.13, respectively. Comparing the 2-month and 4-month periods, the mean difference was 4.04 ± 0.64, and from the 2-month to 6-month periods, it was 9.08 ± 0.83. Lastly, the comparison between 4 months and 6 months showed a mean difference of 5.04 ± 0.35. Statistically significant differences were observed with p-values of less than 0.001 for all comparisons except for baseline to 2 months ($p = 1.000$) and baseline to 4 months ($p = 0.238$).

For the texture marker, the mean differences were minimal and consistent across the different time points. From baseline to 2 months, the difference was 0.47 ± 0.77, while from baseline to 4 months and baseline to 6 months, the differences were 0.78 ± 0.62 and 0.56 ± 0.71, respectively. The differences between 2 months and 4 months, 2 months and 6 months, and 4 months and 6 months were 0.31 ± 0.23, 0.09 ± 0.35, and -0.22 ± 0.33, respectively. However, none of these comparisons showed statistical significance, with p-values of 1.000 across all comparisons.

In summary, Table 4.3 provided a detailed analysis of the mean differences in facial rejuvenation markers over time, with significant changes observed particularly in pore and wrinkle markers, while spot and texture markers showed less variation and no significant changes over the study period.

Table 4.3 The comparison of the mean difference of facial rejuvenation markers (Spot, Pore, Wrinkle, and Texture) measured by Visia® CR system in early middle age group at baseline, 2 months, 4 months, and 6 months (n = 15)

Markers between each timepoint	**Spot (Mean diff ± SD)**	**p-value**	**Pore (Mean diff ± SD)**	**p-value**
Baseline to 2 months	-0.01±0.12	1.000	-0.20±0.38	1.000
Baseline to 4 months	-0.62±0.67	1.000	3.04±0.51	<0.001
Baseline to 6 months	-0.32±0.66	1.000	3.73±0.51	<0.001
2 months to 4 months	-0.62±0.69	1.000	3.24±0.27	<0.001
2 months to 6 months	-0.32±0.68	1.000	3.93±0.27	<0.001
4 months to 6 months	0.30±0.11	0.082	0.69±0.08	<0.001
Markers between each timepoint	**Wrinkle (Mean diff ± SD)**	**p-value**	**Texture (Mean diff ± SD)**	**p-value**
Baseline to 2 months	-1.46±1.51	1.000	0.47±0.77	1.000
Baseline to 4 months	2.57±1.13	0.238	0.78±0.62	1.000
Baseline to 6 months	7.62±1.13	<0.001	0.56±0.71	1.000
2 months to 4 months	4.04±0.64	<0.001	0.31±0.23	1.000
2 months to 6 months	9.08±0.83	<0.001	0.09±0.35	1.000
4 months to 6 months	5.04±0.35	<0.001	-0.22±0.33	1.000

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), Mean diff for (Mean Difference)

4.1.2.3 Mean Value of Sebum level, Elasticity, Skin capacitance, and Transepidermal water loss (TEWL)

Table 4.4 presented the mean values of sebum level, elasticity, skin capacitance, and transepidermal water loss (TEWL) measured in the early middle age group (n = 15) across different time points: baseline, 2 months, 4 months, and 6 months.

The sebum level was recorded at a mean of 18.22 ± 8.12 at baseline, and this value remained unchanged at 2 months (18.22 ± 8.26). At 4 months, a slight decrease was observed, with the mean sebum level at 17.97 ± 7.20, followed by a minor

increase to 18.05 ± 6.62 at the 6-month mark. The data indicated a relative stability in sebum production among the participants throughout the study period.

Elasticity was initially measured at 0.40 ± 0.11 at baseline, reflecting the skin's ability to return to its original shape after stretching. At the 2-month follow-up, the elasticity value remained constant at 0.40 ± 0.10. However, a notable increase in elasticity was recorded at 4 months (0.44 ± 0.11) and further improved to 0.51 ± 0.09 at 6 months. This increase indicated an enhancement in skin elasticity over the duration of the study.

Skin capacitance, which measures the skin's ability to hold moisture, started at a baseline of 44.29 ± 14.34. By the 2-month follow-up, the mean capacitance increased slightly to 46.17 ± 13.90. A more significant increase was observed at 4 months, with a mean capacitance of 50.48 ± 12.57, and it continued to rise to 52.48 ± 10.13 by the 6-month mark. These results suggested a progressive improvement in the skin's moisture retention capacity over the study period.

Lastly, transepidermal water loss (TEWL), which assesses the amount of water that evaporates from the skin, was initially measured at 35.38 ± 8.96 at baseline. This value decreased to 34.61 ± 9.90 at the 2-month follow-up. A more significant reduction in TEWL was recorded at 4 months, with a mean value of 28.88 ± 7.34, and it further decreased to 27.76 ± 6.86 at 6 months. These findings indicated that the skin became more efficient at retaining moisture over time, as reflected by the decrease in water loss.

In conclusion, Table 4.4 provided a comprehensive analysis of various skin parameters in the early middle age group over a 6-month period, highlighting stability in sebum levels, improvements in elasticity, significant increases in skin capacitance, and reductions in transepidermal water loss.

Table 4.4 The mean of sebum level, elasticity, skin capacitance, and transepidermal water loss in early middle age group at baseline, 2 months, 4 months, and 6 months (n = 15)

Markers	Baseline	2 months	4 months	6 months	p-value
Sebum level	18.22±8.12	18.22±8.26	17.97±7.20	18.05±6.62	1.000
Elasticity	0.40±0.11	0.40±0.10	0.44±0.11	0.51±0.09	<0.001
Skin Capacitance	44.29±14.34	46.17±13.90	50.48±12.57	52.48±10.13	<0.001
TEWL	35.38±8.96	34.61±9.90	28.88±7.34	27.76±6.86	<0.001

Note TEWL for (Transepidermal Water Loss)

4.1.2.4 Mean Difference of Sebum level measured by Sebumeter® SM815

Table 4.5 presented the comparison of mean differences in sebum levels measured by Sebumeter® SM815 in the early middle age group (n = 15) at baseline and subsequent follow-ups at 2 months, 4 months, and 6 months. The table documented the changes in sebum levels over time, providing both the mean differences and their corresponding p-values.

At the baseline, the mean difference in sebum levels between baseline and the 2-month follow-up was recorded as -0.01 ± 0.06 with the p-value of 1.000 confirmed that this change was not statistically significant. Similarly, the comparison between baseline and the 4-month follow-up showed a mean difference of 0.26 ± 0.35, also with a p-value of 1.000, indicating no statistically significant change in sebum levels during these intervals. The mean difference between baseline and 6 months was measured at 0.17 ± 0.56, further affirming a lack of significant change in sebum production throughout the study duration.

Comparing sebum levels from 2 months to 4 months, the mean difference was recorded at 0.26 ± 0.40, accompanied by a p-value of 1.000, suggesting no statistically significant change. The comparison from 2 months to 6 months yielded a mean difference of 0.17 ± 0.61, again with a p-value of 1.000, supporting the observation of stable sebum levels. Furthermore, the mean difference between 4

months and 6 months showed a decrease of -0.08 ± 0.34, also yielding a p-value of 1.000, which indicated that the changes were statistically insignificant.

In summary, Table 4.5 demonstrated that the sebum levels remained relatively stable throughout the study period across all measured intervals. The consistent p-values of 1.000 indicated that there were no significant changes in sebum production among participants at various time points. This analysis utilized repeated measures ANOVA and post hoc tests with the Bonferroni correction to ensure the robustness of the statistical findings.

Table 4.5 The comparison of the mean difference of sebum level measured by Sebumeter® SM815 in early middle age group at baseline, 2 months, 4 months, and 6 months (n = 15)

Markers	Sebum Level (μg/cm^2) (Mean diff ± SD)	p-value
Baseline to 2 months	-0.01±0.06	1.000
Baseline to 4 months	0.26±0.35	1.000
Baseline to 6 months	0.17±0.56	1.000
2 months to 4 months	0.26±0.40	1.000
2 months to 6 months	0.17±0.61	1.000
4 months to 6 months	-0.08±0.34	1.000

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), Mean diff for (Mean Difference)

4.1.2.5 Mean Difference of Elasticity measured by Cutometer® MPA580

Table 4.6 provided a comparison of the mean differences in elasticity measured by Cutometer® MPA580 in the early middle age group (n = 15) at baseline and at follow-up intervals of 2 months, 4 months, and 6 months. The table included the mean differences alongside their standard deviations and p-values to evaluate the statistical significance of changes in skin elasticity over the study duration.

At baseline, the mean difference in elasticity from baseline to the 2-month follow-up was recorded at 0.01 ± 0.01, with a p-value of 1.000, indicating no significant change in elasticity during this period. The subsequent comparison from

baseline to the 4-month follow-up showed a mean difference of -0.04 ± 0.02, accompanied by a p-value of 0.361, which suggested a lack of significant alteration in skin elasticity compared to the baseline measurement. In contrast, the difference observed between baseline and the 6-month follow-up was -0.11 ± 0.02, with a p-value of <0.001, demonstrating a statistically significant increase in elasticity over this extended period.

Analyzing the changes from 2 months to 4 months revealed a mean difference of -0.04 ± 0.01, with a p-value of 0.029, indicating a statistically significant increase in elasticity. Furthermore, the comparison from 2 months to 6 months exhibited a more pronounced mean difference of -0.11 ± 0.01, also with a p-value of <0.001, reinforcing the evidence of significant improvement in elasticity over this time span. The final comparison between the 4-month and 6-month follow-ups indicated a mean difference of -0.07 ± 0.01, with a p-value of 0.002, which further confirmed that elasticity continued to decline significantly during the latter part of the study.

Overall, Table 4.6 highlighted the trends in skin elasticity among participants over the study period, indicating a significant increase in elasticity from baseline to 6 months, particularly between 2 and 6 months. The statistical analysis was performed using repeated measures ANOVA and post hoc tests with Bonferroni corrections to ensure the reliability of the results. This comprehensive assessment provided insights into the changes in skin elasticity that occurred in the early middle age group throughout the intervention period.

Table 4.6 The comparison of the mean difference of elasticity measured by Cutometer® MPA580 in early middle age group at baseline, 2 months, 4 months, and 6 months (n=15)

Markers	Elasticity (a.u.) (Mean diff ± SD)	p-value
Baseline to 2 months	0.01±0.01	1.000
Baseline to 4 months	-0.04±0.02	0.361
Baseline to 6 months	-0.11±0.02	<0.001
2 months to 4 months	-0.04±0.01	0.029
2 months to 6 months	-0.11±0.01	<0.001
4 months to 6 months	-0.07±0.01	0.002

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), Mean diff for (Mean Difference)

4.1.2.6 Mean Difference of Skin Capacitance or Skin Hydration measured by Corneometer® CM825

Table 4.7 presented the comparison of mean differences in skin capacitance, a measure of skin hydration, assessed using the Corneometer® CM825 in a early middle age group (n = 15) at baseline and during follow-ups at 2 months, 4 months, and 6 months. The table included mean differences alongside their standard deviations and p-values to evaluate the statistical significance of changes in skin hydration over the study period.

At baseline, the skin capacitance measurement showed a mean difference of -1.87 ± 0.41 when comparing baseline to the 2-month follow-up, with a p-value of 0.003. This indicated a statistically significant increase in skin hydration after 2 months. When analyzing the data from baseline to the 4-month follow-up, the mean difference was found to be -6.19 ± 0.86, accompanied by a p-value of <0.001, demonstrating a significant increase in skin hydration during this time frame. Furthermore, the comparison between baseline and the 6-month follow-up yielded a mean difference of -8.19 ± 1.45, with a p-value of <0.001, indicating a substantial improvement in skin hydration over the entire study period.

In examining the changes from 2 months to 4 months, the mean difference recorded was -4.31 ± 0.65, and the associated p-value was <0.001, suggesting a significant increase in skin capacitance and, therefore, skin hydration during this interval. Similarly, the comparison from 2 months to 6 months revealed a mean difference of -6.32 ± 1.23, with a p-value of 0.001, reinforcing the finding of a significant improvement in hydration from the 2-month mark to the 6-month follow-up. Conversely, the comparison between the 4-month and 6-month follow-ups showed a mean difference of -2.00 ± 0.81, with a p-value of 0.160, indicating no statistically significant change in skin capacitance between these two time points.

Table 4.7 The comparison of the mean difference of skin capacitance or skin hydration measured by Corneometer® CM825 in early middle age group at baseline, 2 months, 4 months, and 6 months (n = 15)

Markers	**Skin Capacitance (a.u.) (Mean diff ± SD)**	**p-value**
Baseline to 2 months	-1.87±0.41	0.003
Baseline to 4 months	-6.19±0.86	<0.001
Baseline to 6 months	-8.19±1.45	<0.001
2 months to 4 months	-4.31±0.65	<0.001
2 months to 6 months	-6.32±1.23	0.001
4 months to 6 months	-2.00±0.81	0.160

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), Mean diff for (Mean Difference)

Overall, Table 4.7 effectively highlighted the trend of increasing skin hydration among the participants over the study duration, with significant changes noted particularly from baseline to 6 months and across subsequent follow-ups. The statistical analyses were conducted using repeated measures ANOVA and post hoc tests with Bonferroni corrections, ensuring that the findings were rigorously evaluated for their statistical significance. This detailed examination provided insight into the changes in skin capacitance, underscoring the impact of the intervention on skin hydration levels in the early middle age group throughout the study.

4.1.2.7 Mean Difference of Transepidermal Water Loss (TEWL) measured by Tewameter® TM300

Table 4.8 provided a comparison of the mean differences in transepidermal water loss (TEWL), measured using the Tewameter® TM300, among participants in the early middle age group (n = 15) at baseline, as well as at 2 months, 4 months, and 6 months. The table presented mean differences, standard deviations, and p-values to assess the significance of changes in TEWL over the specified time intervals.

At baseline, the TEWL measurement revealed a mean difference of 0.77 ± 0.46 when comparing baseline to the 2-month follow-up. The associated p-value of 0.716 indicated no statistically significant change in TEWL during this period. However, when examining the baseline to the 4-month follow-up, the mean difference increased to 6.49 ± 0.68, with a p-value of <0.001, suggesting a significant decrease in transepidermal water loss over this time frame. Similarly, the comparison between baseline and the 6-month follow-up showed a mean difference of 7.62 ± 0.79, accompanied by a p-value of <0.001, indicating a substantial decline in TEWL during the entire study period.

