THE EFFICACY OF INJECTABLE POLYDIOXANONE FOR PERIORBITAL REJUVENATION

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MASTER OF SCIENCE
IN
DERMATOLOGY

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ABSTRACT

Periorbital rejuvenation is a common aesthetic concern among individuals seeking to address signs of aging around the eyes. People have been seeking various kinds of treatment modalities in order to obtain a more youthful appearance. Treatments are attempted from topical applications to varieties of procedures ranging from chemical peeling, topical retinoids and eye creams, botulinum toxin and collagen injection, dermabrasion and laser resurfacing to more invasive techniques such as using threads and plastic surgeries. Polydioxanone (PDO) filler has emerged as an innovative and effective solution in the field of non-surgical facial rejuvenation.

Polydioxanone filler, originally developed for surgical purposes, has gained popularity in the aesthetic industry due to its biocompatibility and biodegradability. The absorbable nature of PDO filler minimizes the risk of adverse reactions, making it a safe option for periorbital rejuvenation. The versatility of PDO filler allows it to address a wide range of concerns, including volume loss, fine lines, and wrinkles around the eyes. Studies have shown that Polydioxanone has outstanding efficacy in stimulating collagen production and has been proven to be more effective than Poly-L-Lactic Acid (PLLA) in inducing collagen formation. Clinical studies have demonstrated the effectiveness of PDO filler in improving periorbital aesthetics, with results lasting up to 6-12 months. The minimally invasive nature of the procedure and the absence of downtime make it an attractive choice for patients seeking quick and natural-looking results. Thus, the use of Polydioxanone filler for periorbital rejuvenation presents a promising non-surgical option for individuals looking to enhance the youthful appearance of their eye area. Its safety, versatility, and effectiveness make it a valuable

tool in the armamentarium of aesthetic practitioners, contributing to patient satisfaction and improved self-confidence.

The purpose of this research taking into consideration of the above studies is to study the efficacy of injectable Polydioxanone for periorbital rejuvenation along with the assessment of safety and side effects of the treatment. There will be a new alternative treatment for periorbital rejuvenation if it is found to be effective. Total of 23 healthy volunteers aged between 35-60 years old who met the criteria will be injected with polydioxanone filler 2 times,4 weeks apart. For the assessment of the efficacy of injectable polydioxanone in the periorbital area is measured by Cutometer, Tewameter and the photography with hVISIA® Complexion Analysis System are compared at Week 0, 4th, 8th and 12th weeks. Physician will assess to detect any side effects and questionnaires are given out to evaluate patients' satisfaction.

Keywords: Periorbital Rejuvenation, Polydioxanone (PDO), Wrinkle Reduction, Skin Elasticity, Minimally Invasive Aesthetics, Collagen Stimulation

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CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

As we age, we must undergo aging process inevitably, which is characterized by loss of skin elasticity, smoothness and skin hydration, appearance of fine lines and wrinkles. Despite the fact that, the skin of young individuals is typically smooth and well hydrated without irregular pigmentations, these skin conditions start to deteriorate as we get older and wrinkles around the eyes become more prominent. This can affect a person's physical and mental state as well as their quality of life (Helfrich et al., 2008).

Rejuvenation refers to the restoration of a youthful appearance by restoring the skin from any damage and stimulating regeneration of the epidermal skin matrix (Ross et al., 2022). Nowadays, many medical and surgical treatment options have become widely available for periorbital rejuvenation. They are mainly focused on increasing skin elasticity, improving skin hydration, reduction of fine lines and promoting collagen synthesis. Multiple rejuvenation treatments have been used to improve rhytids of periorbital area. In order to obtain a more youthful appearance, minor aging signs are usually counteracted with topical methods such as chemical peeling, topical retinoids and eye creams, botulinum toxin and collagen injection, dermabrasion and laser resurfacing (Manaloto & Alster, 1999). Sever and prominent aging signs are usually regulated by using thread and plastic surgeries. With advance in technological innovations, newer methods have become available for the treatment of periorbital rejuvination. Modern preferences are increasingly concentrated on minimally invasive techniques that are just as successful as their more intrusive counterparts (Kim et al., 2019). In the recent days, injection of dermal fillers based on polydioxanone (PDO) has become a popular for stimulation of collagen synthesis and tackling signs of aging (Zhou et al., 2023).

A synthetic polymer called polydioxanone (PDO) is primarily utilized as an absorbable suture material for face lift with little adverse effects. It is also increasingly

being employed as an absorbable thread-lifting substance to stimulate collagen synthesis (Cobo, 2020). Polydioxanone (PDO) in powdered form is anticipated to be an excellent material for collagen-producing fillers (Kim et al., 2019). PDO injection have been proven effective in stimulating collagen synthesis and improving skin elasticity with fewer side effects (Zhou et al., 2023).

The study is aimed to determine the efficacy of injectable polydioxanone (PDO) for periorbital rejuvenation. There has not been previous research regarding this treatment for periorbital area.

1.2 Research Question

Is the injection of polydioxanone filler effective for periorbital rejuvenation?

1.3 Objective

1.3.1 General Objective

To evaluate the efficacy of injectable Polydioxanone for periorbital rejuvenation.

1.3.2 Specific Objective

1.3.2.1 Primary Outcome

To compare the efficacy of Polydioxanone for changes of fine lines, smoothness of skin and trans-epidermal water loss measurement from baseline to 8th weeks after completion of final treatment by using Tewamter and cutometer.

1.3.2.2 Secondary Outcome

To assess the side effects of injection polydioxanone treatment by using research questionnaire.

1.4 Hypothesis

1.4.1 Primary Hypothesis

Injection polydioxanone has a good efficacy and satisfactory evaluation in wrinkle, skin elasticity, and trans epidermal water loss for periorbital rejuvenation.

1.4.2 Secondary Hypothesis

Polydioxanone filler treatment can be used safely, measured by assessing of adverse effects (pain, swelling, erythema and other side effects) after treatment.

1.5 Benefits

1.5.1 As an alternative treatment of periorbital rejuvenation

1.5.2 To use as a data base for further research

1.6 Conceptual Framework

The development of wrinkles, which is an indication of skin aging is linked to decreased skin elasticity and skin thickness, decreased bone density and loss of soft tissue volume. Skin aging can be classified as intrinsic or extrinsic depending on the epidemiological factors affecting the skin aging process. Fine wrinkles, dryness and decreased skin elasticity are a clinical sign of aging. UV radiation, smoking, humidity, wind, inadequate nutrition, lack of sleep as well as stress are extrinsic causes of skin aging. UV rays generate reactive oxygen species (ROS), which in turn trigger a number of signal transduction pathways in skin cell which lead to decrease synthesis of collagen and elastic fibres and causing skin wrinkles (Zhou et al., 2023). On the other hand, genetic predisposition, gender and ethnicity are the intrinsic causes of aging. Both of these will cause collagen degeneration and inhibit collagen production (Baumann, 2007; Zhou et al., 2023). To counteract these, dermal fillers have become actively used, as they can reduce wrinkles and renew skin tissues by injecting safe substances into the facial dermis layer (Zhou et al., 2023). This research is initiated to evaluate the efficacy of polydioxanone (PDO) injections in periorbital rejuvenation.

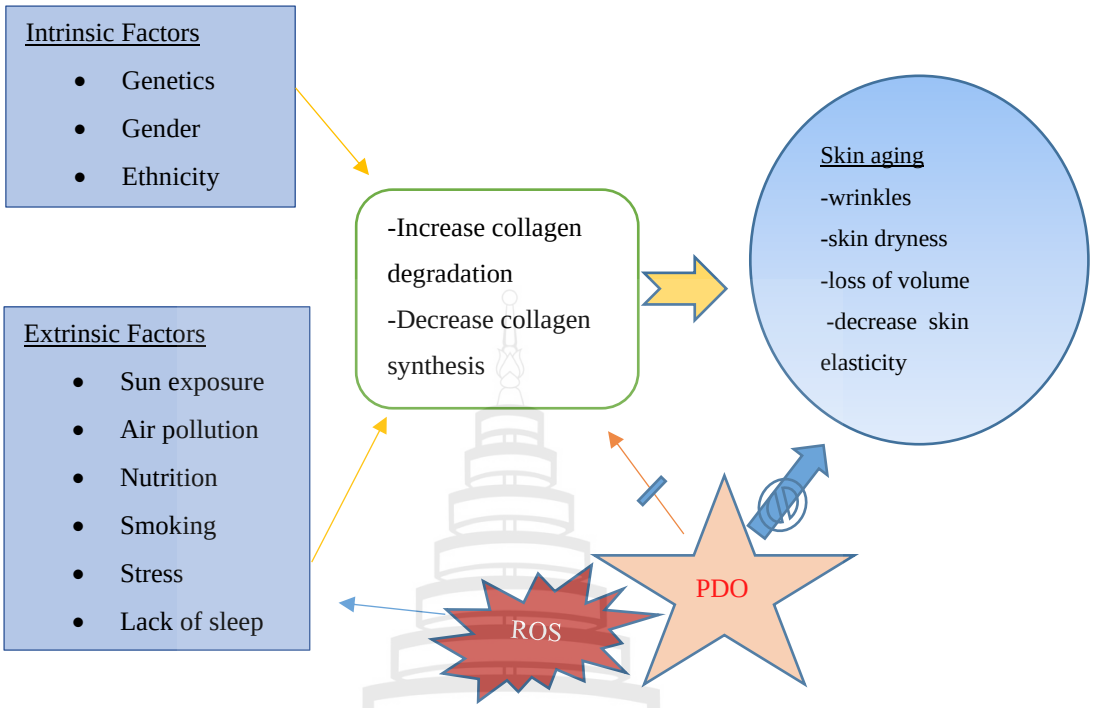


Figure 1.1 Conceptual framework

1.7 The Scope of Research

Twenty-three participants both male and females, age 35-60 who want to get the periorbital rejuvenation treatment, will be done with injection of Polydioxanone filler. Subjects will receive a total of 2 sessions-4 weeks interval apart and will be assessed for efficiency on 4th and 8th weeks later after the last session at Mae Fah Luang Hospital Bangkok.

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

The improvements in periorbital rejuvenation will be independently evaluated by three dermatologists and participants. Viscoelasticity, trans epidermal water loss and wrinkle will be assessed by cutometer and visio scan respectively. Side effects will be assessed by research questionnaire.

1.8 Operation Definition

1.8.1 Physician Global Aesthetic Improvement Scale (GAIS)

Table 1.1 Physician Global Aesthetic Improvement Scale (GAIS)

	Degree	Description
1	Exceptional improvement	Excellent corrective result
2	Very improved patient	Marked improvement of the appearance, but not completely optimal
3	Improved patient	Improvement of the appearance, better compared with the initial condition, but a touch-up is advised
4	Unaltered patient	The appearance substantially remains the same compared with the original condition
5	Worsened patient	The appearance has worsened compared with the original condition

Source DiBernardo and DiBernardo (2018)

1.8.2 Participant Satisfaction Score

Table 1.2 Participant Satisfaction Score

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

Source DiBernardo and DiBernardo (2018)

1.8.3 Classification for wrinkles of the Periorbital region

In this study the Periorbital wrinkles were classified based upon observations of the anatomical details and muscle dynamics of the patients. This photodamage

classification system was developed by Bhertha M. Tamura and Marina Y. Odo. (Surg Cosmet Dermatol 2011;3(2):129-34.) (Tamura & Odo, 2011).

Type I – Wrinkles lateral to the external canthus of the eye, extending from the brow to the zygomatic arch

Type II – Wrinkles lateral to the external canthus of the eye, extending from the line of the external canthus of the eye to the zygomatic arch (absence of wrinkles in the superior lateral region)

Type III – Presence of wrinkles in the line of the external canthus only

These three types of wrinkles can occur together with:

A – Absence of lower eyelid wrinkles

B – Presence of lower eyelid wrinkles, according to the following sub-classification:

B1 – Lateral wrinkles

B2 – Medial wrinkles

B3 – Wrinkles in the internal canthus

1.8.4 Evaluation of Facial Aging by Taking Photograph via VISIA®

Before treatment and each follow up, the researcher takes a photograph to evaluate skin texture, pore, and wrinkle of each patient using VISIA® Complexion Analysis System, of which the following is required:

1. 12-megapixel resolution
2. Automatic focus
3. Automated white balance correction
4. Facial positions: Left 37°, Center 0°, Right 37°
5. Multi-spectral Imaging (standard daylight fluorescent lighting, cross Polarized flash, and ultraviolet lighting)
6. Then skin elasticity was evaluated by Cutometer MPA580 at the same session.
7. Power supply 100-240 V AC, 0.3 A, 50-60 Hz 7
8. Dimensions 26x 25.5 x 7 cm
9. Weight 3.2kg
10. Computer PC with Windows® 98 or higher; Windows® Vista 32-bit version
11. Interface USB (for Windows® NT please ask for serial port)

1.8.5 Skin Elasticity

Skin elasticity is the capacity of the skin to revert to its initial condition. This can be assessed using a device called the Cutometer®, which utilizes a suction method to gauge the viscoelastic characteristics of human skin. As we age, the number and size of visible facial pores tend to increase, while skin elasticity decreases.

1.8.6 Efficacy

Efficacy is a quantitative measure of the clinical trial to indicate whether the intervention made in the trial show desired or intended effect.

1.8.7 Satisfaction

Satisfaction is defined as the pleasant feeling after being fulfillment of wanted desire.

1.8.8 Outcome

Outcome is the way a thing turns out, a consequence.