When assessing the changes from the 2-month to the 4-month follow-up, the mean difference recorded was 5.73 ± 0.87, with a p-value of <0.001. This result indicated a significant decrease in TEWL during this interval, highlighting a notable decline in water loss from the skin. The comparison from 2 months to 6 months yielded a mean difference of 6.85 ± 0.99, with a p-value of 0.001, further reinforcing the trend of decreased water loss over time. The change observed between the 4-month and 6-month follow-ups demonstrated a mean difference of 1.13 ± 0.27, with a p-value of 0.006, suggesting a statistically significant decrease in TEWL between these two time points, albeit to a lesser extent than earlier intervals.

Overall, Table 4.8 effectively illustrated the trend of decreasing transepidermal water loss among the early middle age participants over the study duration, with significant decreases noted particularly from baseline to 4 months and continuing through 6 months. The statistical analyses conducted using repeated measures ANOVA and post hoc tests with Bonferroni corrections provided a thorough evaluation of the data, ensuring the findings were statistically robust. This comprehensive analysis offered valuable insights into changes in TEWL, highlighting

the implications for skin barrier function in the early middle age group throughout the course of the study.

Table 4.8 The comparison of the mean difference of transepidermal water loss measured by Tewameter® TM300 in early middle age group at baseline, 2 months, 4 months, and 6 months (n = 15)

Markers	**TEWL (g/m2/h) (Mean diff ± SD)**	**p-value**
Baseline to 2 months	0.77±0.46	0.716
Baseline to 4 months	6.49±0.68	<0.001
Baseline to 6 months	7.62±0.79	<0.001
2 months to 4 months	5.73±0.87	<0.001
2 months to 6 months	6.85±0.99	0.001
4 months to 6 months	1.13±0.27	0.006

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), Mean diff for (Mean Difference), TEWL for (Transepidermal Water Loss)

4.1.3 Global Aesthetic Improvement Scale Assessment (GAIS)

This section presented the mean scores of the Global Aesthetic Improvement Scale (GAIS) for participants in the early middle age group (n = 15) at 2 months, 4 months, and 6 months post-treatment (Table 4.9). The mean GAIS score at 2 months was recorded as 4.20 ± 0.30, indicating a notable improvement in aesthetic outcomes. At the 4-month mark, the mean GAIS score increased to 4.78 ± 0.27, reflecting further enhancement in perceived aesthetic improvements among the participants. By the 6-month follow-up, the mean GAIS score reached 4.96 ± 0.12, signifying the highest level of improvement recorded during the study period. The accompanying p-value of <0.001 indicated that these differences in mean GAIS scores across the various time points were statistically significant. This statistical analysis was performed using the Friedman Test, which is suitable for analyzing repeated measures on a single group to determine changes over time. The significant increase in GAIS scores over the course of the study suggested a continuous and positive trend in perceived aesthetic improvement in the early middle age group as assessed at multiple intervals following the treatment. These results highlighted the effectiveness of the treatment regimen in enhancing aesthetic outcomes as evaluated by the GAIS, thereby providing valuable insights into the perceived benefits experienced by participants throughout the study duration.

Table 4.9 The mean of GAIS scores in early middle age group at 2 months, 4 months, and 6 months (n = 15)

GAIS score	2 months ($\bar{x}$±SD)	4 months ($\bar{x}$±SD)	6 months ($\bar{x}$±SD)	p-value
	4.20±0.30	4.78±0.27	4.96±0.12	<0.001

Note Data were analyzed using Friedman Test, GAIS for (Global Aesthetic Improvement Scale Assessment), SD for (Standard Deviation), $\bar{x}$ for (Mean)

4.1.4 Patients' Satisfaction Score

This section displayed the patients' satisfaction scores for the early middle age group at the 6-month follow-up, encompassing a total of 15 participants (Table 4.10). The results indicated that no participants reported feeling extremely dissatisfied, dissatisfied, or neutral regarding their treatment outcomes, as evidenced by the 0% frequency for scores 1, 2, and 3. A total of 2 participants, accounting for 13.3%, expressed satisfaction with a score of 4, while a significant majority of 13 participants, representing 86.7%, reported being extremely satisfied with a score of 5. The mean satisfaction score calculated for the group was 4.87 ± 0.35, suggesting a high level of overall satisfaction among the participants. The standard deviation of 0.35 indicated that the scores were closely clustered around the mean, reflecting a consistent experience of satisfaction among those who reported their outcomes. This data highlighted the effectiveness of the treatment administered, with an overwhelming proportion of participants expressing a positive response and a high level of contentment with the results after 6 months. Overall, these satisfaction scores provided valuable insight into the participants' experiences and the perceived effectiveness of the intervention in the context of facial rejuvenation within this demographic.

Table 4.10 Patients' satisfaction scores in early middle age group at 6 months (n = 15)

Satisfaction Scores	Frequency
Extremely Dissatisfied (Score 1)	0
Dissatisfied (Score 2)	0
Neutral (Score 3)	0
Satisfied (Score 4)	2
Extremely Satisfied (Score 5)	13
$\bar{x}$ ± SD	**4.87 ± 0.35**

Note SD for (Standard Deviation), $\bar{x}$ for (Mean)

4.1.5 Side Effects

No serious side effects were observed during the entire study. No significant complaint was reported by the participants.

Figure 4.1 The comparison of clinical photographs of representative case No. 1 at 0, 2, 4, and 6 months, respectively

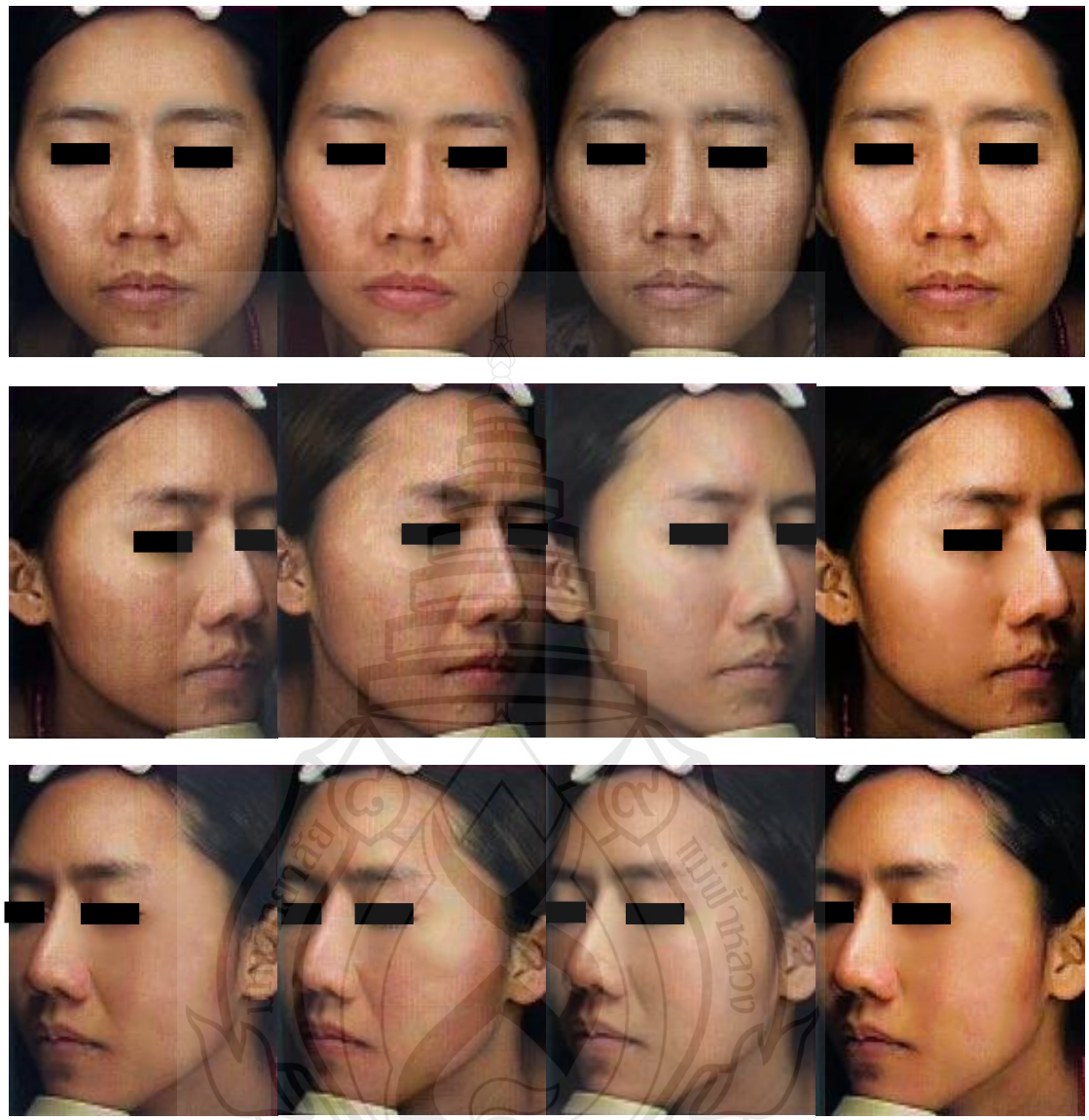


Figure 4.2 The comparison of VISIA CR system photographs of representative case No. 2 at 0, 2, 4, and 6 months, respectively

4.2 Part II: Late Middle Age Group (45-60 Years)

4.2.1 Demographic Data

The baseline characteristics of the 15 participants in the late middle age group was presented in Table 4.11. The average age of the participants was 52.33 years with a standard deviation of 3.44. All 15 participants were female, as no males participated in this group. Regarding occupation, the highest proportion of participants were housekeepers and office employees, each accounting for 33.33% of the group, equating to 5 participants for each occupation. Three participants (20.00%) were therapists, while two participants (13.33%) were nurses. There were no participants who were business owners or unemployed.

In terms of Fitzpatrick skin type, the majority of participants, 66.67%, or 10 individuals, had Type 4 skin, while 33.33%, or 5 participants, had Type 3 skin. No participants in this group had any other skin types. None of the participants reported having any underlying diseases, making the prevalence of such conditions 0%. Additionally, no participants were diagnosed with dyslipidemia (DLP).

Concerning allergies, none of the participants reported a history of food or drug allergies. Similarly, none of the participants were on current medications, including antibiotics or nonsteroidal anti-inflammatory drugs (NSAIDs). There were also no current smokers within the group. However, 3 participants, representing 20.00% of the group, reported consuming alcohol. Overall, the data in Table 4.11 outlined the demographic, occupational, and medical characteristics of the late middle-aged participants, providing a comprehensive overview of their baseline health and lifestyle factors.

Table 4.11 Baseline characteristics of the 15 participants in late middle age group

Characteristic	Mean±SD
Age (year)	52.33±3.44
	Number of cases
Sex	
Male	0
Female	15
Occupation	
Housekeeper	5
Therapist	3
Nurse	2
Office Employee	5
Business Owner	0
Unemployed	0
Fitzpatrick Skin Type	
Type 3	5
Type 4	10
Other	0
Underlying Disease	
None	15
DLP	0
History of Food/Drug Allergy	0
Current Medication (Antibiotic, NSAIDs)	0
Current Smoker	0
Alcohol drinking	3

4.2.2 Facial Rejuvenation Markers

4.2.2.1 Mean Value of Spots, Pores, Wrinkles, and Texture measured by Visia® CR system

Table 4.12 provided data on the mean values of facial rejuvenation markers—spots, pores, wrinkles, and texture—measured by the Visia® CR system in the late middle-aged group at baseline, 2 months, 4 months, and 6 months. The study included 15 participants.

For the marker "spots", the mean value at baseline was 29.65 ± 2.76. After 2 months, this value slightly increased to 29.73 ± 2.77 and then decreased at 4 months to 29.58 ± 2.72. By 6 months, the mean score had further decreased to 29.39 ± 2.51.

For the marker "pores", the baseline mean was 21.45 ± 7.95. After 2 months, there was a significant reduction to 18.23 ± 7.21. This downward trend continued at 4 months, where the value dropped to 13.02 ± 5.45, and at 6 months, the value further decreased to 11.55 ± 5.23.

The "wrinkle" marker also showed a steady decline over time. The baseline mean was 31.38 ± 9.39. After 2 months, the value decreased to 27.55 ± 8.87, and at 4 months, it further dropped to 23.85 ± 8.23. By 6 months, the wrinkle mean was 20.28 ± 8.34, showing the most significant reduction.

For the "texture" marker, the baseline mean was 21.35 ± 13.40. This value remained relatively stable over time. At 2 months, the mean was 21.31 ± 13.38, followed by 21.09 ± 13.07 at 4 months. By 6 months, the value slightly increased to 21.57 ± 13.71, showing minimal fluctuation throughout the study period.

The table 4.12 detailed the progression of these four facial rejuvenation markers across different time points, with changes observed particularly in pores and wrinkles, while spots and texture showed less variation over time.

Table 4.12 The mean of facial rejuvenation markers (Spot, Pore, Wrinkle, and Texture) measured by Visia® CR system in late middle age group at baseline, month 2, month 4, and month 6 (n = 15)

Markers	Baseline ($\bar{x}$±SD)	2 months ($\bar{x}$±SD)	4 months ($\bar{x}$±SD)	6 months ($\bar{x}$±SD)	p-value
Spot	29.65±2.76	29.73±2.77	29.58±2.72	29.39±2.51	0.451
Pore	21.45±7.95	18.23±7.21	13.02±5.45	11.55±5.23	<0.001
Wrinkle	31.38±9.39	27.55±8.87	23.85±8.23	20.28±8.34	<0.001
Texture	21.35±13.40	21.31±13.38	21.09±13.07	21.57±13.71	1.000

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), $\bar{x}$ for (Mean)

4.2.2.2 Mean Difference of Spots, Pores, Wrinkles, and Texture measured by the Visia® CR system

Table 4.13 presented the comparison of the mean difference in facial rejuvenation markers—spots, pores, wrinkles, and texture—measured by the Visia® CR system in the late middle-aged group at various time intervals. The analysis involved 15 participants and compared the mean differences between baseline, 2 months, 4 months, and 6 months.

For the “spot” marker, the mean difference between baseline and 2 months was -0.08 ± 0.07 with a p-value of 1.000, indicating no significant change. The comparison between baseline and 4 months showed a mean difference of 0.7 ± 0.11, also with a p-value of 1.000. The mean difference between baseline and 6 months was 0.26 ± 0.13 with a p-value of 0.451, indicating no statistical significance. The changes between 2 months and 4 months (0.15 ± 0.07), 2 months and 6 months (0.33 ± 0.12), and 4 months and 6 months (0.19 ± 0.11) all had p-values greater than 0.05, showing no significant difference.

For the “pore” marker, a significant difference was observed across the time points. The mean difference between baseline and 2 months was 3.21 ± 0.99 with a p-value of 0.036, showing a significant change. From baseline to 4 months, the mean difference increased to 8.43 ± 1.42, with a p-value of less than 0.001, indicating a

highly significant change. Similarly, the mean difference from baseline to 6 months was 9.90 ± 1.42 with a p-value of less than 0.001, showing a substantial reduction in pore size. The comparisons between 2 months and 4 months (5.21 ± 0.84), 2 months and 6 months (6.69 ± 0.92), and 4 months and 6 months (1.47 ± 0.29) all showed statistically significant differences, with p-values of less than 0.001 and 0.001, respectively.

For the “wrinkle” marker, the mean difference between baseline and 2 months was 3.83 ± 1.06 with a p-value of 0.017, indicating a significant reduction in wrinkles. The difference increased further at 4 months, with a mean difference of 7.53 ± 1.18 and a p-value of less than 0.001. By 6 months, the mean difference was 11.10 ± 1.43, also with a p-value of less than 0.001. The changes between 2 months and 4 months (3.70 ± 0.99), 2 months and 6 months (7.27 ± 1.35), and 4 months and 6 months (3.57 ± 0.73) were statistically significant, with p-values of 0.013, 0.001, and 0.001, respectively.

For the “texture” marker, there was minimal change between baseline and 2 months, with a mean difference of 0.04 ± 0.06 and a p-value of 1.000. From baseline to 4 months, the mean difference was 0.26 ± 0.12, with a p-value of 0.292, and from baseline to 6 months, the mean difference was -0.22 ± 0.26 with a p-value of 1.000. The comparisons between 2 months and 4 months (0.23 ± 0.11), 2 months and 6 months (-0.25 ± 0.26), and 4 months and 6 months (-0.48 ± 0.28) did not show significant differences, with p-values above 0.05.

The data were analyzed using repeated measure ANOVA and post hoc tests with Bonferroni correction, showing significant changes in pore and wrinkle markers, while spots and texture exhibited fewer notable changes over time.

Table 4.13 The comparison of the mean difference of facial rejuvenation markers (Spot, Pore, Wrinkle, and Texture) measured by Visia® CR system in late middle age group at baseline, month 2, month 4, and month 6 (n = 15)

Markers between each timepoint	**Spot (Mean diff ± SD)**	**p-value**	**Pore (Mean diff ± SD)**	**p-value**
Baseline to 2 months	-0.08±0.07	1.000	3.21±0.99	0.036
Baseline to 4 months	0.70±0.11	1.000	8.43±1.42	<0.001
Baseline to 6 months	0.26±0.13	0.451	9.90±1.42	<0.001
2 months to 4 months	0.15±0.07	0.264	5.21±0.84	<0.001
2 months to 6 months	0.33±0.12	0.084	6.69±0.92	<0.001
4 months to 6 months	0.19±0.11	0.761	1.47±0.29	0.001
Markers between each timepoint	**Wrinkle (Mean diff ± SD)**	**p-value**	**Texture (Mean diff ± SD)**	**p-value**
Baseline to 2 months	3.83±1.06	0.017	0.04±0.06	1.000
Baseline to 4 months	7.53±1.18	<0.001	0.26±0.12	0.292
Baseline to 6 months	11.10±1.43	<0.001	-0.22±0.26	1.000
2 months to 4 months	3.70±0.99	0.013	0.23±0.11	0.335
2 months to 6 months	7.27±1.35	0.001	-0.25±0.26	1.000
4 months to 6 months	3.57±0.73	0.001	-0.48±0.28	0.623

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), Mean diff for (Mean Difference)

4.2.2.3 Mean Value of Sebum level, Elasticity, Skin capacitance, and Transepidermal water loss (TEWL)

Table 4.14 summarized the mean values of sebum level, elasticity, skin capacitance, and transepidermal water loss (TEWL) in the late middle-aged group at baseline, 2 months, 4 months, and 6 months among fifteen participants.

The sebum level remained relatively stable throughout the study. At baseline, the mean sebum level was 20.04 ± 9.86, which slightly decreased to 20.03 ± 9.87 at 2 months and further to 19.92 ± 9.88 at 4 months. By 6 months, the sebum level returned to nearly the baseline value at 20.05 ± 9.72.

The elasticity of the skin showed a progressive increase over time. At baseline, the mean elasticity was 0.37 ± 0.11, which increased to 0.44 ± 0.11 at 2 months. At 4 months, the elasticity continued to rise to 0.53 ± 0.13, and by 6 months, it reached 0.57 ± 0.11, indicating an improvement in elasticity during the study period.

Skin capacitance, which reflects skin hydration, also showed a consistent increase. At baseline, the mean skin capacitance was 45.44 ± 14.58, rising to 49.40 ± 15.30 at 2 months. By 4 months, the value further increased to 54.20 ± 14.30, and by 6 months, it reached 56.77 ± 13.49, indicating an improvement in skin hydration over time.

TEWL, which measures the amount of water loss through the skin, demonstrated a decrease across the study period. At baseline, the mean TEWL was 35.01 ± 7.69. By 2 months, it had decreased to 30.72 ± 9.89, and at 4 months, it further decreased to 26.10 ± 8.05. By 6 months, TEWL reached its lowest value of 24.54 ± 7.89, suggesting a reduction in water loss through the skin over time.

Table 4.14 The mean of sebum level, elasticity, skin capacitance, and transepidermal water loss in late middle age group at baseline, month 2, month 4, and month 6 (n = 15)

Markers	Baseline	2 months	4 months	6 months	p-value
Sebum level	20.04±9.86	20.03±9.87	19.92±9.88	20.05±9.72	1.000
Elasticity	0.37±0.11	0.44±0.11	0.53±0.13	0.57±0.11	<0.001
Skin Capacitance	45.44±14.58	49.40±15.30	54.20±14.30	56.77±13.49	<0.001
TEWL	35.01±7.69	30.72±9.89	26.10±8.05	24.54±7.89	<0.001

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), $\bar{x}$ for (Mean), TEWL for (Transepidermal Water Loss)

4.2.2.4 Mean Difference of Sebum level measured by Sebumeter® SM815

Table 4.15 presented the comparison of the mean difference in sebum levels in the late middle-aged group, measured at different time points: baseline, 2 months, 4 months, and 6 months. The mean differences and their respective standard deviations (SD) were calculated between each of these time points, and the p-values were provided to assess the statistical significance.

Between baseline and 2 months, the mean difference in sebum level was 0.01 ± 0.04, with a p-value of 1.000, indicating no statistically significant change in sebum levels during this period. The comparison between baseline and 4 months showed a slightly larger mean difference of 0.13 ± 0.08, but the p-value of 0.870 still indicated no significant change. For baseline to 6 months, the mean difference was -0.01 ± 0.26, with a p-value of 1.000, again showing no significant alteration in sebum levels between these points.

When comparing sebum levels between 2 months and 4 months, the mean difference was 0.11 ± 0.06, with a p-value of 0.506, suggesting no significant change in sebum levels. Between 2 months and 6 months, the mean difference was -0.03 ± 0.27, with a p-value of 1.000, indicating no significant variation in sebum levels during this period. Lastly, between 4 months and 6 months, the mean difference in sebum levels was -0.14 ± 0.26, with a p-value of 1.000, showing no statistically significant change between these two time points.

The data were analyzed using repeated measures ANOVA, and post-hoc tests with Bonferroni correction were applied to account for multiple comparisons. No significant differences were found across any of the time points in terms of sebum level changes, as indicated by the p-values.

Table 4.15 The comparison of the mean difference of sebum level measured by Sebumeter® SM815 in late middle age group at baseline, month 2, month 4, and month 6 (n = 15)

Markers	Sebum Level (μg/cm^2) (Mean diff ± SD)	p-value
Baseline to 2 months	0.01±0.04	1.000
Baseline to 4 months	0.13±0.08	0.870
Baseline to 6 months	-0.01±0.26	1.000
2 months to 4 months	0.11±0.06	0.506
2 months to 6 months	-0.03±0.27	1.000
4 months to 6 months	-0.14±0.26	1.000

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), Mean diff for (Mean Difference)

4.2.2.5 Mean Difference of Elasticity measured by Cutometer® MPA580

Table 4.16 presented the comparison of the mean differences in skin elasticity in the late middle-aged group, measured at baseline, 2 months, 4 months, and 6 months. Elasticity was assessed using the Cutometer® MPA580, and the mean differences (Mean diff ± SD) between the time points were calculated, with corresponding p-values to evaluate statistical significance.

From baseline to 2 months, the mean difference in elasticity was -0.07 ± 0.02, with a p-value of 0.039, indicating a statistically significant increase in skin elasticity during this period. The comparison between baseline and 4 months showed a larger mean difference of -0.16 ± 0.04, with a p-value of 0.003, which also demonstrated a significant increase in elasticity. From baseline to 6 months, the mean difference increased further to -0.20 ± 0.03, with a p-value of <0.001, signifying a highly significant improvement in skin elasticity over the 6-month period.

When comparing elasticity between 2 months and 4 months, the mean difference was -0.09 ± 0.02, with a p-value of 0.006, showing a significant increase in elasticity during this interval. The comparison between 2 months and 6 months revealed a mean difference of -0.13 ± 0.02, with a p-value of <0.001, indicating

another significant increase in elasticity. Finally, the mean difference between 4 months and 6 months was -0.04 ± 0.01, with a p-value of 0.001, demonstrating a statistically significant improvement in elasticity over this period as well.

The data were analyzed using repeated measures ANOVA, and post-hoc tests with Bonferroni correction were applied to account for multiple comparisons. Significant increases in skin elasticity were observed at all time points, which were below the threshold for statistical significance ($p < 0.05$). These findings demonstrated a consistent improvement in elasticity over time within the late middle-aged group.

Table 4.16 The comparison of the mean difference of elasticity measured by Cutometer® MPA580 in late middle age group at baseline, month 2, month 4, and month 6 (n=15)

Markers	Elasticity (a.u.) (Mean diff ± SD)	p-value
Baseline to 2 months	-0.07±0.02	0.039
Baseline to 4 months	-0.16±0.04	0.003
Baseline to 6 months	-0.20±0.03	<0.001
2 months to 4 months	-0.09±0.02	0.006
2 months to 6 months	-0.13±0.02	<0.001
4 months to 6 months	-0.04±0.01	0.001

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), Mean diff for (Mean Difference)

4.2.2.6 Mean Difference of Skin Capacitance or Skin Hydration measured by Corneometer® CM825

Table 4.17 displayed the comparison of the mean differences in skin capacitance, or skin hydration, in the late middle-aged group, measured at baseline, 2 months, 4 months, and 6 months using the Corneometer® CM825. The mean differences (Mean diff ± SD) between these time points were calculated, with p-values indicating statistical significance.

From baseline to 2 months, the mean difference in skin capacitance was -3.96 ± 0.98, with a p-value of 0.007, indicating a statistically significant increase in

skin hydration. The comparison between baseline and 4 months showed a larger mean difference of -8.76 ± 1.69, with a p-value of 0.001, reflecting a further significant increase in skin capacitance. From baseline to 6 months, the mean difference was -11.33 ± 1.65, with a p-value of <0.001, marking a highly significant increase in skin hydration over the 6-month period.

When comparing the change in skin capacitance between 2 months and 4 months, the mean difference was -4.80 ± 1.06, with a p-value of 0.003, indicating a significant increase in hydration during this interval. The comparison between 2 months and 6 months revealed a mean difference of -7.38 ± 1.11, with a p-value of <0.001, suggesting another significant increase. Finally, the mean difference between 4 months and 6 months was -2.58 ± 0.50, with a p-value of 0.001, demonstrating a statistically significant improvement in skin capacitance during this period.

All data were analyzed using repeated measures ANOVA, with post-hoc tests using Bonferroni correction. The p-values, all below the threshold of $p < 0.05$, indicated significant increases in skin capacitance at each comparison point, revealing a consistent improvement in skin hydration over time in the late middle-aged group.

Table 4.17 The comparison of the mean difference of skin capacitance or skin hydration measured by Corneometer® CM825 in late middle age group at baseline, month 2, month 4, and month 6 (n = 15)

Markers	**Skin Capacitance (a.u.) (Mean diff ± SD)**	**p-value**
Baseline to 2 months	-3.96±0.98	0.007
Baseline to 4 months	-8.76±1.69	0.001
Baseline to 6 months	-11.33±1.65	<0.001
2 months to 4 months	-4.80±1.06	0.003
2 months to 6 months	-7.38±1.11	<0.001
4 months to 6 months	-2.58±0.50	0.001

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), Mean diff for (Mean Difference)

4.2.2.7 Mean Difference of Transepidermal Water Loss (TEWL) measured by Tewameter® TM300

Table 4.18 presented the comparison of mean differences in transepidermal water loss (TEWL) measured by the Tewameter® TM300 in the late middle-aged group at baseline, 2 months, 4 months, and 6 months. The mean differences (Mean diff ± SD) between these time points were analyzed, with p-values provided to assess statistical significance.