1.9 Polydioxanone (PDO) Dermal Filler (Ultracol®)

Polydioxanone (PDO) is a synthetic, biocompatible polymer employed in medical settings, notably in surgery and aesthetics. Its primary application is as an absorbable suture material for face lifts, displaying minimal adverse effects. Additionally, PDO is used as an absorbable thread-lifting substance to induce collagen synthesis. In powdered form, it is considered a promising material for collagen-producing fillers. PDO injections have demonstrated efficacy in stimulating collagen synthesis and improving skin elasticity with limited side effects, offering a minimally invasive option for facial enhancements (Zhou et al., 2023).

The PDO dermal filler, Ultracol®, is a microsphere PDO filler, which promotes neocollagen synthesis through foreign body reaction. With a biodegradability of 6-8 months, it has been globally utilized for over 30 years, certified by the Thai Food and Drug Administration (Ultra-V-medical, 2023). PDO microspheres show a uniform size and spherical shape and its collagen stimulation effect and biodegradability is superior compared to hyaluronic acid. Notably, limited human research has been conducted on

injectable polydioxanone, with a single published article serving as a reference for this research (Zhou et al., 2023).

Figure 1.2 Ultracol® polydioxanone (PDO) filler

CHAPTER 2

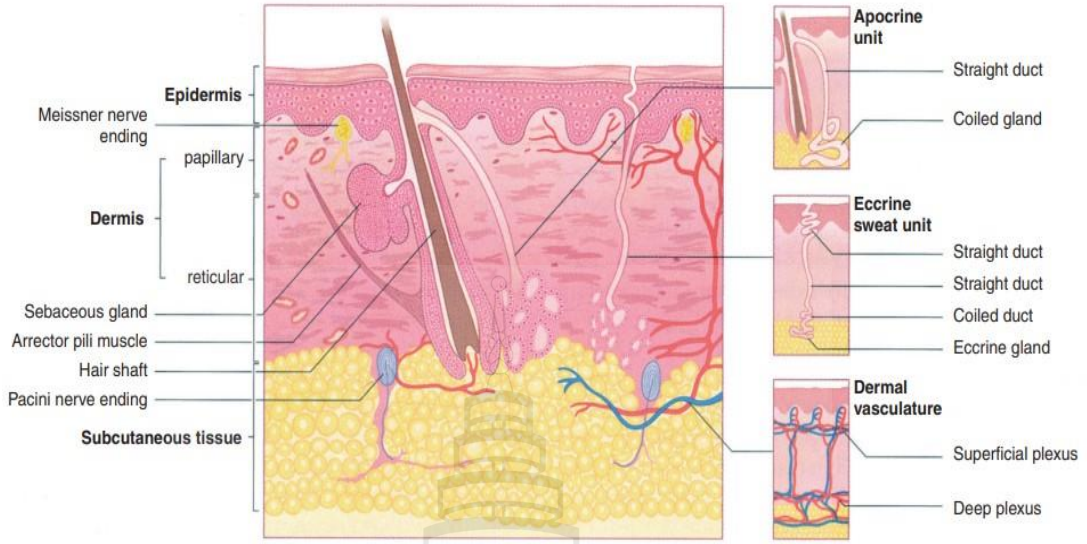
REVIEW OF RELATED LITERATURE

2.1 Skin Anatomy

Theanine, a common non-protein amino acid, was originally discovered in green tea leaves in the 1940s by Sakato (Sakato, 1949). Known chemically as 2-amino-4-ethylcarbamoyl butyric acid by the International Union of Pure and Applied Chemistry (IUPAC) (Li et al., 2022). It naturally occurs predominantly as the L-(S) enantiomer. Theanine is unique in nature, and it's primarily found in the *Camellia genus*, particularly in tea-producing plants such as *Camellia sinensis var. sinensis* and *Camellia sinensis var. assamica*. Theanine contributes significantly to the distinctive taste of tea, with teas containing higher levels of theanine often regarded as of higher quality (Chu, 1997).

Biosynthesis of theanine occurs in tea plants from glutamic acid and ethylamine via the enzyme theanine synthetase, it occurs mainly in the roots and subsequently transferred to the developing shoots (Deng et al., 2008). Theanine levels in tea leaves can vary due to factors such as growing conditions, tea grade, variety, and time of harvest. As shown in some trials, controlled exposure to sunlight can increase theanine levels in tea. And despite post-harvest processing, theanine levels remain consistent across different types of tea (Hara, 2012).

Studies suggest that theanine consumption can positively impact health and well-being of individual taking them, this includes stress reduction, improved learning ability, and potential preventive effects against certain diseases. However, reaching doses associated with positive effects solely through tea consumption may present a challenge due to the significant quantity one would need to consume to reach them. Additionally, the presence of caffeine in tea further complicates achieving these doses, potentially causing side effects before the desired benefits are experienced (Janet et al., 2015).



Source Mehta et al. (2016)

Figure 2.1 Skin anatomy

2.2 Periorbital Structures

2.2.1 Bony Anatomy

The bony structure of the brow and forehead is primarily defined by the supraorbital rims and the frontal bone of the skull. In general, this area tends to be more prominent in men, contributing to a more masculine and angular facial appearance (Kashkouli et al., 2017).

2.2.2 Muscles

The periorbital muscles play a crucial role in facial expression and eyebrow movement. These muscles can be divided into two main groups:

1. Eyebrow Elevators: The frontalis muscle is the primary eyebrow elevator. It's responsible for raising the eyebrows and creating forehead wrinkles when expressing surprise or curiosity.

2. Eyebrow Depressors: Several muscles, including the corrugator, the orbital portion of the orbicularis oculi, depressors supercilii, and procerus, are involved in depressing the eyebrows and creating expressions like frowning or concentration (Kashkouli et al., 2017).

2.2.3 Eyebrow Anatomy

The eyebrow is typically divided into three parts: the head (innermost portion), the body (middle portion), and the tail (outermost portion). The eyebrow often has an upward contour at the head (near the nose) and a downward contour at the tail (toward the temple). The medial brow (inner part) of the eyebrow should start at the supraorbital rim (the bony structure above the eye). The lateral brow (outer part) of the eyebrow should end at an oblique line that extends from the base of the nose (alar base) through the outer corner of the eye (lateral canthus). The peak (highest point) of the eyebrow's arch is typically located at the junction of the middle and lateral thirds, which can vary based on individual facial features (Kashkouli et al., 2017).

2.2.4 Eyelid Structure

The eyelid structure consists of three main layers.

1. Skin Layer: This is the outermost layer that covers the eyelid, including the tarsus (the firm structure supporting the eyelid) and the pre-septal areas (areas in front of the orbital septum).

2. Eyelid Skin: Eyelid skin is known to be the thinnest of all skin on the body and lacks subcutaneous fat.

3. Fat Pads: There are three primary fat pads in the periorbital (around the eye) area, including the preaponeurotic fat pad, pre-septal fat pad, and galea fat pad (also referred to as retro-orbicularis oculi fat or ROOF pad) (Kashkouli et al., 2017).

2.2.5 Periorbital Ligaments

Ligaments around the eye include Suspension ligaments, lateral check ligaments, Medial check ligaments, Lockwood’s ligaments. Tear trough is a true osteocutaneous ligament, which begins medially at the level of the medial canthal tendon insertion and extends to the medial pupil line. There, it continues laterally as the orbicularis retention ligament (Kashkouli et al., 2017).

2.2.6 Motor and Sensory Nerves

All the periorbital muscles are innervated by the facial nerve. The facial nerve's frontalis branch, which also supplies the superficial temporal vessels with blood, runs deep into the frontalis muscle. The zygomatic branches of the facial nerve, which reached the muscle at a straight angle, innervate the pretarsal and preseptal portions of

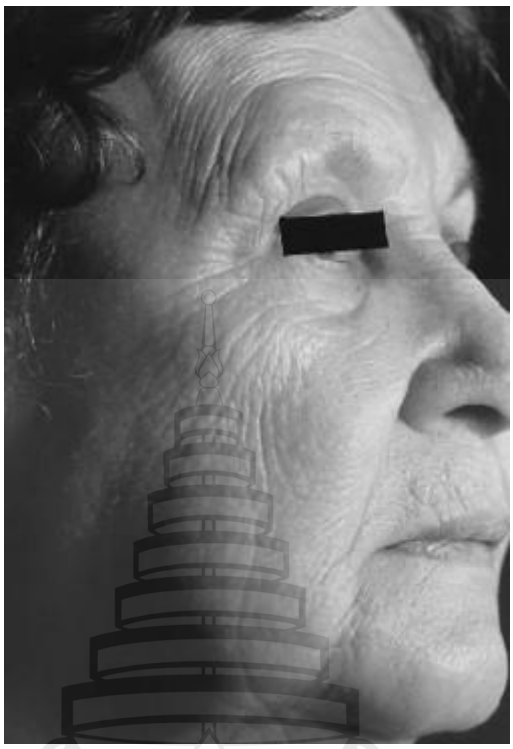
the orbicularis oculi muscle. Additionally, buccal branches take part in the medial innervation of the lower orbicularis oculi muscle (Kashkouli et al., 2017)

2.3 Skin Aging

Aging is a natural and dynamic biological process which is inevitable. It is characterized by a decline in physiologic reserve capacity and a steady degradation of bodily systems. Aging is apparent in all body organs and is easily observable in the skin. Aging is differentiated into Intrinsic aging (Chronological) and Extrinic aging (Photoaging) (Liang et al., 2023).

2.4 Intrinsic Skin Aging

Intrinsic aging refers to an unpreventable physiologic change that happen over time, influenced by genetic and hormonal factors. Alterations such as decrease collagen production, blood flow, amount of lipids and loss of rete ridges occur, may result in dry, pale skin with fine wrinkles, loss of elasticity, sagginess and weakened reparative capacity (Zhang & Duan, 2018).



Source Scharffetter-kochanek et al. (2000)

Figure 2.2 Intrinsic aging is characterized by fine wrinkling, decreased skin elasticity, and sagging

2.5 Extrinsic Skin Aging

Extrinsic aging, primarily driven by ultraviolet (UV) radiation, can lead to various physiologic and histologic changes in the skin. These changes include:

1. Epidermal Thinning: UV radiation can cause the outer layer of the skin (epidermis) to become thinner over time.

2. Loss of Elasticity: UV exposure can break down collagen and elastin fibers in the skin, leading to a loss of skin elasticity.

3. Dyspigmentation: UV rays can cause irregular pigmentation, such as age spots and uneven skin tone.

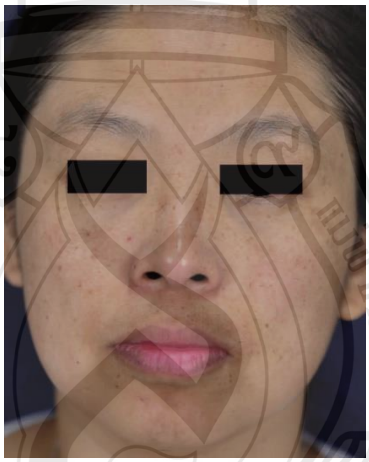
4. Dryness: Skin can become dry and rough due to the damage caused by UV radiation.

5. Wrinkles: Fine and coarse wrinkles are common signs of extrinsic aging, often caused by repetitive UV exposure.

6. Telangiectasis: These are visible blood vessels or "spider veins" that can appear on the skin’s surface.

7. Increased Risk of Growth: Extrinsic skin aging is associated with an increased risk of developing both benign (non-cancerous) and malignant (cancerous) growths, such as skin cancer.

In addition to UV radiation, other factors like cigarette smoking, diet, chemical exposure, trauma, and air pollutants can also contribute to extrinsic skin aging and its clinical features. Proper sun protection and skincare can help mitigate the effects of these environmental factors and promote healthier skin (Callaghan & Wilhelm, 2008; Wang & Dreesen, 2018; Zhang & Duan, 2018).



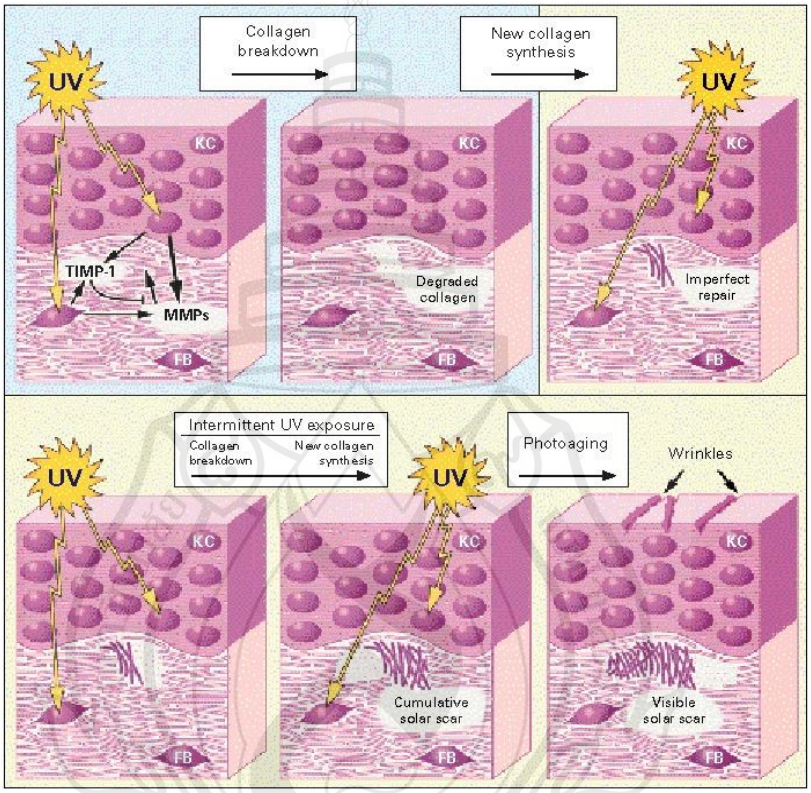
Source Fang et al. (2014)

Figure 2.3 Photoaging

Free radicals can stimulate the release of inflammatory cytokines such as IL-1 and TNF-α that can activate the transcription factor AP-1, which in turn lead to the inhibition of collagen I and collagen III production. Free radicals can also inhibit Transforming Growth Factor (TGF)-β, which is essential for collagen production. Therefore, a decrease in TGF-β can further reduce collagen production. Sunlight, especially UV radiation, can not only directly damage collagen fibers in the skin but also stimulate the secretion of NF-κB, which can further decrease collagen production.