The mean difference in TEWL between baseline and 2 months was 4.29 ± 1.31, with a p-value of 0.034, indicating a statistically significant decrease in water loss during this period. Between baseline and 4 months, the mean difference was 8.91 ± 1.14, with a p-value of <0.001, reflecting a more pronounced decrease in TEWL. The comparison between baseline and 6 months showed the largest mean difference of 10.47 ± 1.17, with a p-value of <0.001, indicating a highly significant decrease in water loss over the 6-month period. When comparing the change in TEWL between 2 months and 4 months, the mean difference was 4.63 ± 0.73, with a p-value of <0.001, indicating a significant decrease in water loss during this interval. The comparison between 2 months and 6 months revealed a mean difference of 6.18 ± 0.79, with a p-value of <0.001, suggesting another statistically significant decline in TEWL. Finally, between 4 months and 6 months, the mean difference was 1.56 ± 0.27, with a p-value of <0.001, marking a significant decrease in water loss during this final period.

The data were analyzed using repeated measures ANOVA, and post-hoc tests were conducted with Bonferroni correction. All comparisons showed significant improvement in TEWL ($p < 0.05$), indicating a consistent decrease in water loss through the 6 months in the late middle-aged group.

Table 4.18 The comparison of the mean difference of transepidermal water loss measured by Tewameter® TM300 in late middle age group at baseline, month 2, month 4, and month 6 (n = 15)

Markers	**TEWL ($g/m^2/h$) (Mean diff ± SD)**	**p-value**
Baseline to 2 months	4.29±1.31	0.034
Baseline to 4 months	8.91±1.14	<0.001
Baseline to 6 months	10.47±1.17	<0.001
2 months to 4 months	4.63±0.73	<0.001
2 months to 6 months	6.18±0.79	<0.001
4 months to 6 months	1.56±0.27	<0.001

Note Data were analyzed using Repeated measure ANOVA and post hoc test with Bonferroni, SD for (Standard Deviation), Mean diff for (Mean Difference), TEWL for (Transepidermal Water Loss)

4.2.3 Global Aesthetic Improvement Scale Assessment (GAIS)

Table 4.19 presented the mean Global Aesthetic Improvement Scale (GAIS) scores for the late middle-aged group at 2 months, 4 months, and 6 months. The scores were reported as Mean ± Standard Deviation (SD) for each time point. At the 2-month mark, the mean GAIS score was 4.44 ± 0.50, reflecting a moderate level of perceived improvement in aesthetic appearance among the participants. By 4 months, this mean score increased to 4.87 ± 0.21, indicating a significant enhancement in perceived aesthetics. At the 6-month assessment, the mean GAIS score further rose to 4.98 ± 0.09, approaching the maximum score on the scale, which suggested a strong overall perception of improvement in aesthetic outcomes. The p-value associated with these results was less than 0.001, as determined by the Friedman Test, indicating that the differences in GAIS scores over the study period were statistically significant. This analysis highlighted a trend of progressive aesthetic improvement in the late middle-aged group throughout the 6-month evaluation period, demonstrating the effectiveness of the intervention being investigated.

Table 4.19 The mean of GAIS scores in late middle age group at month 2, month 4, and month 6 (n = 15)

GAIS score	**2 months ($\bar{x}$ ± SD)**	**4 months ($\bar{x}$ ± SD)**	**6 months ($\bar{x}$ ± SD)**	**p-value**
	4.44±0.50	4.87±0.21	4.98±0.09	<0.001

Note Data were analyzed using Friedman Test, GAIS for (Global Aesthetic Improvement Scale Assessment), SD for (Standard Deviation), $\bar{x}$ for (Mean)

4.2.4 Patients' Satisfaction Score

Table 4.20 displayed the patients' satisfaction scores for the late middle-aged group at the 6-month mark, involving a total of 15 participants. The satisfaction scores were categorized into five distinct levels, ranging from "Extremely Dissatisfied" to "Extremely Satisfied". The data revealed that there were no participants who rated their satisfaction as "Extremely Dissatisfied" (Score 1), "Dissatisfied" (Score 2), or "Neutral" (Score 3), indicating a notably positive response towards the intervention. Among the participants, only one individual (6.67%) reported being "Satisfied" (Score 4), while a substantial majority of 14 participants (93.33%) indicated they were "Extremely Satisfied" (Score 5). The overall mean satisfaction score was calculated as 4.98 ± 0.09, suggesting a very high level of satisfaction among the participants regarding the aesthetic improvements observed. The standard deviation of 0.09 indicated minimal variation in satisfaction scores, further emphasizing the consistency of positive feedback across the group. This table underscored the favorable reception of the treatment by the late middle-aged participants after a six-month evaluation period.

Table 4.20 Patients' satisfaction scores in late middle age group at 6 months (n = 15)

Satisfaction Scores	Frequency
Extremely Dissatisfied (Score 1)	0
Dissatisfied (Score 2)	0
Neutral (Score 3)	0
Satisfied (Score 4)	1
Extremely Satisfied (Score 5)	14
$\bar{x}$ ± SD	**4.98 ± 0.09**

Note SD for (Standard Deviation), $\bar{x}$ for (Mean)

4.5 Side Effects

No serious side effects were observed during the entire process. Complaints were limited to local irritation, typically presenting as mild erythema (6.67%) on the skin, with no systemic effects. They were similar to those observed with other injectables and were fully reversible over time.

Figure 4.3 The comparison of clinical photographs of representative case No. 3 at 0, 2, and 6 months, respectively

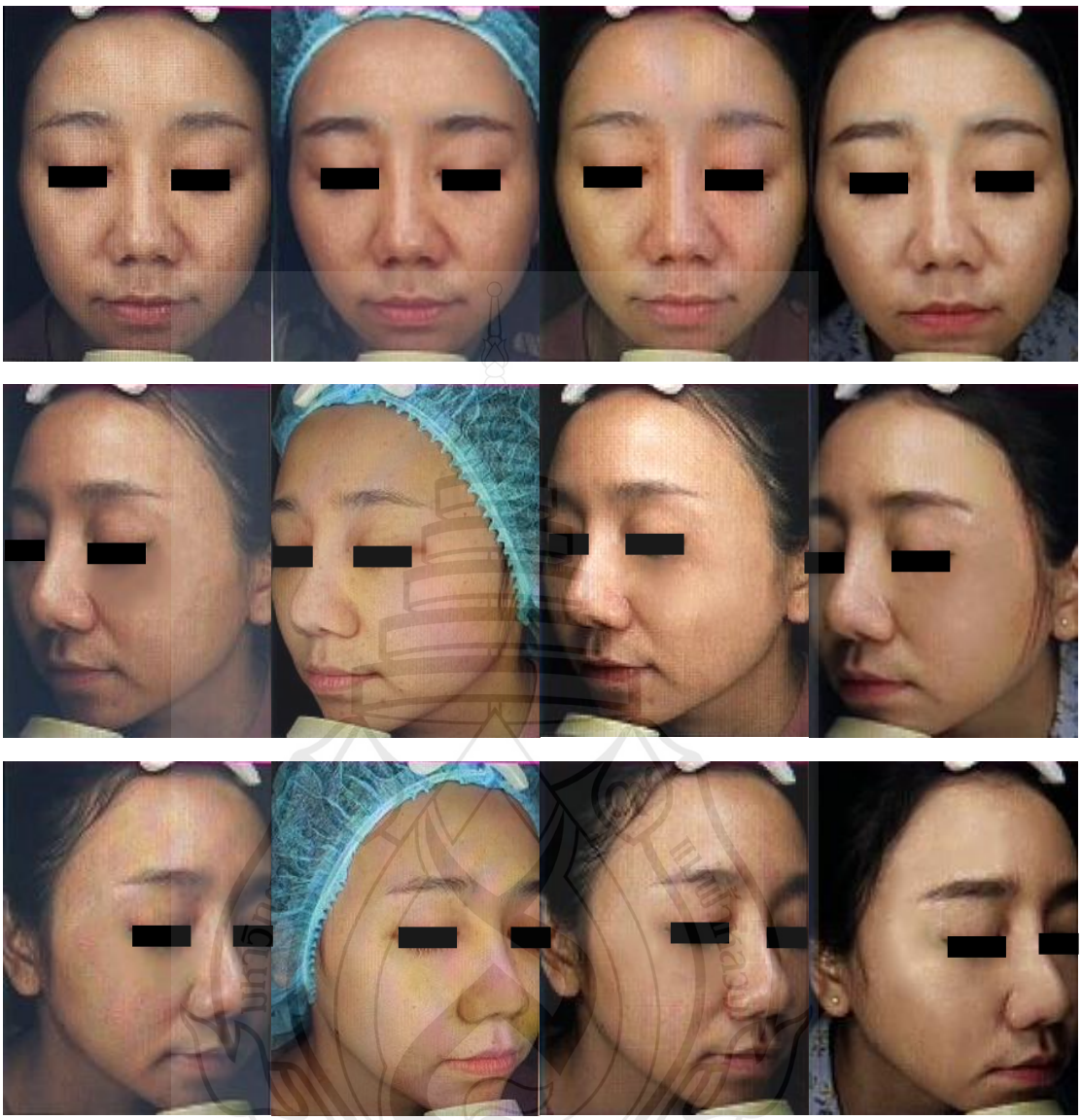


Figure 4.4 The comparison of VISIA CR system photographs of representative case No. 4 at 0, 2, 4, and 6 months, respectively

CHAPTER 5

DISCUSSION

5.1 Discussion

The present study aimed to evaluate the efficacy of poly-D,L-lactic acid (PDLLA) as a biostimulator for facial rejuvenation, focusing on various markers including the subjective data for facial rejuvenation (Global Aesthetic Improvement Scale ,Satisfaction score), objective data for facial rejuvenation as measured by Visia CR system (Spots, Pores, Wrinkles, and Texture), Sebum Level as measured by Sebumeter® SM8 1 5 , Elasticity as measured by Cutometer® MPA5 8 0 , Skin Capacitance as measured by Corneometer® CM8 2 5 , and Transepidermal Water Loss (TEWL) as measured by Tewameter® TM300. They will also be assessed for the Side effect using the side effect record form over the entire study duration. The results presented in Chapter 4 provide a comprehensive overview of the impact of PDLLA treatment on these markers over a six-month period in both early and late middle-aged groups. The findings not only highlight the potential benefits of PDLLA in enhancing facial aesthetics but also contribute to the existing body of literature on biostimulator therapies.

5.1.1 The Efficacy of PDLLA on Facial Rejuvenation Markers

5.1.1.1 Improvements in Elasticity and Skin Hydration

The data indicated a statistically significant increase in skin elasticity and hydration among participants receiving PDLLA treatment. Specifically, Table 4.6 and Table 4.16 demonstrated a notable change in elasticity scores from baseline to each subsequent time point, with the most pronounced improvement observed at six months ($p < 0.001$). Similarly, Table 4.7 and Table 4.17 indicated a significant increase in skin capacitance, which reflects skin hydration levels, throughout the study period. This improvement suggests that PDLLA not only stimulates collagen production but also enhances the skin's moisture-retaining capacity, leading to improved overall skin quality. As a result, improvements in pore and wrinkles were also observed, as shown

in Table 4.3 and Table 4.13. The changes in hydration from the second month onwards may be explained by the immediate filling effect after injection. However, this effect fades after approximately two weeks, and the subsequent changes are likely due to collagen synthesis that occurs afterward. (Kwon et al., 2019)

These findings align with prior research that has investigated the use of biostimulators for facial rejuvenation. For instance, a study by Lin and Lin (2022) reported significant enhancements in skin elasticity and hydration following the nonsurgical lower eyelid rejuvenation involved administering PDLLA (AestheFill) for under-eye rejuvenation. The formulation included 3 mL of SWFI and 1 mL of lidocaine (total 4 mL) and was injected in a single session, with 2 mL per side. This treatment led to improvements in skin texture, elasticity, wrinkle depth, and skin brightness, with no serious side effects reported in the total of 10 participants (Lin & Lin, 2022). However, they also reported significant improvements in skin texture and brightness, unlike our study, which found no change. Given the delicate nature of the periorbital area, adapting our midface injection and dilution technique—such as using a 23G cannula for controlled, precise subdermal deposition—could further optimize outcomes while minimizing risks in periorbital treatments.

Similarly, a recent systematic review by Seo et al. (2024) noted that the use of PDLLA (Juvelook) for skin rejuvenation using a mesotherapy injector with 32G 9-pin needles. The formulation (HA, lidocaine, and saline) was injected over 3 sessions in a study involving 16 participants demonstrated promising results (Seo et al., 2024). All participants underwent two or three treatment sessions spaced four weeks apart, leading to statistically significant improvements in various signs of aging, including skin elasticity, firmness, hydration, reduced wrinkles and fine lines, at 4 months post-treatment. Notably, 50% of the patients reported an overall improvement of more than 50%. Importantly, no severe adverse events were reported, highlighting the treatment's safety. Furthermore, histological examination revealed increases in collagen and elastic fibers in the dermis, suggesting that PDLLA effectively stimulates dermal regeneration, corroborating with our study's outcomes. Given PDLLA’s particle size (30–60 μm), using a 23G cannula, as in our study, offers advantages such as smoother delivery, reduced clogging, and controlled subdermal deposition. It also minimizes trauma, lowers vascular injury risk, and improves patient comfort.

5.1.1.2 Reduction in TEWL

The reduction in transepidermal water loss (TEWL) further supports the efficacy of PDLLA in enhancing skin barrier function, as observed in Table 4.8 and Table 4.18. The results indicated significant reductions in TEWL at each follow-up interval, particularly from baseline to six months ($p < 0.001$). This decrease suggests improved skin barrier integrity, which is critical for maintaining hydration and preventing moisture loss. Furthermore, the data presented in Table 4.5 and Table 4.15 regarding sebum levels revealed negligible changes throughout the study, suggesting that while PDLLA treatment may improve skin hydration and elasticity, it does not significantly alter sebum production.

The findings regarding TEWL reduction are consistent with previous studies, such as the one conducted by Bohnert et al. Participants received 3 treatment sessions, spaced 4 weeks apart, with either PLLA (treatment group) or NSS (control group) and were followed up at months 6, 9, and 12. Participants who received PLLA (poly-L-lactic acid) injections, which have a similar chemical isomer to PDLLA, showed significantly improved skin quality at the 12-month follow-up compared to those who received a saline solution (placebo). Additionally, the PLLA group demonstrated enhanced skin barrier function and experienced a greater reduction in transepidermal water loss, increased skin elasticity, and higher levels of satisfaction throughout all follow-up visits (Bohnert et al., 2019). Notably, it required post-injection massage to prevent nodule formation. In contrast, our study used PDLLA with a 23G cannula in only two sessions without the need for massage, yet still achieved significant improvements in skin elasticity, hydration, and transepidermal water loss (TEWL). The differences in technique highlight PDLLA's potential advantages, including reduced risk of nodules, fewer treatment sessions, and a more controlled deposition, making it a promising alternative for facial biostimulation for middle age group.