Fragments of collagen resulting from these processes can inhibit the production of new collagen, contributing to a decrease in collagen levels (Callaghan & Wilhelm, 2008).

This complex interplay of factors ultimately leads to the inhibition of collagen production, which is a key factor in skin aging, wrinkling, and other skin-related issues. Protecting the skin from UV radiation and oxidative stress is crucial in maintaining healthy collagen levels and skin appearance (Callaghan & Wilhelm, 2008).



Source Fisher et al. (2019)

Figure 2.4 Pathophysiology of skin aging

2.6 Periorbital Rejuvenation

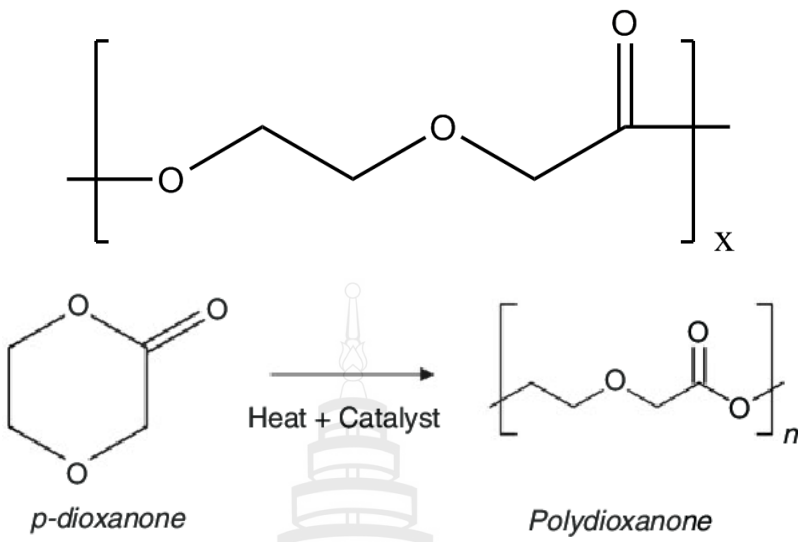
Periorbital aging indeed involves various factors, including genetics and the environment. This aging process is marked by the development of wrinkles, texture irregularities, dryness, and pigmentation changes in the eyebrows, eyelids, glabella, and pericanthal region. Additionally, volume loss and the presence of animation lines around the glabella and lateral canthi contribute to the overall aging appearance in this

region (Shokri & Lighthall, 2018). Rejuvenation involves techniques aimed at removing and regenerating the skin to achieve a more youthful and organized appearance. In recent years, there has been a growing demand for methods to prevent or delay the skin aging process. This has led to the introduction of various medical and surgical interventions designed to address and treat skin aging effectively. In the last decade, injection of collagen stimulating dermal fillers have become popular to achieve the effect of rejuvenation. Dermal fillers are used to replenish lost volume, smooth out lines, and soften wrinkles or enhance facial contours. They are medical devices devoid of any pharmacological action. These fillers can be categorized based on their characteristics and how long they remain in the body, distinguishing between absorbable and non-absorbable types depending on whether they are absorbed within the body (Zhou et al., 2023).

Tissue fillers primarily operate through two mechanisms. First, they fill gaps in the skin to increase volume. Second, they stimulate the surrounding skin tissue to produce new collagen. The former leads to more immediate improvement after the procedure, but the effects may diminish over time. Conversely, collagen synthesis fillers initially provide minor wrinkle improvement but eventually promote collagen and other connective tissue synthesis or regeneration, creating structures for fibroblasts or vascular cells to enter. This results in more significant effectiveness during later stages rather than earlier ones (Zhou et al., 2023).

2.7 Structure of Polydioxanone

Polydioxanone (PDO) comprises numerous repeating ether-ester units and is produced through the ring-opening polymerization of the p-dioxanone monomer. It exhibits a glass transition temperature typically falling between -10 and 0 degrees Celsius and possesses a crystallinity level of about 55%. PDO finds biomedical applications in diverse areas including orthopedic and plastic surgery, drug delivery, tissue engineering and cardiovascular procedures. This polymer undergoes degradation through hydrolysis and is subsequently eliminated from the body (Boland et al., 2005).



Source Boland et al. (2005)

Figure 2.5 Ring-opening polymerization reaction

2.8 Polydioxanone Filler

When applied to the human body, PDO-based fillers exhibit their effects based on their biodegradation and decomposition timeline within the body. As a type of dermal filler, PDO microspheres demonstrate a consistent size and spherical shape. There has been study which revealed that PDO fillers encourage the production of new collagen (Zhou et al., 2023). Furthermore, PDO particles were observed to remain in their original position for a duration of 12 weeks following injection.

Following the injection of PDO filler, the Carboxymethylcellulose gel carrier slowly gets absorbed by macrophages over several weeks. PDO microspheres actively stimulate neo-callogenesis to replace the volume previously occupied by the absorbed Carboxymethylcellulose gel. A notable feature of PDO-based fillers is their capacity for bio-stimulation rather than an immediate peeling effect. Initially, these collagen-stimulating fillers provide a modest improvement in wrinkles. However, they subsequently induce the synthesis or regeneration of collagen and other connective tissues over time. Consequently, PDO's ability to create space and scaffolding for fibroblasts or blood vessels results in a more significant and enduring effect compared to other fillers (Zhou et al., 2023).

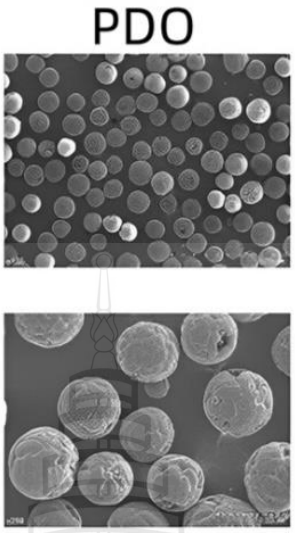


Figure 2.6 Particle morphology of Polydioxanone

2.8.1 Ultracol®

ULTRACOL® manufactured by Ultra V Co., Ltd., Seoul, Republic of Korea, is an FDA approved microparticle dermal filler based on polydioxanone, commonly referred to as PDO (Ultra-V-medical, 2023). This innovative product is not only safe to use due to its excellent biodegradability but also associated with minimal side effects. The outcomes are achieved through a process where over 1400 PDO mono threads are transformed into micro particles and combined with carboxymethylcellulose, a highly tolerable and medically safe carrier substance (Ultra-V-medical, 2023). Carboxymethylcellulose, which is also employed in the food industry and pharmaceuticals, acts as a binding agent, preventing unintentional clumping. Due to it's efficient collagen stimulating effect and biodegradation properties, it is suitable for correction of natural volume reduction and to restore photo aging skin (Zhou et al., 2023).

The composition of UltraCol 200mg includes:

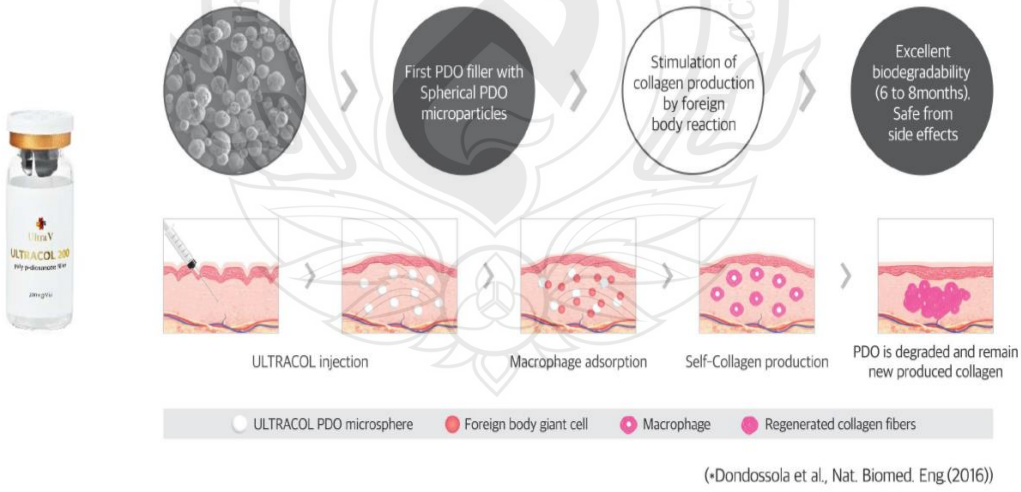
1. 170mg of Polydioxanone
2. 30mg of Carboxymethylcellulose

It is advised to administer this product using a cannula with a gauge ranging from 21G to 25G, depending on the specific indication.

Figure 2.7 Protocol for injection

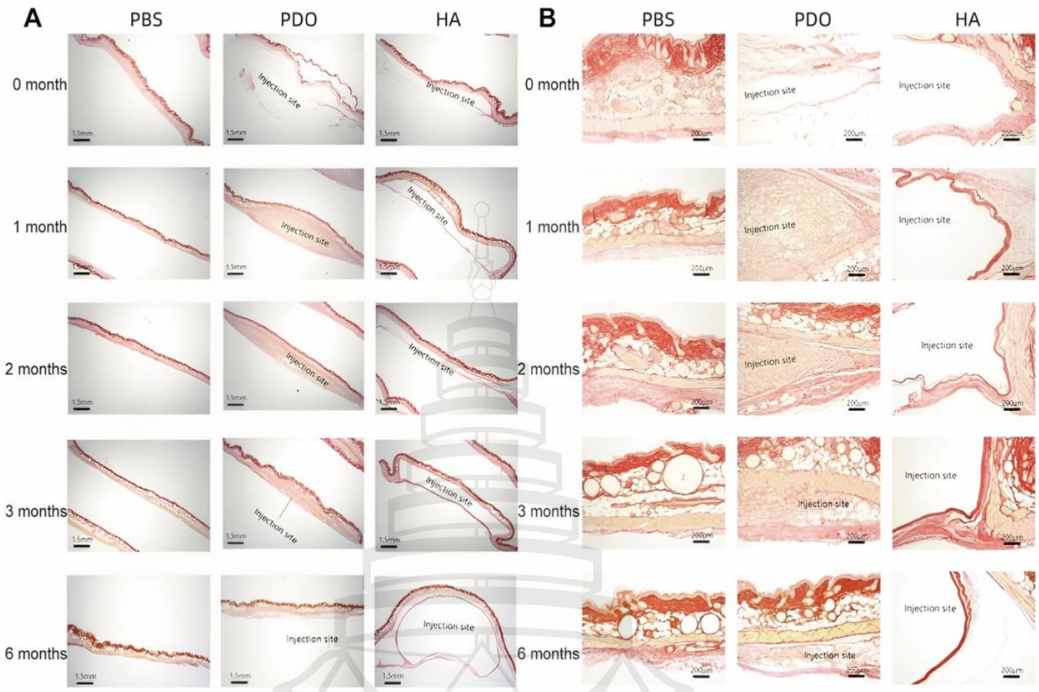
2.8.2 Mechanism of Action

Ultracol® has a uniform spherical shape structure and it stimulates neocollagenesis by foreign body action. The surface area of a PDO microsphere is 0.00785 mm2 and therefore 200mg (1 vial) has 2 million microspheres of PDO equivalent to15,700mm2. It has superlative biodegradability in 6-8 months and is guaranteed to be safe (Ultra-V-medical, 2023).



Source Ultra-V-medical (2023)

Figure 2.8 Mechanism of action



Source Zhou et al. (2023)

Figure 2.9 Comparison on regeneration of tissue

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

Open label Quasi-Experimental, Clinical Study

3.2 Study Population

3.2.1 Sample Population

Twenty-three subjects, aged 35-60 years having Periorbital aging signs with either Type1,2 or 3 of the classification of periorbital wrinkles as mentioned above, who live in Bangkok.

3.2.2 Sample Size Determination

Similar article: Bio-characteristics and Efficacy analysis of biodegradable Poly Dioxanone Dermal Filler in a Mouse Model and Humans which used sample size population of 10(5 men and 5 women) (Zhou et al., 2023).

The results of wrinkle index in this study showed that 38.59 ± 8.05 at baseline and 33.14 ± 6.18 at 12^{th} week after the treatment.

There is a significant change in the pre and post test result.

From the formula, $\alpha = 0.05$ (two-tailed) $Z_{0.025} = 1.96$, $Z_{0.1} = 1.28$

$n_1 = 10$ $n_2 = 10$

$S_1 = 8.05$ $S_2 = 6.18$

$m_1 = 38.59$, $m_2 = 33.14$

$S_p^2 = [(n_1-1)\ S_1^2+(n_2-1)\ S_2^2]/n_1+n_2-2$

S_p^2 (pooled variance) = 51.4976

$n=(Za_{/2}+Zb)^2s^2/\ (m_1-m_2)^2$

$=(1.96+1.28)^2\ 51.4976/(38.59-33.14)^2$

$=18.200$

Rounding up to the nearest whole number= 19 subjects

Note

n=sample number

s^2 =variance

Estimated dropout rate is 20%, thus 23 subjects (n=23) are recruited.