5.1.1.3 Patient Satisfaction and Aesthetic Improvement

Patient satisfaction scores, as reflected in Tables 4.9 and 4.10, underscored the subjective efficacy of PDLLA treatment. The mean Global Aesthetic Improvement Scale (GAIS) scores increased significantly over the study period, with a p-value of <0.001, indicating that patients perceived marked improvements in their facial

aesthetics. The overwhelming majority of participants (93.33%) reported being "Extremely Satisfied" with the results at the six-month follow-up, reflecting the treatment's acceptability and effectiveness.

These findings are in line with the previous studies (Bohnert et al., 2019; Lin & Lin, 2022; Seo et al., 2024), which reported high satisfaction rates among patients receiving PLA treatments for facial rejuvenation. The combination of objective improvements in skin quality and subjective satisfaction points to the multifaceted benefits of PDLLA as a biostimulator.

5.1.2 Comparison Between Early and Late Middle Age Groups

The findings from this study revealed notable differences in the response to PDLLA treatment between the early middle age group and the late middle age group, providing valuable insights into the efficacy of the biostimulator across varying age demographics.

In terms of facial rejuvenation markers, the late middle age group (ages approximately 45 to 60) exhibited a more pronounced improvement in skin elasticity and hydration compared to the early middle age group (ages approximately 30 to 45). For instance, as illustrated in Table 4.14, the late middle age group demonstrated a significant increase in elasticity scores over the six-month period, suggesting that aging skin may be more responsive to PDLLA treatment. Conversely, while the early middle age group also showed improvements in elasticity and skin hydration, the magnitude of these changes was comparatively less pronounced. The results from Table 4.15 further indicated that while both groups benefited from reduced transepidermal water loss (TEWL), the late middle age group achieved lower TEWL values at the six-month follow-up, reflecting more enhanced barrier function.

The effects of PDLLA (Poly-D,L-lactic acid) injections are more pronounced in older adults due to age-related changes in the immune system, chronic inflammation, and the natural decline in collagen production. With aging, individuals experience "inflammaging", a state of chronic, low-grade inflammation caused by a weakened and dysregulated immune system (Chung et al., 2019). This persistent inflammatory environment can influence biological processes such as wound healing and tissue repair. PDLLA's mechanism involves inducing a controlled inflammatory response that stimulates fibroblasts and promotes collagen production. Older adults generally

have a higher baseline level of systemic inflammation compared to younger individuals (Kale & Yende, 2011), potentially enhancing and prolonging the collagen-stimulating effects of PDLLA. This sustained inflammatory response contributes to more noticeable and lasting results from PDLLA injections, as the body maintains the response over an extended period. Additionally, older individuals face natural declines in collagen production, resulting in volume loss, skin laxity, and wrinkles (Lorenc & Lee, 2016). PDLLA, similar to other collagen stimulators, gradually promotes new collagen growth, restoring volume and firmness and improving skin structure and elasticity, making it more suitable for older adults than for teenagers, who generally have sufficient collagen levels.

In contrast, younger individuals usually have sufficient collagen (Reilly & Lozano, 2021) and have a more robust and efficient immune system with lower baseline levels of inflammation (Adeva-Andany et al., 2016). This may lead to a quicker resolution of the inflammatory response induced by PDLLA, resulting in a shorter and less pronounced period of collagen stimulation. Consequently, younger patients may not experience the same level of skin rejuvenation as older patients, whose prolonged inflammatory response supports more extensive collagen synthesis. Therefore, they might not see significant benefits from PDLLA injections. In cases where mild, preventive skin biostimulation is desired, hyper-diluted PDLLA might be used, but it would not yield the same visible impact as it does in mature skin (Chao et al., 2018; Lin & Lin, 2021).

Understanding the role of age-related inflammation and immune function provides valuable insights into the differential efficacy of PDLLA in various age groups. This highlights the importance of tailoring treatment protocols to patient age and inflammatory status to optimize clinical outcomes in collagen-stimulating therapies.

5.1.3 Safety

In the present study, PDLLA (Poly-D,L-lactic acid) injections demonstrated a favorable safety profile with minimal side effects reported. No serious adverse events or long-term complications were observed (e.g., granulomas or vascular compromise), aligning with the established safety profile of PDLLA as a biodegradable, biostimulatory agent. These results reinforce PDLLA's suitability for safe, minimally

invasive facial rejuvenation, especially for older adults seeking volume restoration and skin elasticity improvement.

The study by Ianhez et al. (2024) highlights complications associated with collagen biostimulators, particularly poly-L-lactic acid (PLLA), calcium hydroxyapatite (CaHA), and polycaprolactone (PCL). Among the 55 identified cases, PLLA (Elleva® and Sculptra®) accounted for 69.1% of complications, with nodules being the most common adverse effect, occurring in 89.1% of cases, often manifesting after one month. Despite multiple treatment approaches, complete resolution was achieved in only five cases, indicating the persistent nature of some complications (Ianhez et al., 2024). Compared to these biostimulators, poly-D, L-lactic acid (PDLLA) presents a potentially safer alternative due to its different molecular properties and injection technique. PDLLA has a smaller particle size and a more uniform degradation process, which may contribute to a lower risk of nodule formation and delayed adverse effects. Additionally, PDLLA's formulation allows for smoother tissue integration, reducing the need for post-injection massage, which is often required for PLLA to minimize nodule risk. Moreover, the use of a 23G cannula for PDLLA administration, as in our study, enhances safety by enabling controlled product placement while minimizing vascular compromise and injection trauma. Unlike CaHA, which cannot be reversed, and PLLA, which requires extensive management if complications arise, PDLLA appears to offer a favorable balance of efficacy and safety. Future research directly comparing these biostimulators may provide further insights into their respective safety profiles and long-term outcomes.

PDLLA's safety has been further validated in multiple studies, indicating a low incidence of adverse events when used appropriately. Reported side effects are usually mild and transient, including temporary swelling, redness, or discomfort at the injection site, which typically resolve without intervention (Achtnich et al., 2014; No et al., 2015). Importantly, PDLLA's gradual breakdown into lactic acid minimizes risks of complications associated with permanent fillers, making it particularly favorable for aging skin (Kwon et al, 2018; Lin et al., 2019). Moreover, PDLLA injections are administered over multiple sessions, allowing clinicians to monitor patient responses and adjust treatments as needed, which further enhances safety outcomes. This combination of biodegradability, controlled inflammatory response,

and minimal side effects underpins PDLLA's reputation as a safe option for non-surgical facial rejuvenation, especially in older adults. A study by Seo et al. (2024): No serious adverse events occurred, though some patients experienced facial erythema (N = 4) and procedural pain (N = 3), likely due to angiogenesis or transient irritation from the technique using 32G 9-pin needles. The study by Hyun et al. (2015) reported three patients experiencing adverse events at the injection site, including discoloration, nodules, and vesicles. Hyun et al. used a needle-based injection method, which may increase the risk of localized trauma and uneven product distribution, leading to complications such as nodules.

Although PDLLA is safer than PLLA, there is a case report by Choi Min et al. (2024) of granulomas in the infraorbital area. A 58-year-old woman developed granulomas after her third PDLLA injection, which were unresponsive to TCA and light therapy, requiring surgery (Choi Min et al., 2024). This emphasizes the importance of careful injection technique, especially in thin-skinned areas like the infraorbital region. Proper injection technique, including precise placement and appropriate dosage, is crucial to minimizing risks, as demonstrated in our study.

5.1.3 Limitations, Suggestions, and Integration

While the results of this study are promising, certain limitations should be acknowledged. Although PDLLA injections have proven effective in facial rejuvenation, the study did not assess PDLLA's impact in combination with other procedures or on other body parts, where skin structure, thickness, and tissue response to substances may vary significantly.

To enhance the understanding of PDLLA's efficacy, future studies should explore its impact on different skin types and demographics. Investigating the synergistic effects of combining PDLLA with other aesthetic procedures, such as laser treatments or microneedling, could yield valuable insights into optimizing facial rejuvenation protocols. Moreover, expanding research to include other body areas, such as the neck, décolletage, and hands, could clarify PDLLA's efficacy and safety across different anatomical sites, providing broader guidance for clinical applications.

Integrating the findings from this study with existing literature on facial rejuvenation therapies can inform clinical practice and guide aesthetic treatment protocols. The demonstrated efficacy of PDLLA in improving key facial rejuvenation

markers highlights its potential as a valuable addition to the aesthetic dermatologist's toolkit. Clinicians should consider PDLLA not only for its ability to enhance skin elasticity and hydration but also for its capacity to improve patient satisfaction outcomes. Considering the combination of PDLLA injections with treatments like lasers, microneedling, or chemical peels might enhance overall rejuvenation by targeting different skin layers—PDLLA promotes collagen in the subdermal layer, while lasers and microneedling improve surface texture. Additionally, pairing PDLLA with hyaluronic acid fillers provides both immediate volume and long-term structural support. Further research could establish optimal protocols for combining these procedures, enhancing patient outcomes and satisfaction. Finally, examining PDLLA use on various body areas could inform customized treatment protocols, optimizing injection techniques and dosages for each area based on skin thickness and desired outcomes. This integrated approach could significantly refine PDLLA's application in aesthetic medicine, broadening its use beyond facial rejuvenation.

Our study introduces a new innovation, highlighting the ideal candidates for PDLLA injection. These include the middle-aged group with poor skin quality, such as loose skin, dry skin, large pore size, and fine wrinkles, particularly in the middle face area. Additionally, PDLLA is indicated for lower eyelid rejuvenation, addressing concerns like tear trough deformities, infraorbital hollows, nasojugal grooves, dark circles, and mild eyebags.

5.2 Conclusion

In conclusion, this study demonstrated the efficacy of Poly-D,L-lactic acid (PDLLA) as a biostimulator for facial rejuvenation, particularly highlighting the significant improvements in facial rejuvenation markers such as skin elasticity, hydration, and overall patient satisfaction across both early and late middle age groups with no serious side effect. The findings suggest that older individuals experience more pronounced benefits from PDLLA treatment, likely due to the inherent biological factors associated with skin aging. Additionally, while both age groups showed positive results, the variations in treatment responsiveness underscore the necessity for tailored aesthetic approaches based on age demographics.

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APPENDIX A

HUMAN ETHICS DOCUMENT

The Mae Fah Luang University Ethics Committee on Human Research
333 Moo 1, Thasud, Muang, ChiangRai 57100
Tel: (053) 917-170 to 71 Fax: (053) 917-170 E-mail: rec.human@mfu.ac.th

CERTIFICATE OF APPROVAL

COA: 97/2024 **Protocol No: EC 24011-20**

Title: The efficacy of poly-D, L-lactic acid (PDLLA) biostimulator and facial rejuvenation markers

Principal investigator: Chaichana Srituravanich, M.D

School: Anti-Aging and Regenerative Medicine

Funding support: Personal funding

Approval:

1) Research protocol Version 2 Date March 22, 2024
2) Information sheet and informed consent documents Version 2 Date March 22, 2024
3) Advertisement Version 2 Date March 22, 2024
4) Assessment form and case record form Version 2 Date March 22, 2024
5) Satisfaction assessment form Version 1 Date January 5, 2024
6) Principal investigator and Co-investigators
   - Chaichana Srituravanich, M.D.

The aforementioned documents have been reviewed and approved by the Mae Fah Luang University Ethics Committee on Human Research in compliance with international guidelines such as Declaration of Helsinki, the Belmont Report, CIOMS Guidelines and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use - Good Clinical Practice (ICH - GCP)

Date of Approval: May 17, 2024
Date of Expiration: May 16, 2025
Frequency of Continuing Review: 1 year

Sanphoe

(Assoc. Prof., Maj. Gen. Sangkae Chamnanvanakij, M.D.)
Chairperson of the Mae Fah Luang Ethics Committee on Human Research

APPENDIX B

INFORMATION SHEET AND INFORMED CONSENT

Version...01... Date ...05/01/2024.... AP 03_1/2022

หนังสือแสดงความยินยอมเข้าร่วมการวิจัย

ข้าพเจ้า _____ ตัดสินใจเข้าร่วมการวิจัยเรื่อง "การศึกษาประสิทธิภาพของการฉีดพอลิดิแอล-แลคติกแอซิด (พีดีแอลแอลเอ) สารกระตุ้นทางชีวภาพ และการฟื้นฟูคุณภาพผิวหน้าด้วยหัวชี้วัดต่างๆ" ซึ่งข้าพเจ้าได้รับข้อมูลและคำอธิบายเกี่ยวกับการวิจัยนี้แล้ว และได้มีโอกาสซักถามและได้รับคำตอบเป็นที่พอใจแล้ว ข้าพเจ้ามีเวลาเพียงพอในการอ่านและทำความเข้าใจข้อมูลในเอกสารให้ข้อมูลสำหรับผู้เข้าร่วมการวิจัยอย่างถี่ถ้วน และได้รับเวลาเพียงพอในการตัดสินใจว่าจะเข้าร่วมการวิจัยนี้

ข้าพเจ้ารับทราบว่าข้าพเจ้าสามารถปฏิเสธการเข้าร่วมการวิจัยนี้ได้โดยอิสระ และระหว่างการเข้าร่วมการวิจัย ข้าพเจ้ายังสามารถถอนตัวออกจากการวิจัยได้ทุกเมื่อ โดยไม่ส่งผลกระทบต่อการดูแลรักษา หรือสิทธิที่ข้าพเจ้าพึงมี

ข้าพเจ้าได้รับทราบจากผู้วิจัยแล้วว่า หากเกิดอันตรายใดๆจากการวิจัย ผู้วิจัยจะรับผิดชอบค่ารักษาพยาบาลที่เป็นผลสืบเนื่องจากการวิจัยนี้

ข้าพเจ้ารับทราบว่าข้าพเจ้าสามารถปฏิเสธการเข้าร่วมการวิจัยนี้ได้โดยอิสระ และระหว่างการเข้าร่วมการวิจัย ข้าพเจ้ายังสามารถถอนตัวออกจากการวิจัยได้ทุกเมื่อ โดยไม่ส่งผลกระทบต่อการดูแลรักษา หรือสิทธิที่ข้าพเจ้าพึงมี

โดยการลงนามนี้ ข้าพเจ้าไม่ได้สละสิทธิใด ๆ ที่ข้าพเจ้าพึงมีตามกฎหมาย และหลังจากลงนามแล้ว ข้าพเจ้าจะได้รับเอกสารข้อมูลและขอความยินยอมไว้จำนวน 1 ชุด

ลายมือชื่อผู้เข้าร่วมการวิจัย _____ วัน-เดือน-ปี _____
(_____)

ลายมือชื่อผู้ขอความยินยอม _____ วัน-เดือน-ปี _____
(_____)

APPENDIX C

CASE RECORD FORM

แบบเก็บข้อมูลโครงการวิจัย

ลำดับที่.....

วันที่เริ่มประเมิน.....

<u>ส่วนที่ 1</u>

ข้อมูลส่วนตัว

อายุ.....ปี น้ำหนัก.....กิโลกรัม ส่วนสูง.....เซนติเมตร

เพศ ☐ ชาย☐ หญิง

สถานภาพ ☐ โสด ☐ สมรส ☐ หย่าร้าง

อาชีพ ☐ นักศึกษา ☐ รับราชการ

☐ พนักงานบริษัท ☐ ธุรกิจส่วนตัว

☐ รับจ้าง ☐ แม่บ้าน

☐ อื่นๆ โปรดระบุ.....

โรคประจำตัว ☐ ไม่มี ☐ มี โปรดระบุ.....

ประวัติแพ้ยา/อาหาร ☐ ไม่เคย ☐ เคย ชื่อยา/อาหารที่แพ้.....

อาการที่แพ้.....

ประวัติยาที่ใช้ประจำ ☐ ไม่มี ☐ มี โปรดระบุชื่อยา.....

ประวัติโรคในครอบครัว.....

ประวัติสูบบุหรี่.....

Fitzpatrick skin type.....

สำหรับเพศหญิง

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

ประวัติการคุมกำเนิด ☐ ไม่ได้คุมกำเนิด

☐ คุมกำเนิด โปรดระบุวิธี.....

<u>ส่วนที่ 2</u>

ข้อมูลพารามิเตอร์ต่างๆ

ครั้งที่ 1 วันที่บันทึกข้อมูล.....(เดือนที่ 0)

ค่าจากเครื่อง VISIA

ค่า Spot..... ค่า Pore.....ค่า Wrinkle.....ค่า Texture.....

ค่าจากเครื่อง Sebumeter (Sebum level).....

ค่าจากเครื่อง Cutometer (Elasticity).....

ค่าจากเครื่อง Corneometer (Skin capacitance).....

ค่าจากเครื่อง Tewameter (TEWL).....

ครั้งที่ 2 วันที่บันทึกข้อมูล.....(เดือนที่ 2)

ค่าจากเครื่อง VISIA

ค่า Spot..... ค่า Pore.....ค่า Wrinkle.....ค่า Texture.....

ค่าจากเครื่อง Sebumeter (Sebum level).....

ค่าจากเครื่อง Cutometer (Elasticity).....

ค่าจากเครื่อง Corneometer (Skin capacitance).....

ค่าจากเครื่อง Tewameter (TEWL).....

ครั้งที่ 3 วันที่บันทึกข้อมูล.....(เดือนที่ 4)

ค่าจากเครื่อง VISIA

ค่า Spot..... ค่า Pore.....ค่า Wrinkle.....ค่า Texture.....

ค่าจากเครื่อง Sebumeter (Sebum level).....

ค่าจากเครื่อง Cutometer (Elasticity).....

ค่าจากเครื่อง Corneometer (Skin capacitance).....

ค่าจากเครื่อง Tewameter (TEWL).....

ครั้งที่ 4 วันที่บันทึกข้อมูล.....(เดือนที่ 6)

ค่าจากเครื่อง VISIA

ค่า Spot..... ค่า Pore.....ค่า Wrinkle.....ค่า Texture.....

ค่าจากเครื่อง Sebumeter (Sebum level).....

ค่าจากเครื่อง Cutometer (Elasticity).....

ค่าจากเครื่อง Corneometer (Skin capacitance).....

ค่าจากเครื่อง Tewameter (TEWL).....

ท่านอาสาสมัครกรุณาประเมินระดับคะแนนความพึงพอใจของผลการรักษาหลังจากการฉีด PDLLA ครบ 2 ครั้ง ไปแล้ว โดยเลือกช่องที่ตรงกับความคิดของท่าน

- ☐ 5 = พอใจมากที่สุด
- ☐ 4 = พอใจมาก
- ☐ 3 = พอใจปานกลาง
- ☐ 2 = ไม่พอใจ
- ☐ 1 = ไม่พอใจเป็นอย่างมาก

แบบสัมภาษณ์ของแพทย์ผู้ประเมินผลการฉีดPDLLA

กรุณาประเมินผลการรักษาหลังจากการฉีด PDLLA ด้วย Global Aesthetic Improvement Score (GAIS)

โดย 5= ดีขึ้นอย่างมาก

4= ดีขึ้นมาก

3= ดีขึ้น

2= ไม่เปลี่ยนแปลง

1= แย่ลง

ครั้งที่	คะแนน GAIS score
1 (เดือนที่ 0)	-
2 (เดือนที่ 2)	
3 (เดือนที่ 4)	
4 (เดือนที่ 6)	

แบบบันทึกอาการข้างเคียง (Side Effect record Form)

ครั้งที่ 1 วันที่..... (เดือนที่ 0)

☐ ไม่มี ☐ มี โปรดระบุอาการ.....

การรักษา.....

ระยะเวลาการรักษา.....

ความสัมพันธ์ระหว่างอาการข้างเคียงกับPDLLA โดยใช้ Naranjo's algorhithm

☐ 3 = ใช่แน่

☐ 2 = น่าจะใช่

☐ 1 = อาจจะใช่

☐ 0 = น่าสงสัย (ไม่น่าใช่)

ครั้งที่ 2 วันที่..... (เดือนที่ 2)

☐ ไม่มี ☐ มี โปรดระบุอาการ.....

การรักษา.....

ระยะเวลาการรักษา.....

ความสัมพันธ์ระหว่างอาการข้างเคียงกับPDLLA โดยใช้ Naranjo's algorhithm

☐ 3 = ใช่แน่

☐ 2 = น่าจะใช่

☐ 1 = อาจจะใช่

☐ 0 = น่าสงสัย (ไม่น่าใช่)

ครั้งที่ 3 วันที่..... (เดือนที่ 4)

☐ ไม่มี ☐ มี โปรดระบุอาการ.....

การรักษา.....

ระยะเวลาการรักษา.....

ความสัมพันธ์ระหว่างอาการข้างเคียงกับPDLLA โดยใช้ Naranjo's algorhithm

☐ 3 = ใช่แน่

☐ 2 = น่าจะใช่

☐ 1 = อาจจะใช่

☐ 0 = น่าสงสัย (ไม่น่าใช่)

ครั้งที่ 4 วันที่..... (เดือนที่ 6)

☐ ไม่มี ☐ มี โปรดระบุอาการ.....

การรักษา.....

ระยะเวลาการรักษา.....

ความสัมพันธ์ระหว่างอาการข้างเคียงกับPDLLA โดยใช้ Naranjo's algorhithm

☐ 3 = ใช่แน่

☐ 2 = น่าจะใช่

☐ 1 = อาจจะใช่

☐ 0 = น่าสงสัย (ไม่น่าใช่)

APPENDIX D

THAI FDA APPROVAL OF THE INTERVENTION

แบบ บ.น.พ. ๑

ใบอนุญาตนำเข้าเครื่องมือแพทย์

ใบอนุญาตที่ 66-2-1-2-0004634

ใบอนุญาตฉบับนี้ให้ไว้แก่

บริษัท ควอลเทค คอนซัลติ้ง (ไทยแลนด์) จำกัด

ผู้จดทะเบียนสถานประกอบการนำเข้าเครื่องมือแพทย์ ใบจดทะเบียนที่ **กท. สน. 88/2562**

เพื่อแสดงว่าเป็นผู้รับอนุญาตนำเข้าเครื่องมือแพทย์ตามมาตรา ๑๗ แห่งพระราชบัญญัติเครื่องมือแพทย์ พ.ศ. ๒๕๕๑ และที่แก้ไขเพิ่มเติม สำหรับเครื่องมือแพทย์

AestheFill

รายละเอียดเครื่องมือแพทย์ **ตามเอกสารแนบท้าย**

ชื่อและที่ตั้งของสถานที่ผลิตเครื่องมือแพทย์ **ตามเอกสารแนบท้าย**

ณ สถานที่นำเข้าเครื่องมือแพทย์ชื่อ **บริษัท ควอลเทค คอนซัลติ้ง (ไทยแลนด์) จำกัด**

ตั้งอยู่เลขที่ **1 อาคารพร้อมพันธุ์ 2 ชั้น 6 ห้องเลขที่ 603**

ตรอก/ซอย **ลาดพร้าว 3** ถนน - หมู่ที่ -

ตำบล/แขวง **จอมพล** อำเภอ/เขต **จตุจักร**

จังหวัด **กรุงเทพมหานคร** รหัสไปรษณีย์ 10900 โทรศัพท์ 0 2116 4959 โทรสาร -

ชื่อและที่ตั้งของเจ้าของผลิตภัณฑ์

REGEN Biotech, Inc., 1F, 20, Daehwa-ro 139beon-gil, Daedeok-gu, Daejeon, Korea

ใบอนุญาตนำเข้าฉบับนี้ใช้ได้จนถึงวันที่ 31 ธันวาคม พ.ศ. 2570 และให้ใช้เฉพาะสถานที่ซึ่งระบุไว้ในใบอนุญาตเท่านั้น

ออกให้ ณ วันที่ 27 เดือน **มีนาคม** พ.ศ 2566

สำนักงานคณะกรรมการอาหารและยา

กระทรวงสาธารณสุข

ผู้อนุญาต

APPENDIX E

THAI FDA APPROVAL OF THE MATERIALS AND EQUIPMENTS

เครื่องมือแพทย์	USA6205860	VISIA-CR VISIA-CR RESEARCH SYSTEMS	บริษัท ฟิลเทค เอ็นเตอร์ไพรส์ 1994 จำกัด (มหาชน)	U1MC00010262400760000111C	คงอยู่

24 พฤศจิกายน 2566 18:40 น.

สถานะ	:	คงอยู่
ประเภทผลิตภัณฑ์	:	เครื่องมือแพทย์
ใบสำคัญ/เลขที่อนุญาต	:	USA6205860
ชื่อผลิตภัณฑ์ (TH)	:	VISIA Complexion Analysis
ชื่อผลิตภัณฑ์ (EN)	:	VISIA Complexion Analysis FACIAL SYSTEMS
ชื่อผู้รับอนุญาต	:	บริษัท ฟิลเทค เอ็นเตอร์ไพรส์ 1994 จำกัด (มหาชน)
สถานที่ผลิต	:	บริษัท ฟิลเทค เอ็นเตอร์ไพรส์ 1994 จำกัด (มหาชน)
ที่อยู่สถานที่ผลิต	:	บ้านเลขที่429 ถนนบอนด์สตรีท ตำบลบางพูด อำเภอปากเกร็ด จังหวัดนนทบุรี 11120
Newcode	:	U1MC00010262400760000011C

APPENDIX F

LINE CHARTS

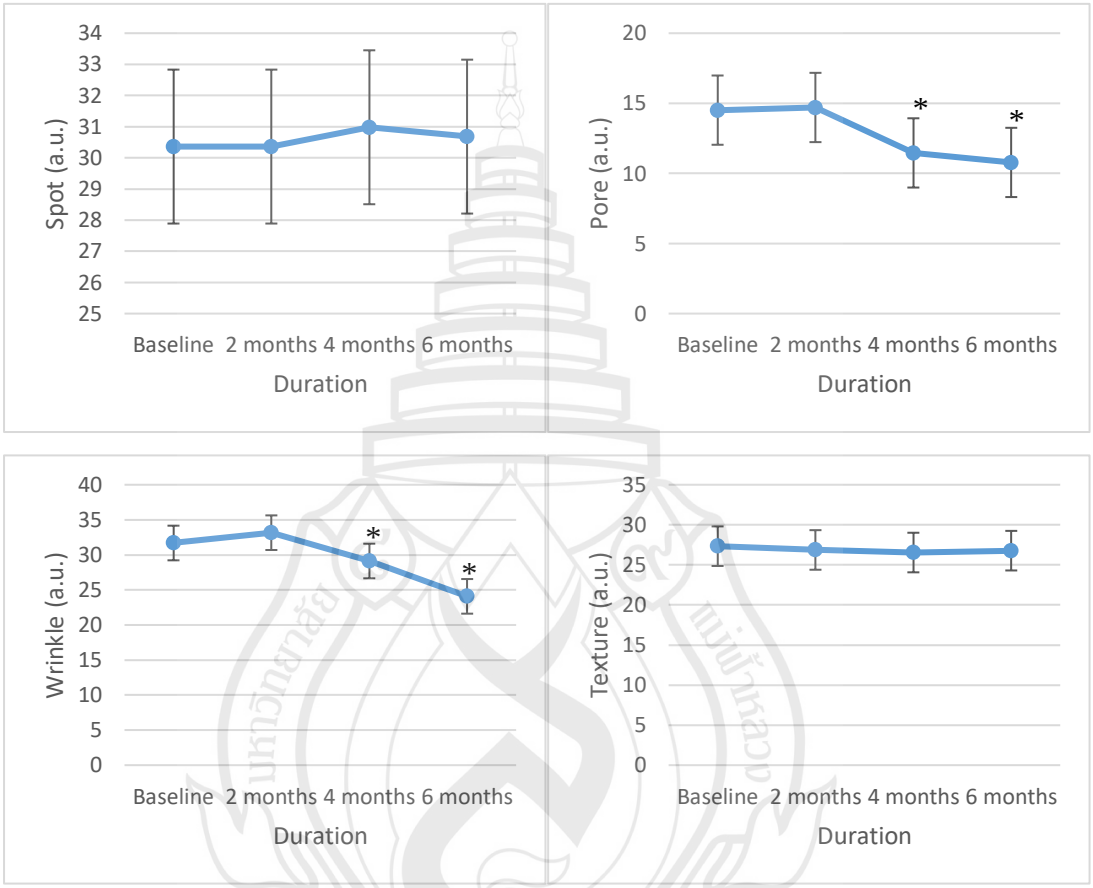

Figure F1 The comparison of the mean of facial rejuvenation markers (Spot, Pore, Wrinkle, and Texture) measured by Visia ® CR system in early middle age group at each timepoints, * indicates significant difference at p value < 0.05