3.2.3 Inclusion Criteria

1. Participants aged 35-60 with signs of periorbital aging.
2. Subjects who accept the treatment of Periorbital rejuvenation with injection of polydioxanone dermal filler.
3. Subjects who willing to give consent and come every appointment.
4. Subjects who can come to Mae Fah Luang Hospital.

3.2.4 Exclusion Criteria

1. Pregnant and breastfeeding woman
2. Woman who intending to have pregnancy during the trial
3. Dermabrasion or chemical peel treatment within 3 months
4. Any device and/or neurotoxin treatment within 6 months
5. Collagen/fat injections within 9 months
6. The allergy of PDO(Polydioxanone) and anesthetic agent (Ultracol, Lidocaine)
7. Participants who are possessed skin disorder
8. History of skin disease i.e., Keloids, Excessive scarring
9. History of using the anticoagulant and suffering from coagulopathy

3.2.5 Discontinuation Criteria

1. Subjects who want to discontinue the treatment with any reasons
2. Subjects who get severe complication
3. Subjects who get pregnant during program

3.2.6 Early termination Criteria

1. The efficacy of the study is better or worse than anticipated
2. Too high dropout rate
3. Severe delay to the planned schedule
4. Technical issues that cannot be resolved
5. Increase in cost for the research

6. More than 30 % of the volunteers develop serious adverse events from the injection of PDO

The subjects will be selected according to inclusion and exclusion reference. The subjects will be invited to the study by giving investigator voucher and advertising at MFU clinic and MFU school at Asok.

3.3 Study Location

Mae Fah Luang University Hospital, Bangkok

3.4 Research Instrument

3.4.1 Patient record form
3.4.2 Description of the trial objectives and purpose
3.4.3 Descriptive of the trial design
3.4.4 Informed consent form
3.4.5 Clinical evaluation record form
3.4.6 Polydioxanone Dermal filler
3.4.7 Questionnaires and Satisfaction Assessment Form
3.4.8 Adverse effect record form

3.5 Variables of the Study

3.5.1 Independent Variable:
Polydioxanone Dermal filler
3.5.2 Dependent Variable:
Physician Global Aesthetic Scale
Participant Satisfaction Score
Adverse effect
Skin Viscoelasticity by Cutometer

Wrinkle by Visioscan

TWEL by Tewameter

3.6 Study Procedures

3.6.1 Selection of Research Subjects

3.6.1.1 Participants were recruited by fulfilled criteria.

3.6.1.2 The researcher gave explanations of the intention and step of procedures during the study including the benefit and possible side effects after treatment.

3.6.1.3 Participants signed voluntarily an informed consent form for research participation.

3.6.1.4 The information of the subjects was recorded.

3.6.2 Treatment Process

3.6.2.1 Standardized photos will be taken before treatment (baseline) and at each follow up visits 4th, 8th and 12th week using Visia® with identical setting, same light and same position. The participants will be photographed locally on the face and cropped particularly over the eyes.

3.6.2.2The researcher will also record skin moisture and elasticity of the participants by using Cutometer MPA 580 and Tewameter at baseline, 4th, 8th and 12th week respectively. The landmark of the measurement will be two points (1) 1.5cm from lateral canthus horizontally outward and (2) 1.5 cm downward from lower eyelid at the mid-papillary line.

3.6.2.3 The face will be cleaned and Local anesthesia 2% lidocaine without adrenaline 0.1 cc per site will be injected at the injection point of dermal filler.

3.6.2.4 Polydioxanone dermal filler 0.5 cc will be injected at Periorbital region of 2 eyes for a total of 2 sessions 4 week apart, week 0 and week 4. Injection will be done 2cm below the eye superficially in a fanning method using a 25G cannula in subdermal region, 0.1 cc per stroke for a total of 5 strokes in a retrograde manner.

3.6.2.5 The procedure will be carried out by a well-trained experienced Thai Doctor and main advisor will help and closely supervise during the whole process.

3.6.2.6 All post treatment adverse effects such as pain, swelling, burn and other side effects will be assessed and given treatment with steroid if they were to occur.

3.6.2.7 All post treatment care procedures such as avoiding sporting activities an direct sunlight exposure will be advised to all subjects.

Figure 3.1 Landmarks to measure skin moisturization and elasticity

Figure 3.2 Protocol for injection

3.6.3 Follow Up Visit (4th 8th and 12th Week After Treatment)

3.6.3.1 At each follow up visit, all subjects will be taken a photograph under identical camera setting as baseline. Facial position for photograph: Left 45°, Center 0° and Right 45°.

3.6.3.2 All subjects will be assessed by cutometer, tewameter and visioscan.

3.6.3.3 If any adverse reaction occurs, they will be evaluated.

3.7 Outcome Measurements and Data Collection

3.7.1 Subjective Evaluation

3.7.1.1 The improvement will be assessed by comparing digital photographs at baseline and each follow up visits (4th ,8th and 12th week) by Global Aesthetic Improvement Scale (GAIS)

1 = worse

2 = no change

3 = improve

4 = very improve

5 = Exceptional improve

3.7.1.2 Satisfaction will be evaluated by participant's satisfaction score by using grading scale at baseline each follow up visits (4th ,8th and 12th week)

-1 = unsatisfied

0 = indifferent

1 = somewhat satisfied

2 = moderately satisfied

3 = very satisfied

4 = completely satisfied

3.7.2 Objective Evaluation

3.7.2.1 Wrinkles will be assessed by Visioscan at baseline and each 4th, 8th and 12th week follow up visit accordingly.

3.7.2.2 Skin elasticity will be assessed by Cutometer at baseline and each 4th, 8th and 12th week visit accordingly.

3.7.2.3 Trans-epidermal water loss (TEWL) will be assessed by Tewameter at baseline and each 4th, 8th and 12th week visit accordingly.

3.7.2.4 All complications will be assessed at baseline and each 4th, 8th and 12th week visit accordingly.

3.7.2.5 Dermatologists also evaluate the periorbital wrinkle score according to Rao-Goldman 5point scoring scale at baseline, 4th, 8th and 12th week as follows:

1= Wrinkle absent

2=Shallow but visible

3=Moderately deep

4= Deep wrinkle with well-defined edges

5= Very deep with redundant folds

3.8 Research Materials

3.8.1 Cutometer® MPA 580

Figure 3.3 Cutometer® MPA 580

The measuring principle of cutometer is based on suction method. It can be calculated the related to elastic and cisco- elastic properties of skin surface and skin aging. (Courage + Khazaka Electronic, Köln - Skin Testing Equipment, n.d.)

3.8.2 Tewameter® TM 300

Source Medical Search (n.d.)

Figure 3.4 Tewameter® TM 300

With the "open chamber" principle, the Tewameter® TM 300 (previously the Tewameter® TM Hex) is the world's most widely used measuring device for the assessment of transdermal water loss (TEWL). It is an essential parameter for assessing the moisture barrier function of the skin and is a basic measure for all types of applications. Even minor damage to the skin's moisture barrier can be identified at an early stage.

3.8.3 VISIA Complexion Analysis System

Figure 3.5 VISIA® Complexion Analysis System

IntelliFlash®, cross-polarized light, ultraviolet light is used to record and measure skin conditions above and below the surface. UV photography provides the most comprehensive data set available for sun damage assessment and analysis, including UV fluorescence imaging to reveal porphyrins. Canfield's RBX® technology separates the unique color signatures of red and brown skin components for unparalleled visualization of conditions that cause color concentration, such as spider veins, hyperpigmentation, inflammation and other conditions.

3.8.4 Polydioxanone Dermal filler (Ultracol®)

For this research, we will use FDA approved Polydioxanone dermal filler Ultracol® (Korea) which is manufactured by the company Ultra V (Ultra-V-medical, 2023).

Figure 3.6 Ultracol®

3.9 Data Analysis

3.9.1 Participants' eligibility for this study is determined by applying inclusion and exclusion criteria, and personal information is highly confidential in data analysis.

3.9.2 The medical record data of the participants and the results of this study trial conducted at Mae Fah Luang University Dermatology Clinic, we will analyze using SPSS 18 and Microsoft Excel 2010 software.

3.9.3 General demographic data of the participants is recorded using descriptive statistical analysis to provide descriptive information with the mean and standard deviation for numeric data and with percentage in categorical data.

3.9.4 Physician's Global Aesthetic Improvement Scale Outcomes and Participant Satisfaction Scores at each follow-up visit and before treatment with Friedman Test.

3.9.5 Statistical significance is obtained by comparing the mean values of wrinkle score, viscoelasticity score, and transdermal water loss scores at each follow-up visit and before treatment using repeated measures ANOVA for normal distribution data and use the Wilcoxon signed-rank test for non-normal distribution data can be used.

3.9.6 Adverse events after injection are analyzed using descriptive statistical analysis.

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

3.10 Ethical Considerations

3.10.1 All protocols of the research will be conducted only after approval by the human research committee at Mae Fah Luang University and this study will be conducted by following the guideline of Good Clinical Practice (GCP). The researcher has completed Good Clinical Practice and the Human Research Ethics training course. Participants will gain assurance that the rights, safety and welfare of study subjects are respected consistent with ethical considerations.

For general understanding, the considerations were as follows.

3.10.2 An evaluation and approval of the research by ethics committee.

3.10.3 Volunteers totally understand the objective, methodology and possible risks and adverse effects of the research.

3.10.4 Prior to participating in the study, volunteers will provide their informed consent by signing a consent form. Individuals have the option to depart at any given moment without incurring any negative consequences.

3.10.5 The event of the occurrence of a problem, the researcher will assume responsibility and fully help the volunteers.

3.10.6 This research will be free of charge.

3.10.7 Respect the confidentiality of the volunteers' personal information by keeping it highly confidential.

3.10.8 During the course of the research, serious adverse events will be reported to the ethic committee.

3.10.9 For the benefits, the participants will receive an injection of Polydioxanone dermal filler at the periorbital area for rejuvenation effects. This is an opportunity to learn the effects of collagen production stimulated by Polydioxanone and induce remodeling with healing in less time and little patient discomfort that result in the development of medical knowledge and a good chance for the other researchers to conduct further studies in the future.

CHAPTER 4

RESULTS

4.1 Demographic Data of the Participants

Table 4.1 Demographic data of the participants

Demographic data	n=23
Gender, n	
Male	6
Female	17
Age group (years), mean ± SD	41.0 ± 5.58
Occupation, n	
Student	8
Employee	15
Sunlight Exposure Duration, n	
Less than 1 hour	12
1 - 1.5 hours	7
2 hours	4
Underlying disease, n	
No	23
Personal Medication, n	
No	23
History of allergy, n	
No	23

According to Table 4.1, showing the demographic data of the 23 participants, 17 were female and 6 were male. The mean age group was 41.0±5.58 years. 8 were students and 15 were employees. All research participants had no underlying disease, history of medication, or allergies.

4.2 Clinical Evaluation

4.2.1 Wrinkle Score by VISIA

Table 4.2 Statistical analysis of wrinkle score at left, right front on baseline, follow-up 4th, 8th, and 12th week (n=23)

Follow-up	Left (mean ± SD)	Right (mean ± SD)	Front (mean ± SD)
Baseline	34.79 ± 6.29	29.87 ± 5.99	7.19 ± 3.63
4th week	27.78 ± 6.66	22.32 ± 5.78	4.47 ± 2.82
8th week	23.40 ± 7.96	17.42 ± 6.77	2.75 ± 1.86
12th week	21.41 ± 17.44	11.64 ± 6.13	1.69 ± 1.42
p-value	<0.0001*	<0.0001*	<0.0001*

Note Data were analyzed with Repeated measure ANOVA

* Statistically significant at the 0.05 level

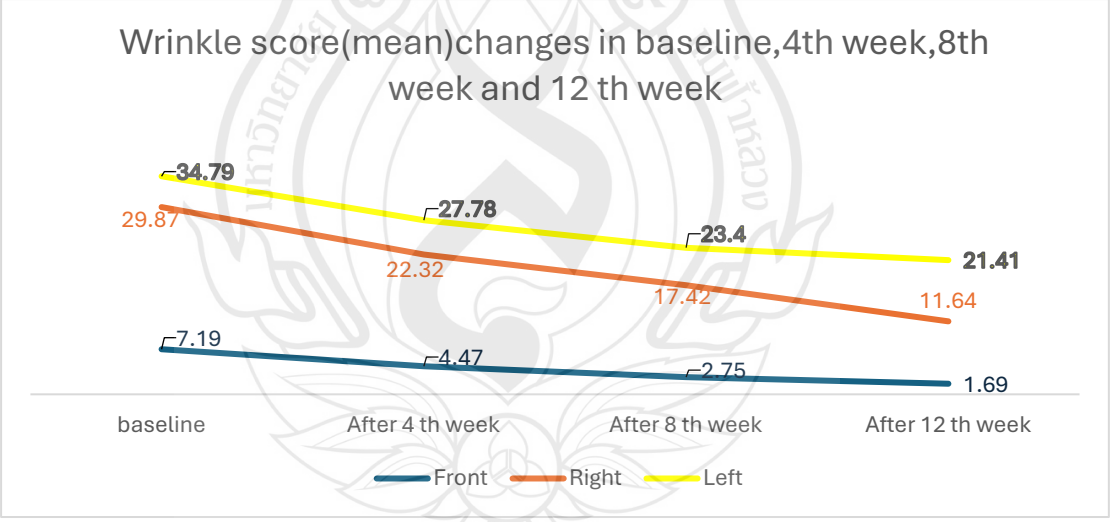


Figure 4.1 Demonstrates line graph revealing the wrinkle score at the Front, left and the right side from baseline to the follow-up 12th week

Based on the data presented in Table 4.2, which displays the statistical analysis of the wrinkle scores for both the left and right sides, the average wrinkle score on the left at baseline and follow-up at 4th, 8th and 12th week were 34.79 ± 6.29, 27.78 ± 6.66, 23.40 ± 7.96, 21.41 ± 17.44 respectively. The mean wrinkle score at the right on

baseline, follow-up at 4th, 8th and 12th week were 29.87 ± 5.99, 22.32 ± 5.78, 17.42 ± 6.77, 11.64 ± 6.13 respectively. The mean wrinkle score at the front on baseline, follow up at 4th, 8th, 12th week were 7.19 ± 3.63, 4.47 ± 2.82, 2.75 ± 1.86 and 1.69 ± 1.42 respectively.

The average wrinkle score on the left side at each visit showed a statistically significant decrease at the 0.05 level (partial $\eta^2 = 0.925$, $p < 0.0001$). This indicates that the effect of the Polydioxanone injection on the wrinkle score at the left side was 92.5%.

Similarly, the average wrinkle score at the front also decreased significantly at the 0.05 level (partial $\eta^2 = 0.925$, $p < 0.0001$), reflecting a 92.5% treatment effect.

On the other hand, the average wrinkle score at the front of each visit showed a significant reduction at the 0.05 level (partial $\eta^2 = 0.744$, $p < 0.0001$), corresponding to a 74.4% treatment effect from the Polydioxanone injection.

Table 4.3 Multiple comparison (Post hoc) of wrinkle score at left and right (n=23)

Pairewise	**Left**		**Right**	
	Mean Difference	**P-value**	**Mean difference**	**P-value**
Baseline-4th week	-7.55	<0.0001*	-7.55	<0.0001*
Baseline-8th week	-12.45	<0.0001*	-12.45	<0.0001*
Baseline-12thweek	-18.23	<0.0001*	-18.23	<0.0001*

Note Data were analyzed with Wilcon Sign Rank test

* The mean difference is statistically significant at the 0.05 level

As shown in Table 4.3, which presents the results of the multiple comparisons, the wrinkle scores on both the left and right sides at the 4th, 8th, and 12th weeks were significantly lower than at baseline, with statistical significance at the 0.05 level ($p < 0.05$). The overall reduction in the wrinkle scores across the entire face from baseline to the 12th-week follow-up was 18.23.

4.2.2 Cutometer Score

Table 4.4 Statistical analysis of cutometer score at under eye (left and right side), crow feet (left and right side) on baseline, follow-up 4th, 8th, and 12th week (n=23)

Follow up	Left (Mean ± SD)		Right (Mean ± SD)	
	Under eye	**Crow feet**	**Under eye**	**Crow feet**
Baseline	0.667 ± 0.197	0.770 ± 0.249	0.746 ± 0.202	0.744 ± 0.228
After 4 weeks	0.673 ± 0.194	0.810 ± 0.206	0.750 ± 0.184	0.755 ± 0.210
After 6 weeks	0.717 ± 0.191	0.826 ± 0.190	0.763 ± 0.174	0.774 ± 0.196
After 12 weeks	0.749 ± 0.213	0.853 ± 0.170	0.790 ± 0.151	0.828 ± 0.162
p-value	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Note Data were analyzed with Repeated measure ANOVA

* Statistically significant at the 0.05 level

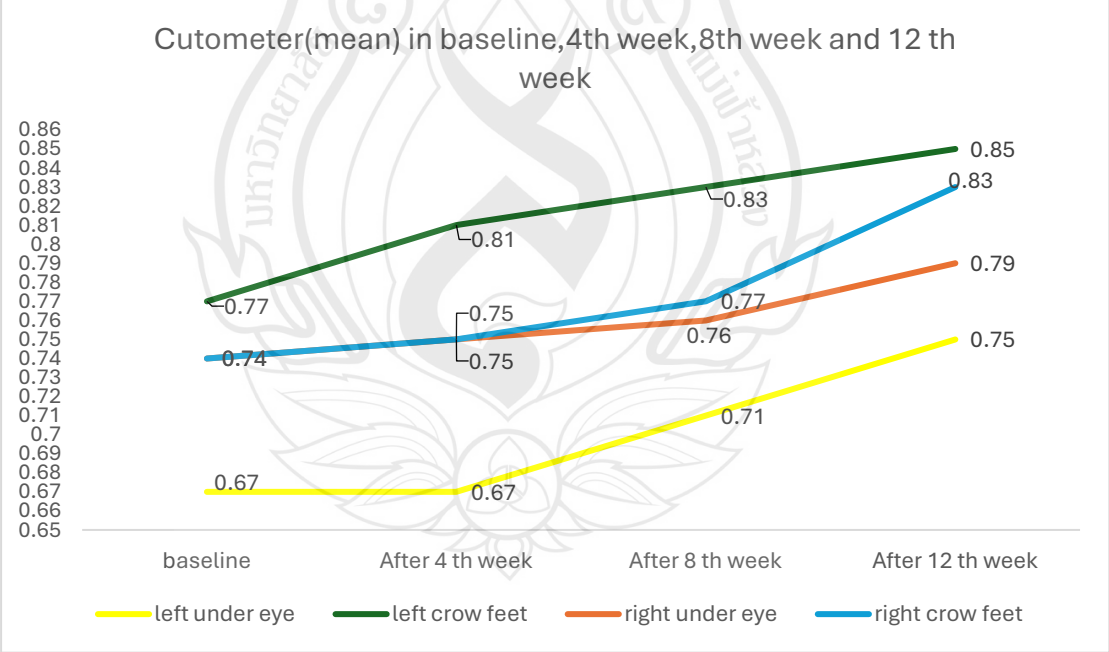


Figure 4.2 Displays line graph showing the cutometer score under eye (left and right side), crow feet (left and right side) from baseline to the follow-up 12th week

According to Table 4.4, which shows the statistical analysis of the cutometer scores at the left and right under eye and crow feet. The mean of the cutometer score of

left under eye on baseline, follow-up 4th, 8th and 12th week were 0.667 ± 0.197, 0.673 ± 0.194, 0.717 ± 0.191, 0.749 ± 0.213 respectively and the mean of the cutometer score of left crow feet on baseline, follow-up 4th, 8th and 12th week were 0.770 ± 0.249, 0.810 ± 0.206, 0.826 ± 0.190, 0.853 ± 0.170 respectively. The mean cutometer score at left under eye and crow feet in each visit increased statistically significantly at the level of 0.05 (Partial η^2 0.936,η^2 0.964, p<0.001) respectively. In other words, the treatment effect of the injection Polydioxanone on the cutometer score at the left under eye and crow feet were 93.6% and 96.4% respectively.

The mean of the cutometer score of right under eye on baseline, follow-up 4th, 8th and 12th week were 0.746 ± 0.202, 0.750 ± 0.184, 0.763 ± 0.174, 0.790 ± 0.151 respectively and the mean of the cutometer score of right crow feet on baseline, follow-up 4th, 8th and 12th week were 0.744 ± 0.228, 0.755 ± 0.210, 0.774 ± 0.196, 0.828 ± 0.162 respectively. The mean cutometer score at right under eye and crow feet in each visit increased statistically significantly at the level of 0.05 (Partial η^2 0.936,η^2 0.947, p<0.001) respectively. In other words, the treatment effect of the injection Polydioxanone on the cutometer score at the right under eye and crow feet were 93.6% and 94.7% respectively.

Table 4.5 Multiple comparison (Post hoc) of cutometer score of under eye (left and right side), crow feet (left and right side) (n=23)

Follow up		Left		Right	
		Under eye	**Crow feet**	**Under eye**	**Crow feet**
4 weeks-Baseline	Mean difference	0.006	0.041	0.004	0.012
	p value	0.689	0.066 [a]	0.810	0.512
8 weeks-Baseline	Mean difference	0.050	0.057	0.017	0.030
	p value	0.001	0.003 [a]	0.712	0.160
12 weeks-Baseline	Mean difference	0.046	0.083	0.043	0.084
	p value	0.152	0.001 [a]	0.217	0.016

Note Data were analysis with a Wilcon Sign Rank test, others were done with T test

Based on the multiple comparison results presented in Table 4.5, the cutometer score at left and right under eye and crow feet. The post hoc analysis indicates the paired difference in mean cutometer. This table shows that significant improvement in skin elasticity was observed for the left undereye region at 8 weeks (p=0.001), left crow feet on the 8th week (p=0.003) and 12th weeks (p=0.001) and Right crow feet at 12th week(p=0.016) which are statistically significant (p <0.05).

4.2.3 Tewameter Score

Table 4.6 Statistical analysis of tewameter score of under eye (left and right side), crow feet (left and right side) on baseline, follow-up, 4th, 8th, and 12th week (n=23)

Follow up	Left (Mean ± SD)		Right (Mean ± SD)	
	Under eye	Crow feet	Under eye	Crow feet
Baseline	16.730 ± 6.790	16.021 ± 8.482	16.266 ± 8.063	17.439 ± 6.764
After 4 weeks	15.4 ± 6.830	15.400 ± 8.119	15.970 ± 7.182	16.644 ± 6.561
After 6 weeks	15.060 ± 6.625	14.874 ± 7.435	15.5 ± 5.876	15.804 ± 5.047
After 12 weeks	14.345 ± 6.473	14.135 ± 7.118	14.704 ± 5.602	15.674 ± 5.372
p-value	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Note Data were analyzed with Repeated measure ANOVA

* Statistically significant at the 0.05 level

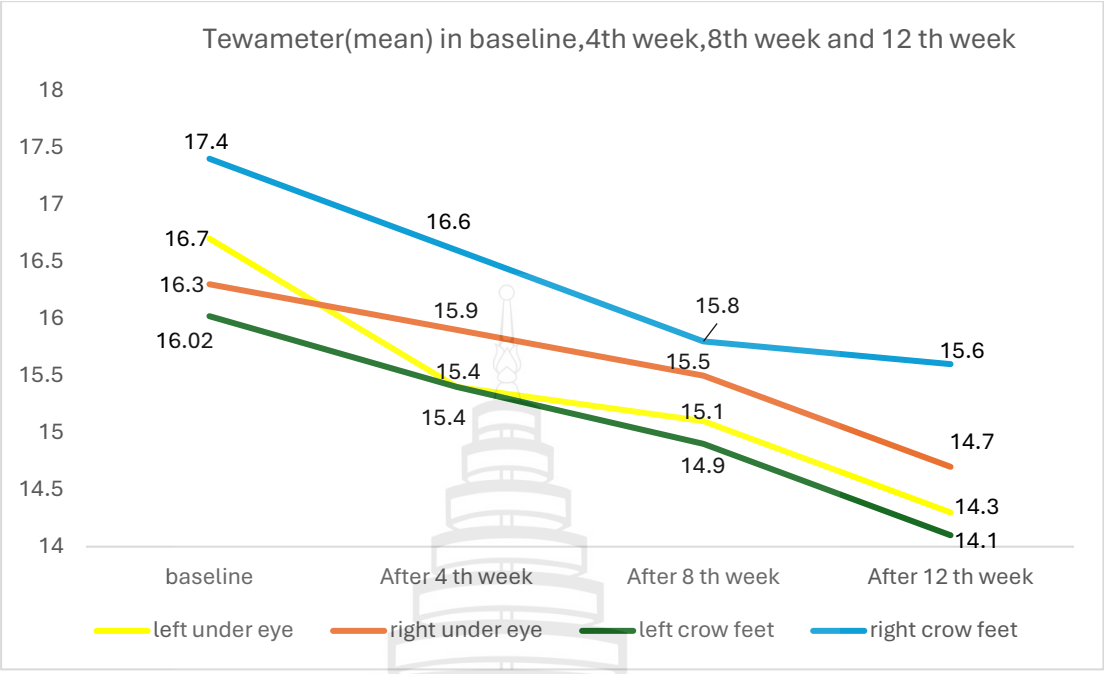


Figure 4.3 Displays line graph showing the tewameter score at under eye (left and right side), crow feet (left and right side) from baseline to the follow-up 12th week

According to Table 4.6, which shows the statistical analysis of the tewameter score of the left and right under eye and crow feet, the mean of the tewameter score of left under eye on baseline, follow-up 4th, 8th, and 12th week were 16.730 ± 6.790, 15.4 ± 6.830, 15.060 ± 6.625 and14.345 ± 6.473 respectively and left crow feet on baseline, follow-up 4th, 8th, and 12th week were 16.021 ± 8.482, 15.400 ± 8.119, 14.874 ± 7.435, 14.135 ± 7.118 respectively. The mean tewameter score at left under eye and crow feet in each visit decreased statistically significantly at the level of 0.05 (Partial η^2 0.85,η^2 0.799, $p<0.0001$). In other words, the treatment effect of the injection Polydioxanone on the tewameter score at the left under eye and crow feet was 85% and 79.9% respectively.