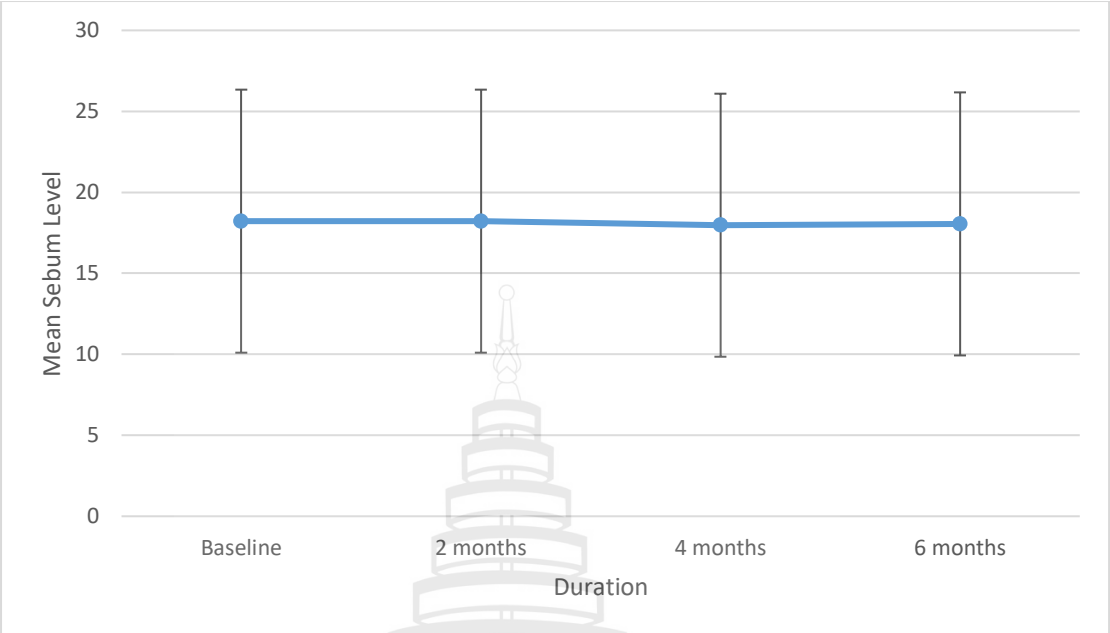

Figure F2 The comparison of the mean of sebum level measured by Sebumeter® SM815 in early middle age group at each timepoints

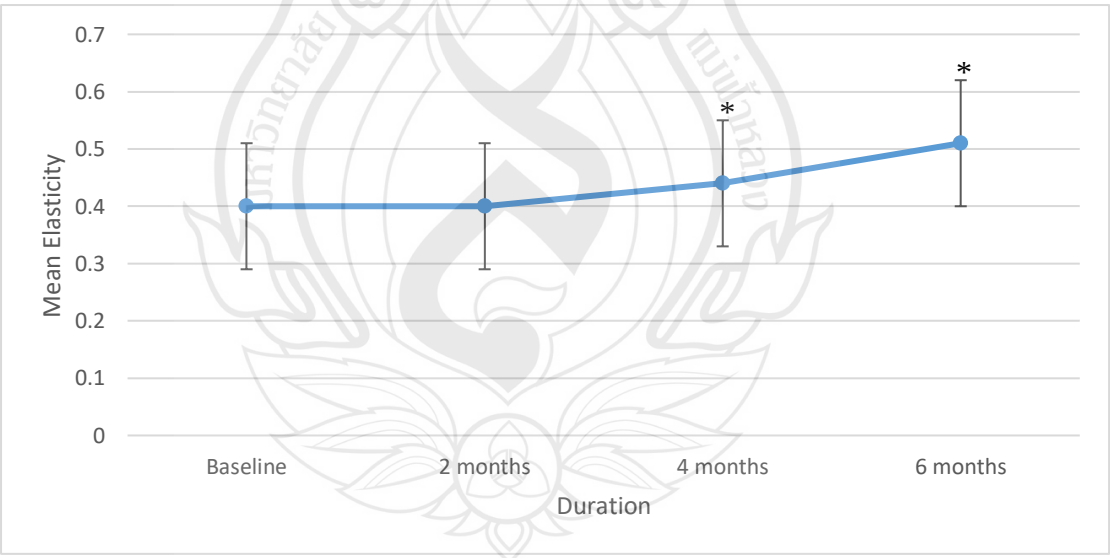

Figure F3 The comparison of the mean of elasticity measured by Cutometer® MPA580 in early middle age group at each timepoints, * indicates significant difference at p value < 0.05

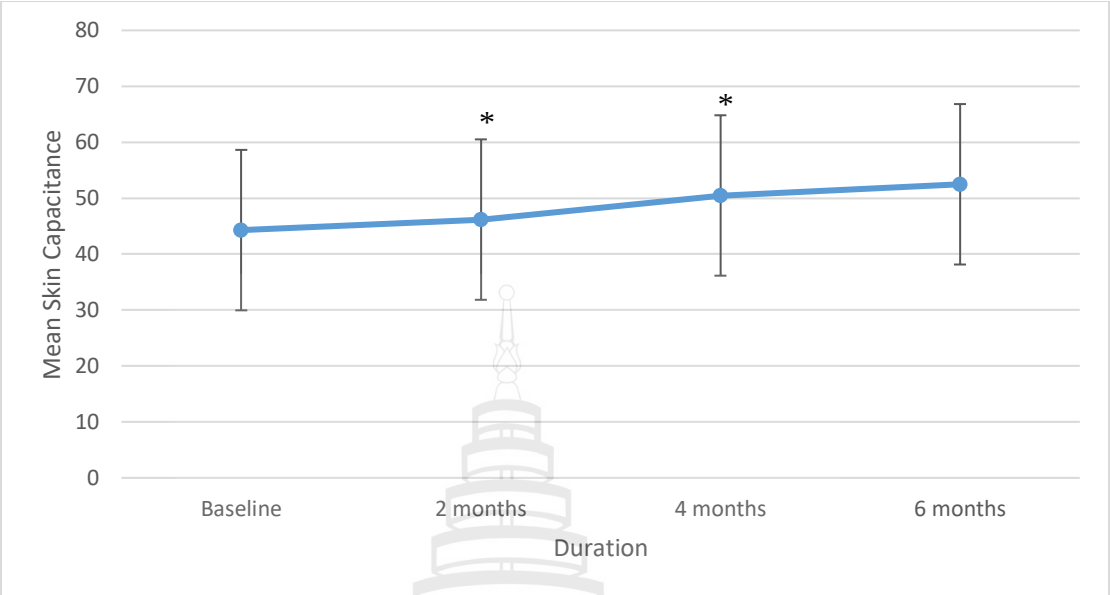

Figure F4 The comparison of the mean of skin capacitance measured by Corneometer® CM825 in early middle age group at each timepoints, * indicates significant difference at p value < 0.05

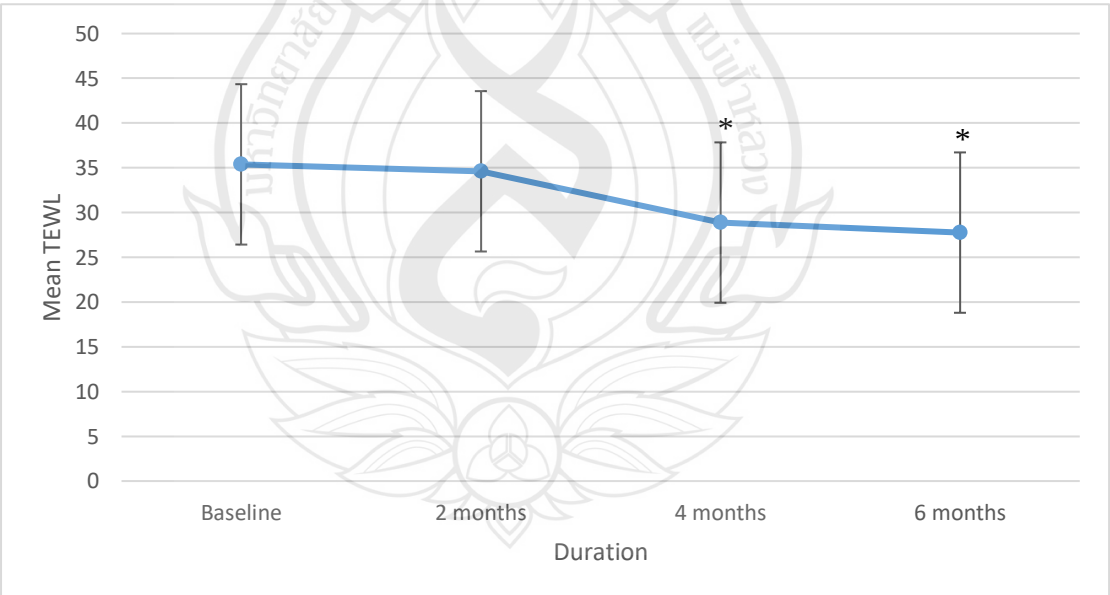

Figure F5 The comparison of the mean of transepidermal water loss (TEWL) measured by Tewameter® TM300 in early middle age group at each timepoints, * indicates significant difference at p value < 0.05

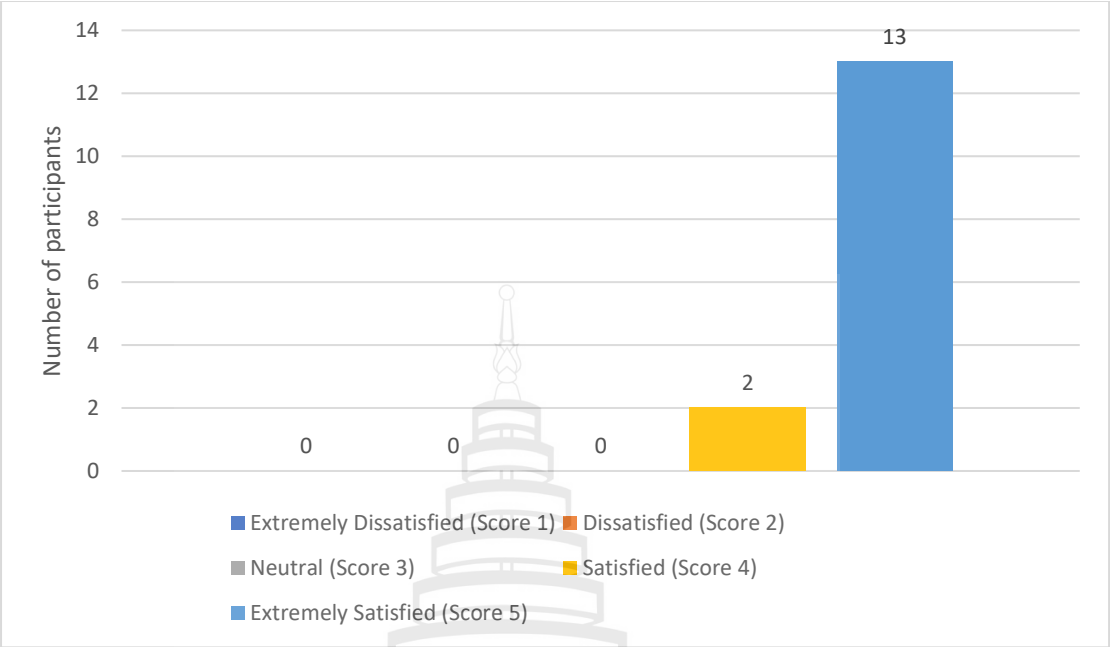

Figure F6 Bar chart of patients' satisfaction scores in early middle age group at the end of the study