The mean of the tewameter score of right under eye on baseline, follow-up 4th, 8th, and 12th week were 16.266 ± 8.063, 15.970 ± 7.182, 15.5 ± 5.876, 14.704 ± 5.602 respectively and right crow feet on baseline, follow-up 4th, 8th, and 12th week were 17.439 ± 6.764, 16.644 ± 6.561, 15.804 ± 5.047, 15.674 ± 5.372 respectively. The mean tewameter score at left under eye and crow feet in each visit decreased statistically

significantly at the level of 0.05 (Partial η^2 0.85,η^2 0.897, p<0.0001). In other words, the treatment effect of the injection Polydioxanone on the tewameter score at the left under eye and crow feet was 85.9% and 89.7% respectively.

Table 4.7 Multiple comparison (Post hoc) of tewameter score at under eye (left and right side), crow feet (left and right side) (n=23)

Follow up		Left		Right	
		Under eye	**Crow feet**	**Under eye**	**Crow feet**
4 weeks-Baseline	Mean difference	-1.330	-0.626	-0.296	-0.796
	p value	0.009	0.017 [a]	0.460	0.000
8 weeks-Baseline	Mean difference	-1.669	-1.148	-0.766	-1.635
	p value	0.002	0.002 [a]	0.384	0.027
12 weeks-Baseline	Mean difference	-2.386	-1.887	-1.561	-1.765
	p value	0.000	0.001 [a]	0.096	0.050

Note Data were analysis with a Wilcon Sign Rank test, others were done with T test

* The mean difference is statistically significant at the 0.05 level

According to Table 4.7, which displays the multiple comparison result of the left and right under eye and crow feet, the tewameter score at left (under eye and crow feet) and right (under eye and crow feet) on the 4th, 8th, and 12th week was lower than at baseline, which were statistically significant at the level of 0.05 (p<0.05). The decrease in the tewameter score on the left and right under eye and crow feet from baseline to the follow-up 12th week was left under eye (2.386) crow feet (1.887) and right under eye (1.561) crow feet (1.765).

4.2.4 Physician Global Aesthetic Improvement Scale (GAIS)

Table 4.8 Frequencies of Dermatologists' evaluation score (n=23)

	4th week		8th week		12th week	
	Frequency	**Percent**	**Frequency**	**Percent**	**Frequency**	**Percent**
Fair improvement	15	65.2	-	-	-	-
Moderate improvement	8	34.8	12	52.2	1	4.3
Good improvement	-	-	11	47.8	14	60.9
Excellent improvement	-	-	-	-	8	34.8
Total	**23**	**100.0**	**23**	**100.0**	**23**	**100.0**

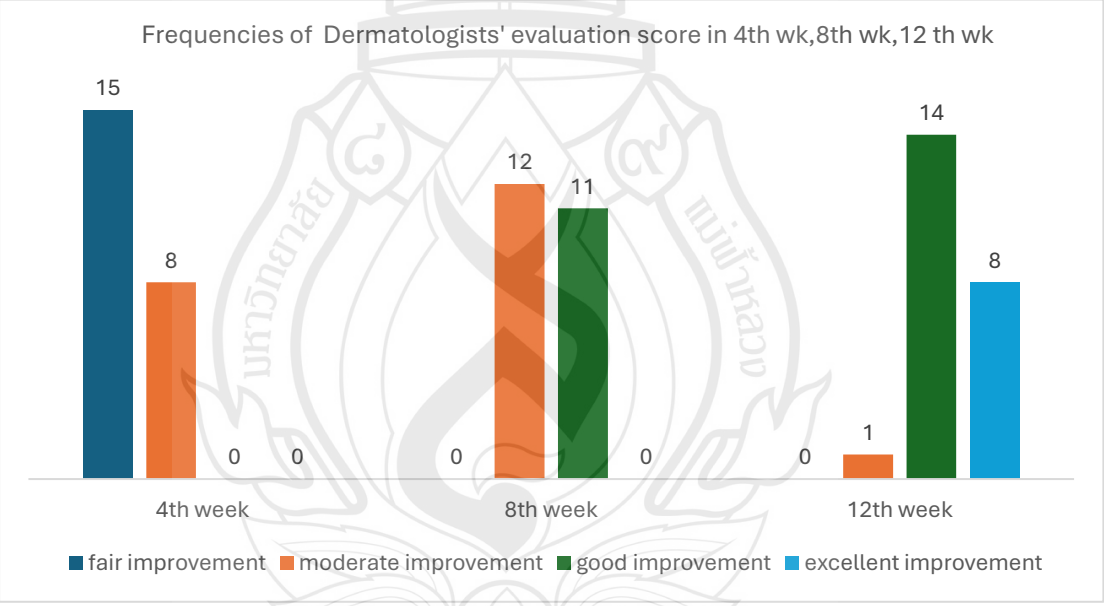


Figure 4.4 Displays a bar graph showing frequency of Dermatologists' evaluation score from the follow up 4th to 12th week

As shown in table 4.8, dermatologist evaluation for both sides of the eyes (left and right) on the 4th week showed 65.2% as fair improvement in 15 volunteers and 34.8% of moderately improved in 8 volunteers. On 8th week of evaluation, there was more improvement than 4th week showing 12 participants (52.2%) as moderate improvement,11 participants (47.8%) as good improvement. Finally, on the 12th week,

a moderate improvement in 1 volunteer (4.3%), good improvement in 14 volunteers (60.9%) and excellent improvement in8 volunteers (34.8%) was seen.

4.2.5 Patients' Satisfactory Score

Table 4.9 Frequency of patients' satisfactory score

	4th week		8th week		12th week	
	Frequency	**Percent**	**Frequency**	**Percent**	**Frequency**	**Percent**
Somewhat satisfied	11	47.8	7	30.4	-	
Moderately satisfied	12	52.2	11	47.8	8	34.8
Very satisfied	-	-	5	21.7	9	39.1
Completely satisfied	-	-	-	-	6	26.1
Total	**23**	**100.0**	**23**	**100.0**	**23**	**100.0**

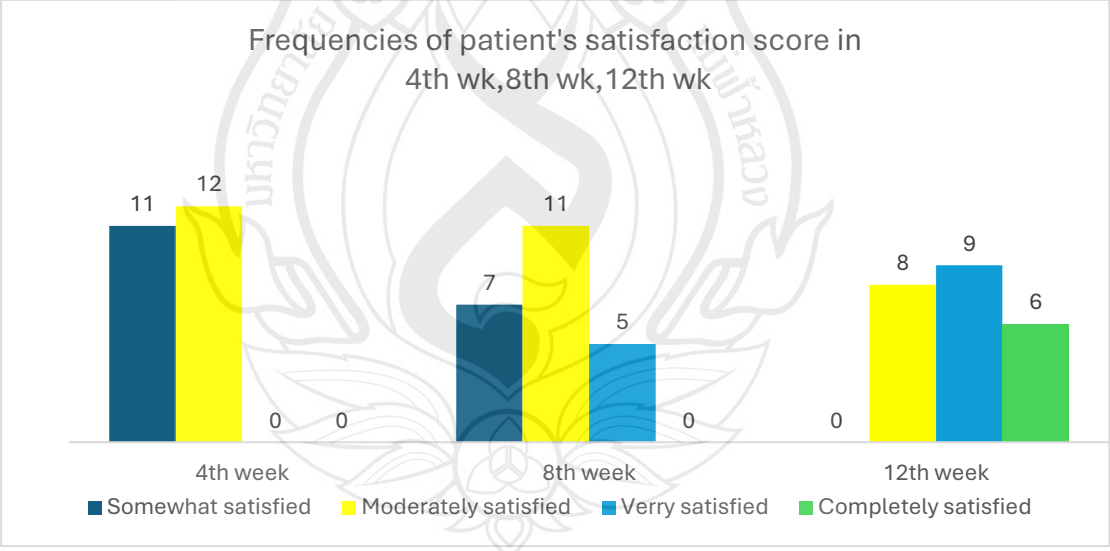


Figure 4.5 Displays a bar graph showing the frequencies of patients' satisfaction score

According to the table 4.9, on the 4th week,11 participants were somewhat satisfied,12 were moderately satisfied. On the 8th week & participants showed somewhat satisfied, 11 were moderately satisfied and 5 were very satisfied. On the 12th

week, the participants' satisfaction showed an increase with 8 volunteers being moderately satisfied, 9 were very satisfied and 6 were completely satisfied.

Before (right) After (right)

Before (left) After (left)

Figure 4.6 Comparison of before and after the treatment

4.3 Adverse Effects

During the study, no serious adverse effect was noted in all participants after the procedure and during the follow ups.

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 Discussion

Rejuvenation involves the removal and regeneration of skin to create a more structured, “youthful” dermal matrix and restore epidermal balance. This study evaluated the effectiveness of injectable Polydioxanone (PDO) for rejuvenating the periorbital area. To our knowledge, this is a clinical experimental study to investigate the efficacy, satisfaction, and adverse effects The study was done in Mae Fah Luang Hospital, Bangkok with, twenty-three participants, aged 35-60, seeking rejuvenation of the periorbital region, were recruited. All subjects attended regular treatment and follow-up sessions.

Participants received two PDO injection treatments, with assessments of wrinkles, skin elasticity, and transepidermal water loss at baseline, and at 4, 8, and 12 weeks. Effectiveness was measured using a Cutometer, Tewameter, VISIA, GAIS, and patient satisfaction surveys, while adverse events were also monitored. Wrinkle scores, measured at each visit, showed a statistically significant reduction in mean scores at the front, left, and right. Skin elasticity, measured using the Cutometer, showed a significant increase in scores at the left and right under-eye areas and crow’s feet. The GAIS score, evaluated by three dermatologists using VISIA® photographs, also showed a significant improvement. Most patients expressed high satisfaction with the results. No serious adverse effects were reported apart from pain at injection site.

Polydioxanone (PDO) is a biodegradable synthetic polymer that is commonly used in medical applications, including as a thread for non-surgical facelifts and as an injectable treatment for skin rejuvenation. PDO has a long history of use in surgical sutures due to its biocompatibility, safety, and ability to gradually degrade in the body.

When used in injectable treatments, PDO stimulates collagen production in the skin. This leads to tightening and rejuvenation of the treated area. PDO injections stimulate the body’s natural collagen production. As PDO degrades, it triggers the

body’s healing response, encouraging collagen synthesis and improving skin structure over time. They are often used in aesthetic medicine to improve skin elasticity, reduce wrinkles, and promote a more youthful appearance, particularly in areas like the face, including the periorbital region (around the eyes), cheeks, and neck. PDO is considered safe, with a low risk of adverse reactions, as it is biocompatible and resorbs into the body after a few months. However, like any procedure, side effects can occur, such as swelling, bruising, or infection, although these are typically mild and temporary. The effects of PDO injections can last from several months to a year, depending on the individual and the treatment area. Collagen stimulation continues for months after the procedure, which can lead to a gradual improvement in skin texture and appearance.

5.2 Conclusion

To sum up, the results significantly proved that the intradermal injection of Polydioxanone (PDO) was effective for rejuvenation of the periorbital area.

Furthermore, dermatologists and the subjects were also satisfied with the improvement in skin quality around the eye area showing wrinkle improvement and visible reduction in the darkening of the under eye area without serious complications. Therefore, it can be concluded that the injection Polydioxanone can be used safely and effectively for periorbital rejuvenation.

5.3 Suggestion

This study can be used as a reference paper for the treatment of facial rejuvenation in the future. However, the duration of the study is only 12 weeks, so we cannot evaluate the effectiveness of the treatment in long term. Longer follow up time may be needed to observe the maximum effect.

REFERENCES

Baumann, L. (2007). Skin ageing and its treatment. The Journal of Pathology: A *Journal of the Pathological Society of Great Britain and Ireland, 211*(2), 241-251.

Boland, E. D., Coleman, B. D., Barnes, C. P., Simpson, D. G., Wnek, G. E., & Bowlin, G. L. (2005). Electrospinning polydioxanone for biomedical applications. *Acta Biomaterialia, 1*(1), 115-123.

Brown, T., & Krishnamurthy, K. (2020). *Histology, Dermis.* StatPearls.

Callaghan, T., & Wilhelm, K. P. (2008). A review of ageing and an examination of clinical methods in the assessment of ageing skin. Part I: Cellular and molecular perspectives of skin ageing. *International Journal of Cosmetic Science, 30*(5), 313-322.

Cobo, R. (2020). Use of polydioxanone threads as an alternative in nonsurgical procedures in facial rejuvenation. *Facial Plastic Surgery, 36*(04), 447-452.

DiBernardo, G. A., & DiBernardo, B. E. (2018). Prediction of treatment outcomes for neck rejuvenation utilizing a unique classification system of treatment approach using a 1440-nm side-firing laser. *Aesthetic Surgery Journal, 38*(suppl_2), S43-S51.

Fang, L., Gold, M., & Huang, L. (2014). Melasma-like Hyperpigmentation Induced by Intense Pulsed Light Treatment in Chinese Individuals. *Journal of cosmetic and laser therapy : official publication of the European Society for Laser Dermatology, 16,* 1-24. https://doi.org/10.3109/14764172.2014.953417

Fisher, G. J., Wang, Z., Datta, S. C., Varani, J., Kang, S., & Voorhees, J. J. (1997). Pathophysiology of premature skin aging induced by ultraviolet light. *New England Journal of Medicine, 337*(20), 1419-1429.

Helfrich, Y. R., Sachs, D. L., & Voorhees, J. J. (2008). Overview of skin aging and photoaging. *Dermatology Nursing, 20*(3).

Kang, S. (2019). *Fitzpatrick's dermatology*. McGraw-Hill.

Kashkouli, M. B., Abdolalizadeh, P., Abolfathzadeh, N., Sianati, H., Sharepour, M., & Hadi, Y. (2017). Periorbital facial rejuvenation; applied anatomy and pre-operative assessment. *Journal of Current Ophthalmology, 29*(3), 154-168.

Kim, C. M., Kim, B. Y., Hye Suh, D., Lee, S. J., Moon, H. R., & Ryu, H. J. (2019). The efficacy of powdered polydioxanone in terms of collagen production compared with poly-L-lactic acid in a murine model. *Journal of Cosmetic Dermatology, 18*(6), 1893-1898.

Liang, Y., Su, W., & Wang, F. (2023). Skin Ageing: A Progressive, Multi-Factorial Condition Demanding an Integrated, Multilayer-Targeted Remedy. *Clinical, Cosmetic and Investigational Dermatology*, 1215-1229.

Manaloto, R. M. P., & Alster, T. S. (1999). Periorbital rejuvenation: a review of dermatologic treatments. *Dermatologic Surgery, 25*(1), 1-9.

Medical Search. (n.d.). *Tewameter Tm 300 Transepidermal Water Loss -Skin Analyser*. https://www.medicalsearch.com.au/tewameter-tm-300 transepidermal-water-loss-skin-analyser/p/221659

Mehta, C., Bhatt, G., & Kothiyal, P. (2016). A Review on organogel for skin aging. *Indian Journal of Pharmaceutical and Biological Research, 4*(3), 28.

Ross, E. V., Tidwell, W. J., Guss, L., & Sutton, A. V. (2022). Study of a 532/1064 fractional picosecond laser for facial rejuvenation. *Dermatologic Surgery, 48*(1), 109-113.

scharffetter-kochanek, K., Brenneisen, P., Wenk, J., Herrmann, G., Ma, W. J., Kuhr, L., Meewes, C., & Wlaschek, M. (2000). Photoaging of skin from phenotype to mechanism. *Experimental Gerontology, 35*, 307-316. https://doi.org/10.1016/S0531-5565(00)00098-X

Shokri, T., & Lighthall, J. G. (2018). Antibiotic use in facial plastic surgery. *Current Opinion in Otolaryngology & Head and Neck Surgery, 26*(4), 242-247.

Tamura, B. M., & Odo, M. Y. (2011). Classification of periorbital wrinkles and treatment with Botulinum Toxin Type A. *Surg Cosmet Dermatol, 3*(2), 129-134.

Ultra-V-medical. (2023). *UltraCol*. https://ultrav-medical.com/ultracol/

Wang, A. S., & Dreesen, O. (2018). Biomarkers of cellular senescence and skin aging. *Frontiers in Genetics, 9*, 247.

Wickett, R. R., & Visscher, M. O. (2006). Structure and function of the epidermal barrier. *American Journal of Infection Control, 34*(10), S98-S110.

Zhang, S., & Duan, E. (2018). Fighting against Skin Aging: The Way from Bench to Bedside. *Cell Transplantation, 27*(5), 729-738. https://doi.org/10.1177/0963689717725755

Zhou, S.-Y., Kang, S. M., Gu, Y. J., Zhang, X. R., Yon, D. K., Shin, B. H., . . . Kwon, H. J. (2023). Bio-characteristics and Efficacy Analysis of Biodegradable Poly Dioxanone Dermal Filler in a Mouse Model and Humans. *In Vivo, 37*(3), 1093-1102.

APPENDIX A

INFORMED CONSENT FORM

Research Project: THE EFFICACY OF INJECTABLE POLYDIOXANONE(PDO) FOR PERIORBITAL REJUVENATION IN THE POPULATION AGED BETWEEN 35-60 YEARS OLD AT MAE FAH LUANG UNIVERSITY HOSPITAL, BANGKOK BY USING OPEN LABEL QUASI EXPERIMENTAL CLINICAL STUDY DESIGN.

I,Mr./Mrs./Miss…….……..……..…..

Address……..…..……

have read thoroughly the foregoing information and all the details from the documents explaining the information to the volunteers who participate in the research project.

Issue date……

I have received a copy of the Research Information Sheet for the volunteers who participate in the research project and a copy of Informed Consent Letter to participate in the research project which I have signed and dated. I have been given a thorough and detailed explanation before signing and the researcher have described in detail about the purpose of the research, discomfort or potential risks, expected benefits from the research, and other options. I have had the opportunity and sufficient time to ask questions about anything related to the research project and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research. I have accepted the fact that the researcher cannot guarantee a definite significant end result as it is subjective to every volunteer's skin. I am aware and I have accepted the fact that there is a chance of side effects such as infection, redness, pain or other symptoms. I have acknowledged from the researcher that if any side effects or hazards arise from the research, I will receive a medical treatment as stated in the Research Information Sheet volunteers who participated in the research project. I have the right to withdraw from the research project at any time. This withdrawal will not affect the medical treatment and any other rights that I shall have. The researcher certifies that my personal information will be kept confidential. Any disclosure of my personal information to some departments associated to the research

project will be done for academic reasons and purposes only. I have read everything above and have understood clearly. I consent voluntarily to participate as a participant in this research.

Signature of the participant ……

Written name of the participant (……)

Date……Month……Year……

I, Dr. Phyu Phyu Thin Khaing, as the Researcher and Principal Investigator have accurately read out and explain in details the Research Information Sheet to the potential participant, and to the best of my ability made sure that the participant understands the objectives, methods of research, discomfort or potential risks, expected benefits of the research, and other options for the research participant. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily. I will continue providing the product to the volunteer until the result is balance on both sides of the face and no irregularities are visible.

Signature of the researcher …….

Written name of the researcher (……)

Date……Month……Year……

APPENDIX B

RESEARCH PROFILE (CONFIDENTIAL)

Volunteer number……..

General information

1. Date :……

2. Sex

O Male…….

O Female……: Pregnancy or Lactation? ...1. Yes.....2. No

3. Age……year

4. Occupation .....

1.Government officer .....

2.Employe…....

3.Housewife.....

4.Student.....

5.Employee .....

6.Others:……

5. Underlying disease :…….

6. Photosensitivity or drug induced photosensitivity:……..

7. Personal medication and supplement:……

a. Chemo-radiotherapy

b. Active inflammatory skin disease, open wound in the treatment area

c. History of malignant or premalignant lesions in the treatment area

8. History of food or drug allergy:…….

9. Current facial product use:…….

10. History of following treatment before the study .....

1. Yes (Identify) .....

2. No ……..

O Ablative and/or Non-Ablative Laser Treatment (within 6 months)

O Radiofrequency Facial Treatment (within 12 months)

O Filler Injection (Periorbital area; within 12 months)

O Microdermabrasion (within 4 months)

O Botulinum Toxin Injection (Periorbital area; within 12 months)

O Other rejuvenation treatment……

11. Average time exposure to the sunlight during 10 am – 4 pm…….minutes/hours

APPENDIX C

CLINICAL EVALUATION

Table C1 Cutometer score at crow's feet for Injectable Polydioxanone at baseline, 4th week, 8th week and 12th week.

No	Week 0		Week 4		Week 8		Week 12	
	Right	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								

Table C2 Cutometer score of undereye wrinkles for Injectable Polydioxanone at baseline, 4th week, 8th week and 12th week.

No	Week 0		Week 4		Week 8		Week 12	
	Right	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								

Table C3 Tewameter score at crow's feet for Injectable Polydioxanone at baseline and follow up 12th week.

Tewameter Scores						
	Baseline			12th week		
PARAMETER	**Left**	**Right**	**Mean**	**Left**	**Right**	**Mean**
TEWL (Transepidermal water loss)						

Table C4 Tewameter score of undereye wrinkles for Injectable Polydioxanone at baseline and follow up 12th week.

Tewameter Scores						
	Baseline			12th week		
PARAMETER	**Left**	**Right**	**Mean**	**Left**	**Right**	**Mean**
TEWL (Transepidermal water loss)						

Table C5 Wrinkle evaluation of Injectable Polydioxanone by VISIA

No	Week 0	Week 4	Week 8	Week 12
1	-			
2	-			
3	-			
4	-			
5	-			
6	-			
7	-			
8	-			
9	-			
10	-			

Table C5 (continued)

No	Week 0	Week 4	Week 8	Week 12
11	-			
12	-			
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				

Table C6 Dermatologists' evaluation score for Injectable Polydioxanone at baseline, 4th week, 8th week and 12th week.

No	Week 0			Week 4			Week 8			Week 12		
	Dr1	Dr2	Dr3	Dr1	Dr2	Dr3	Dr1	Dr2	Dr3	Dr1	Dr2	Dr3
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												

Table C6 (continued)

No	Week 0			Week 4			Week 8			Week 12		
	Dr1	**Dr2**	**Dr3**	**Dr1**	**Dr2**	**Dr3**	**Dr1**	**Dr2**	**Dr3**	**Dr1**	**Dr2**	**Dr3**
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												

Table C7 Patients' satisfactory score on 12th week for Injectable Polydioxanone

No	Injectable Polydioxanone
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	

Table C7 (continued)

No	Injectable Polydioxanone
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	

APPENDIX D

MATERIAL

Figure D1 Photos showing Injectable Polydioxanone (Ultracol®)

APPENDIX E

RESULTS

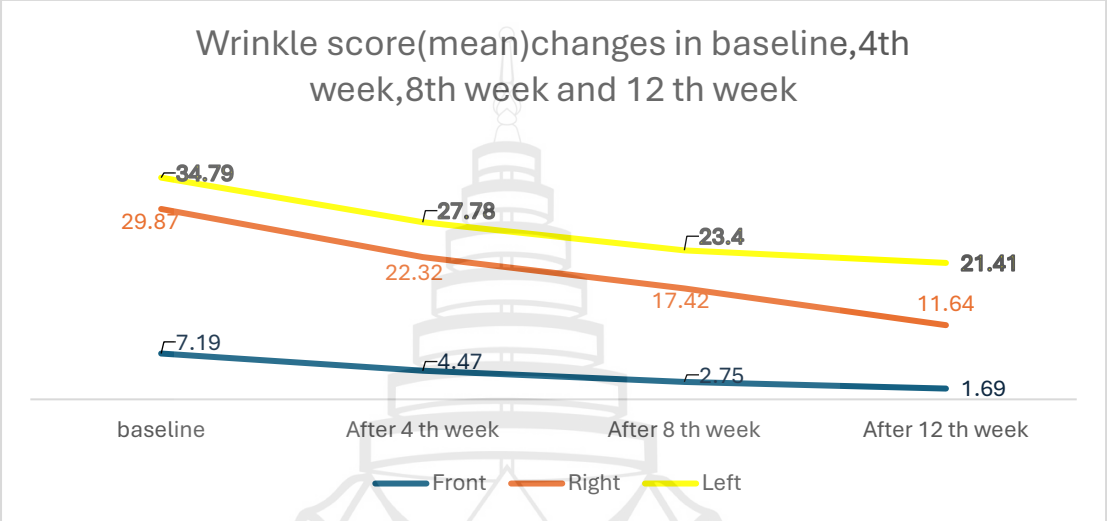


Figure E1 Demonstrates line graph revealing the wrinkle score at the Front, left and the right side from baseline to the follow-up 12th week

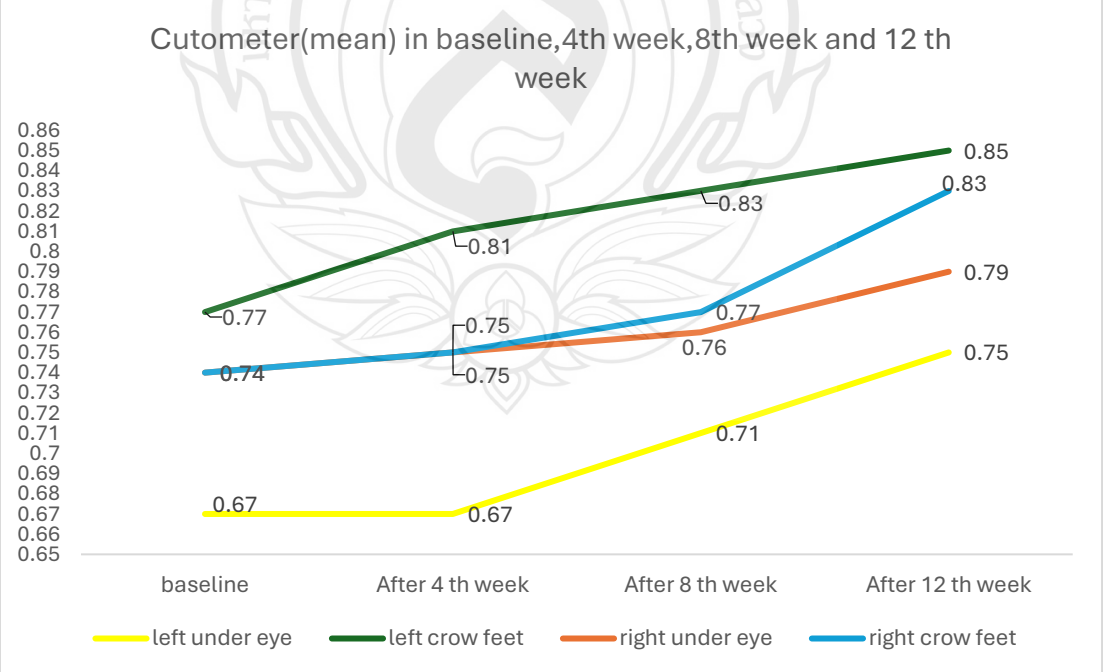


Figure E2 Line graph showing the cutometer score under eye (left and right side), crow feet (left and right side) from baseline to the follow-up 12th week