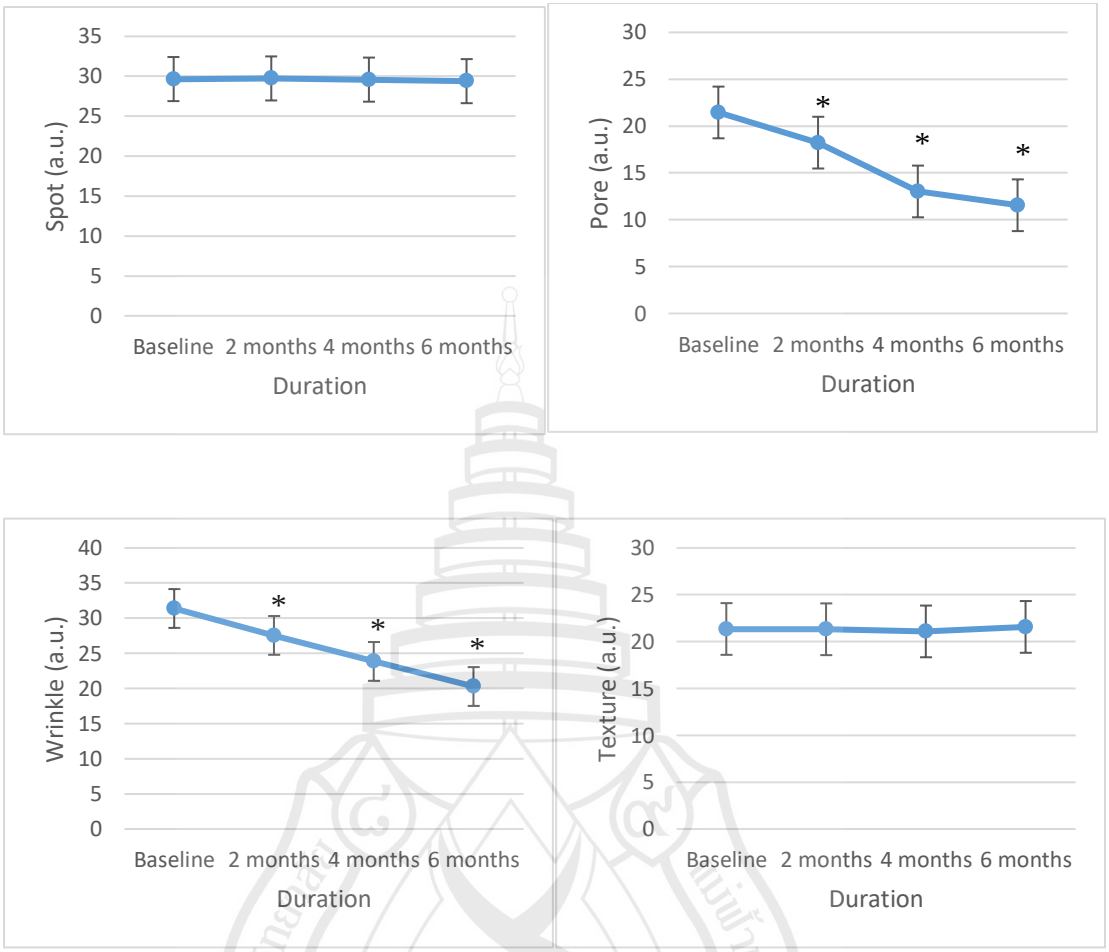

Figure F7 The comparison of the mean of facial rejuvenation markers (Spot, Pore, Wrinkle, and Texture) measured by Visia ® CR system in late middle age group at each timepoints, * indicates significant difference at p value < 0.05

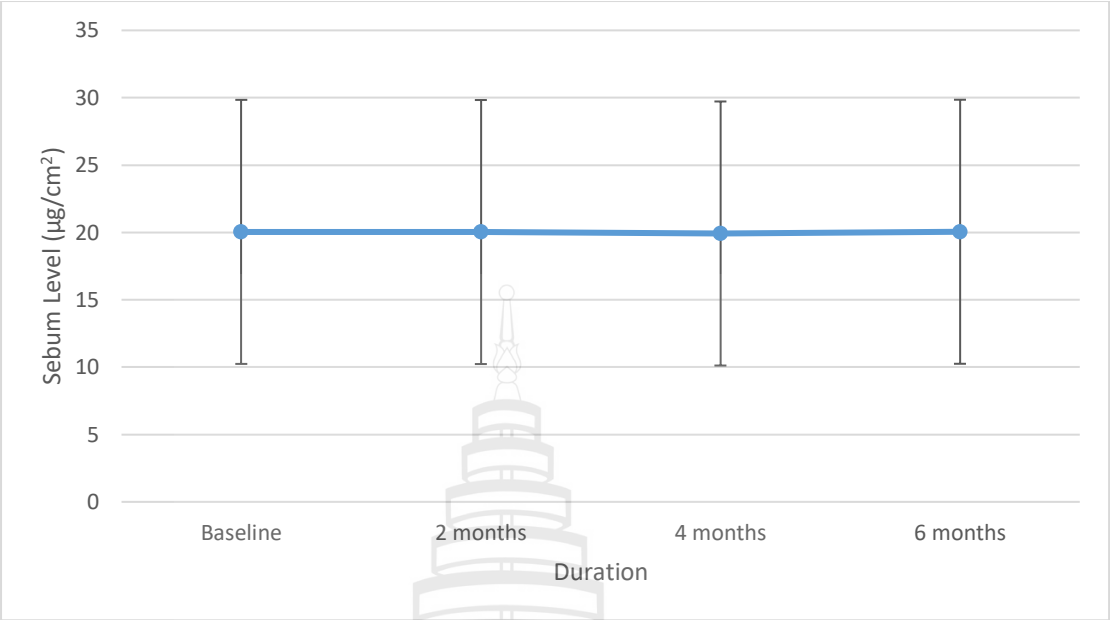

Figure F8 The comparison of the mean of sebum level measured by Sebumeter® SM815 in late middle age group at each timepoints

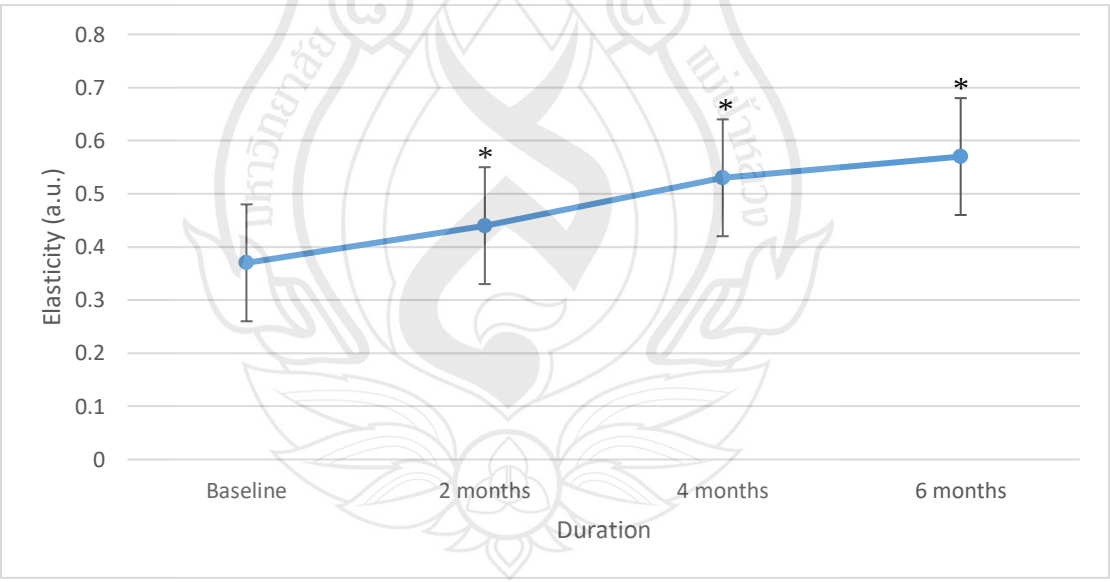

Figure F9 The comparison of the mean of elasticity measured by Cutometer® MPA580 in late middle age group at each timepoints, * indicates significant difference at p value < 0.05

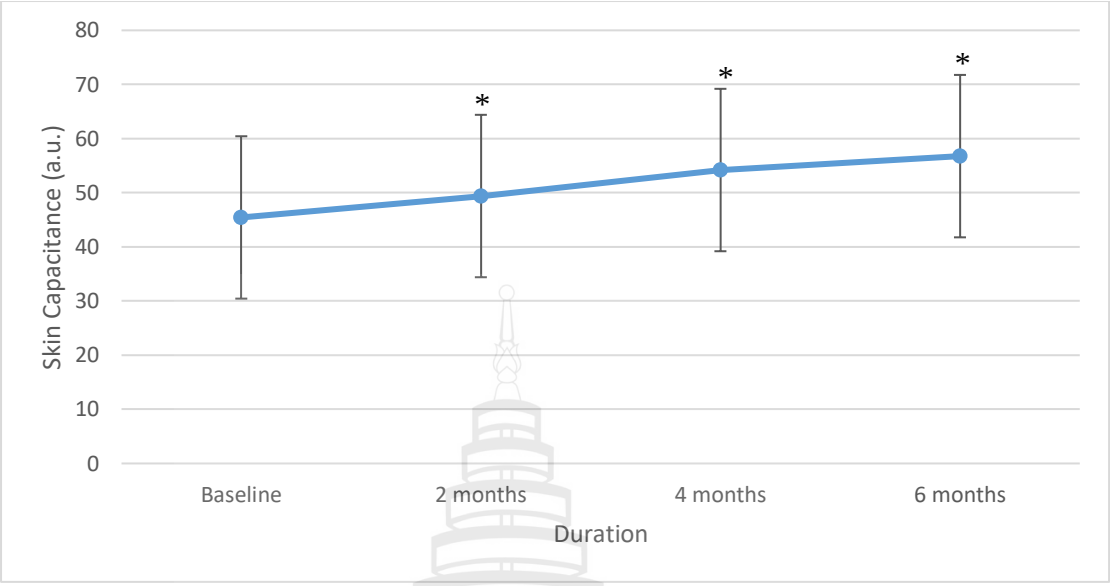

Figure F10 The comparison of the mean of skin capacitance measured by Corneometer® CM825 in late middle age group at each timepoints, * indicates significant difference at p value < 0.05

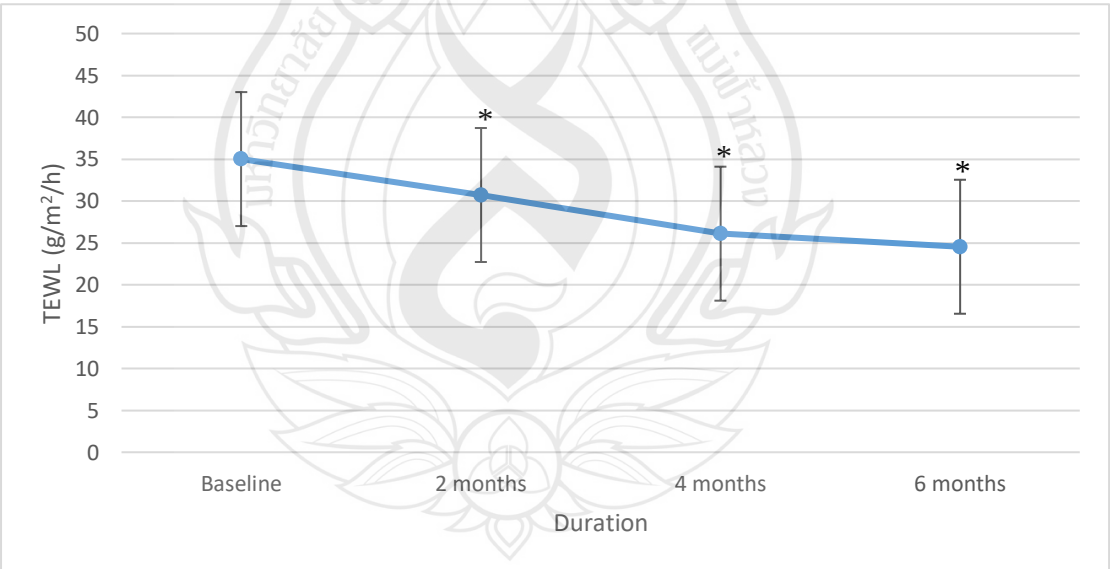

Figure F11 The comparison of the mean of transepidermal water loss (TEWL) measured by Tewameter® TM300 in late middle age group at each timepoints, * indicates significant difference at p value < 0.05

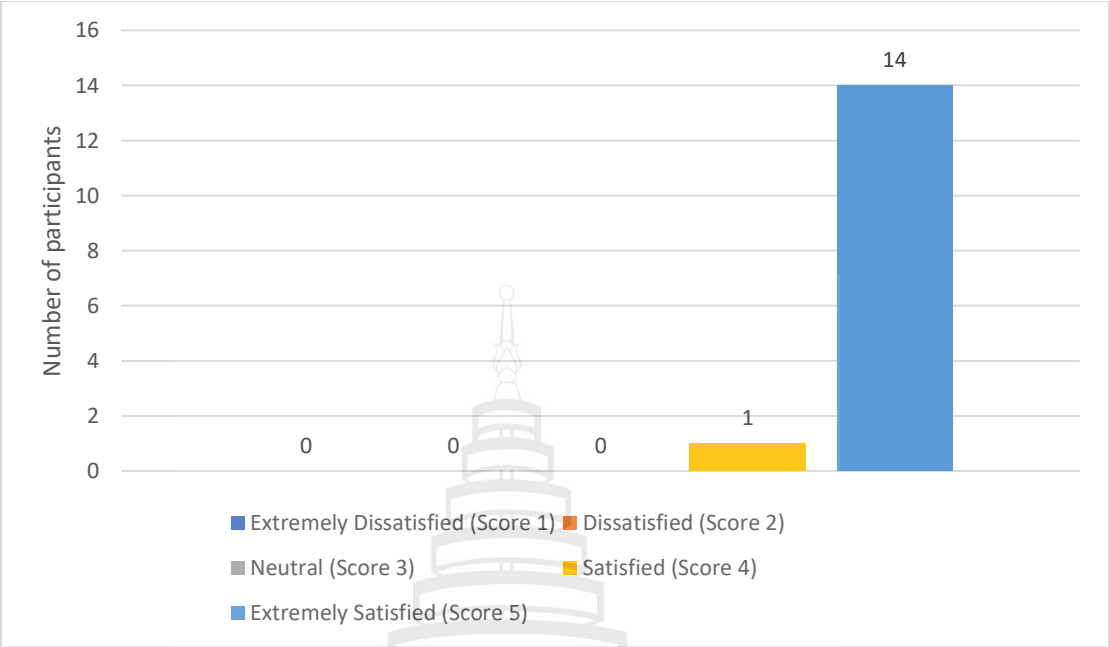

Figure F12 Bar chart of patients' satisfaction scores in late middle age group at the end of the study

CURRICULUM VITAE

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EDUCATIONAL BACKGROUND	
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2021	Master of Science, Anti-Aging and Regenerative Medicine Mae Fah Laung University, Bangkok, Thailand
2022	Diploma of the Thai Board of Preventive Medicine (Public Health)
WORK EXPERIENCE	
2024-Present	Special lecturer and guest speaker Mae Fah Luang University, Bangkok, Thailand
2018-Present	Co-founder & Head of Aesthetic Trainer for Aesthetic and Anti-Aging department MALI Inter Hospital
2019-Present	CEO Baan Bangna Clinic (BBC)
2017-2019	Director & Physician Public Health Center, Department of Health (Bangkok Metropolitan Administration)
2015-2017	Physician Banphaeo Hospital (Public Organization)