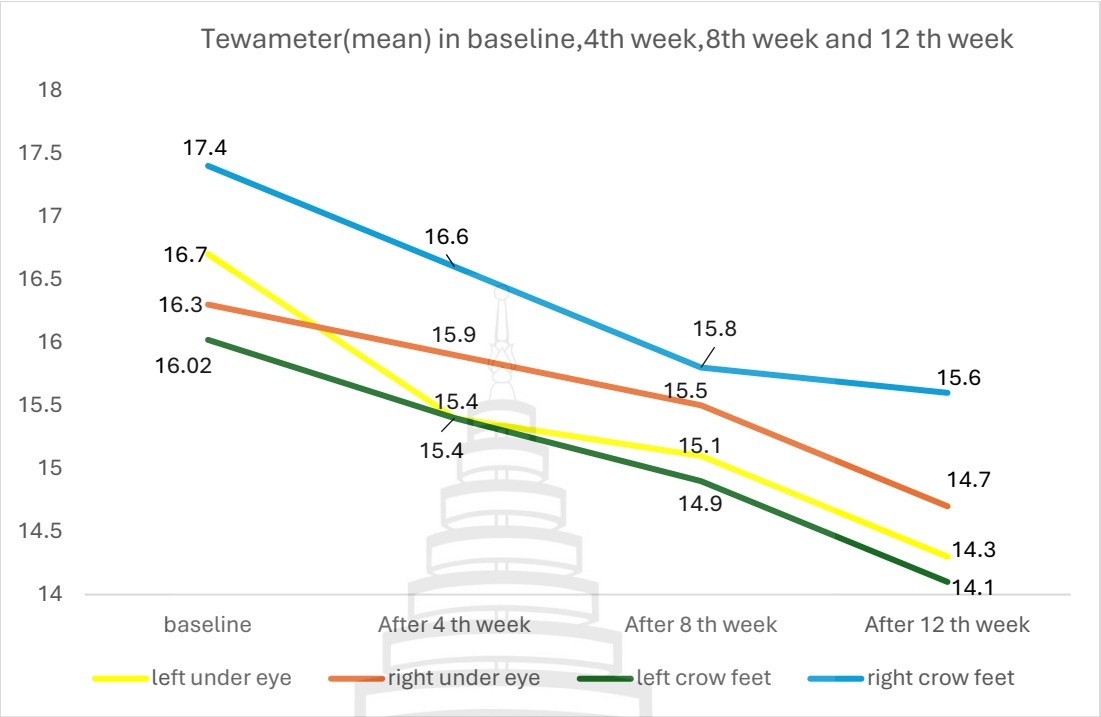


Figure E3 Displays line graph showing the tewameter score at under eye (left and right side), crow feet (left and right side) from baseline to the follow-up 12th week

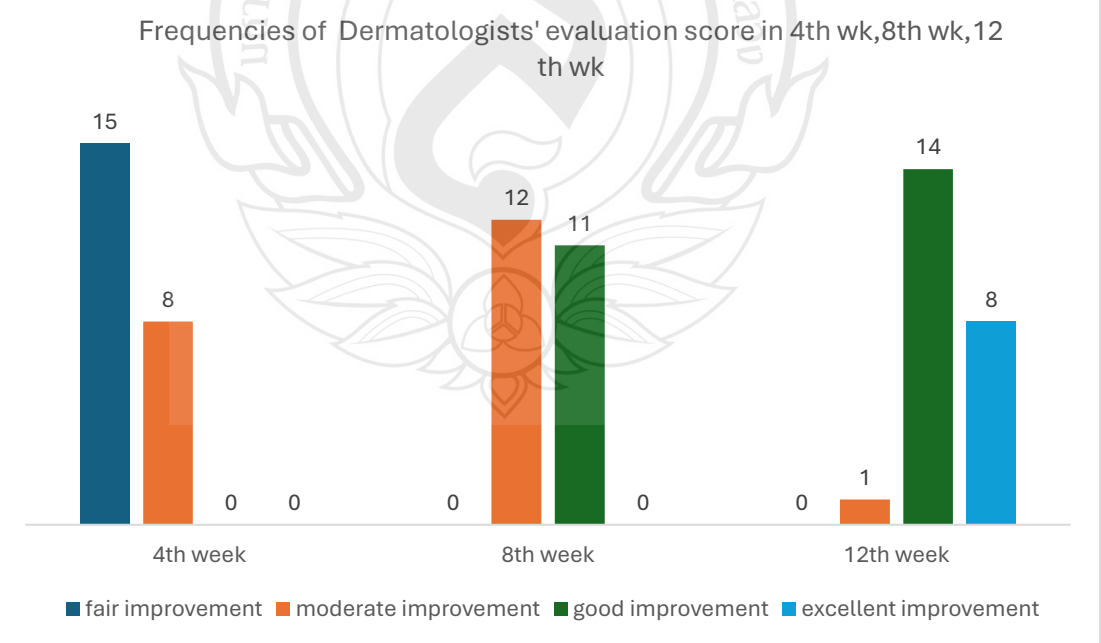


Figure E4 Displays a bar graph showing frequency of Dermatologists' evaluation score from the follow up 4th to 12th week

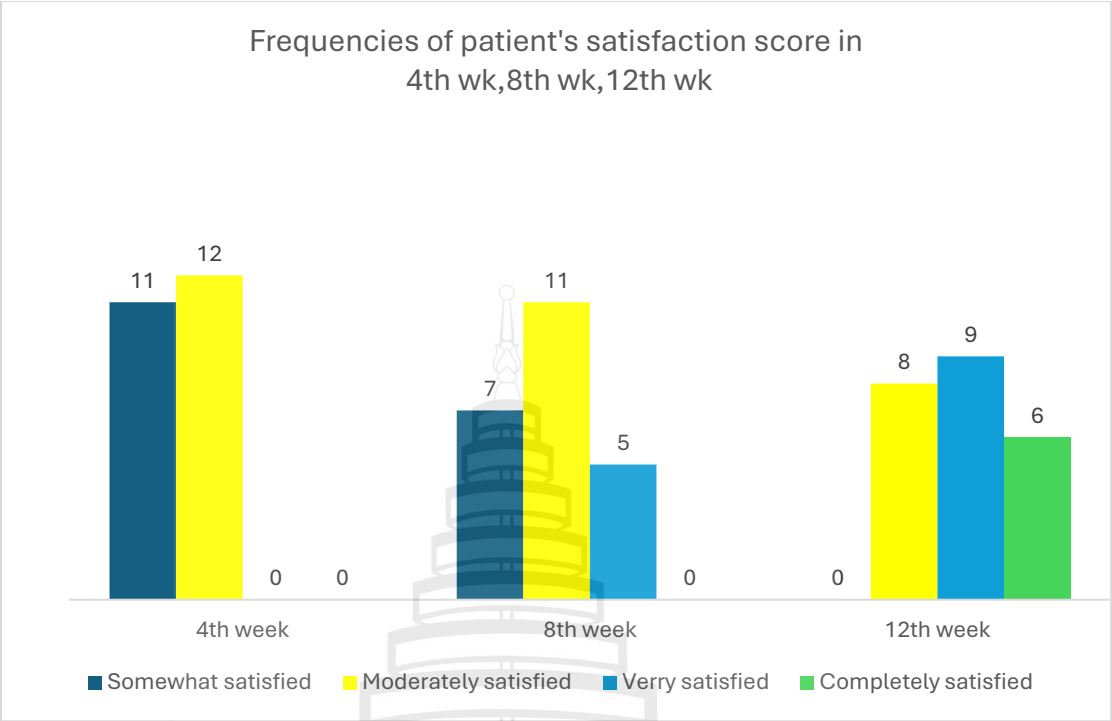


Figure E5 Displays a bar graph showing the frequencies of patients' satisfaction score

RESEARCH RECORD: RESEARCHER'S PART

CASE RECORD FORM (CRF)

1. Cutometer® MP 580

Cutometer	**No**	**Right**	**Left**
Week 0	1		
	2		
	3		
	Total		
Week 4	1		
	2		
	3		
	Total		
Week 8	1		
	2		
	3		
	Total		
Week 12	1		
	2		
	3		
	Total		

2. Tewameter

Tewameter Scores						
	Baseline			12^{th} week		
PARAMETER	Left	Right	Mean	Left	Right	Mean
TEWL (Transepidermal water loss)						

3. Facial Assessment Wrinkle Score by Dermatologists

Side	Baseline	Week 4	Week 8	Week 12
Left				
Right				

-1= worse

0= no changes

1= 1-25% fair improvement

2= >25-50% moderate improvement

3= >50-75% Good improvement

4= >75-100% excellent improvement

4. Treatment Satisfaction Score

Satisfactory evaluation by Volunteers (Please draw the circle on Week 12)

-1	0	1	2	3	4

Score -1= Unsatisfied

Score 0= Indifferent

Score 1= Somewhat satisfied

Score 2= Moderately satisfied

Score 3= Verry satisfied

Score 4= Completely satisfied

5. Side Effect Record (for researcher evaluation)

Side effect in week (if any) ……in week……

Treatment…….

Result…….

Cutometer Score (At Baseline)

Volunteer No	Left (Undereye)	Left (Crow feet)	Right (Undereye)	Right (Crow feet)
PP01				
PP02				
PP03				
PP04				
PP05				
PP06				
PP07				
PP08				
PP09				
PP10				
PP11				
PP12				
PP13				
PP14				
PP15				
PP16				
PP17				
PP18				
PP19				
PP20				
PP21				
PP22				
PP23				

Cutometer Score (At 4th week)

Volunteer No	Left (Undereye)	Left (Crow feet)	Right (Undereye)	Right (Crow feet)
PP01				
PP02				
PP03				
PP04				
PP05				
PP06				
PP07				
PP08				
PP09				
PP10				
PP11				
PP12				
PP13				
PP14				
PP15				
PP16				
PP17				
PP18				
PP19				
PP20				
PP21				
PP22				
PP23				

Cutometer Score (At 8th week)

Volunteer No	Left (Undereye)	Left (Crow feet)	Right (Undereye)	Right (Crow feet)
PP01				
PP02				
PP03				
PP04				
PP05				
PP06				
PP07				
PP08				
PP09				
PP10				
PP11				
PP12				
PP13				
PP14				
PP15				
PP16				
PP17				
PP18				
PP19				
PP20				
PP21				
PP22				
PP23				

Cutometer Score (At 12th Week)

Volunteer No	Left (Undereye)	Left (Crow feet)	Right (Undereye)	Right (Crow feet)
PP01				
PP02				
PP03				
PP04				
PP05				
PP06				
PP07				
PP08				
PP09				
PP10				
PP11				
PP12				
PP13				
PP14				
PP15				
PP16				
PP17				
PP18				
PP19				
PP20				
PP21				
PP22				
PP23				

Tewameter Score (At baseline)

Volunteer No	Left (Undereye)	Left (Crow feet)	Right (Undereye)	Right (Crow feet)
PP01				
PP02				
PP03				
PP04				
PP05				
PP06				
PP07				
PP08				
PP09				
PP10				
PP11				
PP12				
PP13				
PP14				
PP15				
PP16				
PP17				
PP18				
PP19				
PP20				
PP21				
PP22				
PP23				

Tewameter Score (At 12th week)

Volunteer No	Left (Undereye)	Left (Crow feet)	Right (Undereye)	Right (Crow feet)
PP01				
PP02				
PP03				
PP04				
PP05				
PP06				
PP07				
PP08				
PP09				
PP10				
PP11				
PP12				
PP13				
PP14				
PP15				
PP16				
PP17				
PP18				
PP19				
PP20				
PP21				
PP22				
PP23				

Satisfaction Score (At 12th week)

Volunteer No	Score
PP01	
PP02	
PP03	
PP04	
PP05	
PP06	
PP07	
PP08	
PP09	
PP10	
PP11	
PP12	
PP13	
PP14	
PP15	
PP16	
PP17	
PP18	
PP19	
PP20	
PP21	
PP22	
PP23	

Score -1= Unsatisfied

Score 0= Indifferent

Score 1= Somewhat satisfied

Score 2= Moderately satisfied

Score 3= Verry satisfied

Score 4= Completely satisfied

Dermatologists' evaluation score for treatment with injectable Polydioxanone at 4th, 8th and 12th week

-1= Worse

0= No changes

1= 1-25% fair improvement

2= >25-50% moderate improvement

3= >50-75% Good improvement

4= >75-100% Excellent improvement

At 4th week

Volunteer No	Dr 1	Dr 2	Dr 3
PP01			
PP02			
PP03			
PP04			
PP05			
PP06			
PP07			
PP08			
PP09			
PP10			

Volunteer No	Dr 1	Dr 2	Dr 3
PP11			
PP12			
PP13			
PP14			
PP15			
PP16			
PP17			
PP18			
PP19			
PP20			
PP21			
PP22			
PP23			

At 8th week

Volunteer No	Dr 1	Dr 2	Dr 3
PP01			
PP02			
PP03			
PP04			
PP05			
PP06			
PP07			
PP08			
PP09			
PP10			

Volunteer No	Dr 1	Dr 2	Dr 3
PP11			
PP12			
PP13			
PP14			
PP15			
PP16			
PP17			
PP18			
PP19			
PP20			
PP21			
PP22			
PP23			

At 12th week

Volunteer No	Dr 1	Dr 2	Dr 3
PP01			
PP02			
PP03			
PP04			
PP05			
PP06			
PP07			
PP08			
PP09			
PP10			

Volunteer No	Dr 1	Dr 2	Dr 3
PP11			
PP12			
PP13			
PP14			
PP15			
PP16			
PP17			
PP18			
PP19			
PP20			
PP21			
PP22			
PP23			