THE EFFECTIVENESS OF PERIPHERAL BLOOD STEM CELL THERAPY ON CROW'S FEET WRINKLES

WORAMON DULLAYAPHAT

THIS THESIS IS A PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
ANTI-AGING AND REGENERATIVE MEDICINE

SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE
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ABSTRACT

Peripheral blood stem cell therapy (PBSC) has long been known as an effective treatment in various medical conditions. The effectiveness of peripheral blood stem cell therapy on crow's feet wrinkles is an application that is being explored in this thesis. The study is an open labeled study aiming to evaluate the efficacy and safety of PBSC in the treatment of crow's feet wrinkles.

In a 7 wk, 25 healthy volunteers, 21 females and 4 males were recruited. All subjects gave their written consent prior to participate in the study. Rao-Goldman 5 point visual scoring scale and VisioScan® VC 98 were used for evaluating crow's feet wrinkles at wk0, wk2, wk4, and wk6 of the study. On day 5th, PBSCs were harvested from 20 ml of the blood samples and subsequently injected in the lateral periorbital areas. The peripheral blood stem cell mobilization was done by Filgrastim (human granulocyte colony stimulating factor (Hu-G-CSF)), administration for 4 consecutive days.

The improvement in crow's feet wrinkles were demonstrated at wk2 and more improvement were observed at wk4 and wk6. Statistical differences ($p<0.001$) were shown by repeated measure ANOVA. The side effects were mild and included mild pain, redness and skin irritation at the sites of injection.

This study clearly demonstrated the effectiveness of PBSC in the treatment of crow's feet wrinkles. It is recommended that further study should be conducted in order to compare the effectiveness of this therapy with PRP and study the long term effect by following up the subjects at least two years.

Keywords: Peripheral Blood Stem Cell/Crow's Feet Wrinkles/Filgrastim (human Granulocyte Colony-stimulating Factor [Hu-G-CSF])

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CHAPTER 1

INTRODUCTION

1.1 Background and Rational of the Study

Facial rejuvenation is the cosmetic procedure or medical procedure used to increase or restore the appearance of younger and healthier looking skin. To be more precise, it aims to restore skin appearance of youth by using a combination of brow lift, elimination of eye bags, elimination of aging spots and decreasing wrinkles. In this thesis, crow's feet wrinkles are selected as the main indicator of facial rejuvenation.

There are various treatments available including chemical peels, microdermabrasion, topical cream applications, dermal fillers and injections of various substances and cosmetic surgery. Botox and collagen are most commonly used. These agents work to plum up wrinkles or immobilize muscles and prevent the appearance of wrinkles. There have been several reports on the adverse effects of most of these procedures (พรทิพย์ ภูวบัณฑิตสิน, 2551; Alster & Garg, 1996; Bailin, P. L. & Bailin, M. D., 1988; Carruthers, A. & Carruthers, J., 1997; Carruthers, A., Kiene & Carruthers, J., 1996; Coleman & Brody, 1997; Ditre, Griffin & Murphy, 1996; Draelos, 1997; Goh & Khool, 2002; Guerrissi & Sarkissian, 1997; Kligman, D & Kligman, A. M., 1998; Kligman, Grove, Hirose & Leyden, 1986; Leyden, Lavker, Grove & Kaidbey, 1995; Manaloto & Alster, 1999; Nelson, Fader, Gillard, Majmudar & Johnson, 1995; Papadavid & Katsambas, 2003; Tanzi & Alster, 2003; Weinstein, Nigra & Pochi, 1991; Weiss, Ellis & Headington, 1988). During the last decades cell-based therapies including platelet rich plasma (PRP), growth factors and cytokines have received much attention due to the fact that they are likely to provide longer lasting effects with minimal side effects (Eming, Krieg & Davisin, 2007; Fitzpatrick & Rostan, 2003; Gold, Goldman & Biron, 2007; Hussain, Phelps & Goldber, 2008; Kiristy & Lynch, 1993; McCartney-Francis, Mizel, Wong, Wahl, L. & Wahl, S., 1990; Mehta & Fitzpatrick, 2007; Meteo, Reichner & Albina, 1994; Moulin, 1995; Rappolee, Mark, Banda & Werb, 1988; Werner & Grose, 2003; Yaar & Gilchrest, 2008).

PRP has been widely used in facial rejuvenation and aesthetic medicine (Ebisawa et al., 2008; Kakudo, 2008; Rutkowski, 2008; Anderson & Baker, 2003; Marx, 2004; Foster, Mandelbaum, Gerhardt & Rodeo, 2009; Boyan, 2010). It is an extract of the patient's own blood that contains three to five times more platelets and numerous Platelet Derived Growth Factors (PDGF) (Bolta, 2007; Arora, Ren & Romanos, 2009). These growth factors stimulate proliferation of fibroblasts and keratinocytes, which produce collagen and keratin (Marx, Carlson & Eichstaedt, 1998; Kevy & Jacobson, 2001; Weibrich & Kleis, 2002; Don, Wayne, Morkel, Erick & Benedict, 2007; Foster et al., 2009; Redaelli, Romano & Marciano, 2010). PDGF have a significant role in forming new blood vessels, promoting the cellular division of

fibroblasts and the synthesis of collagen and extracellular matrix, including hyaluronic acid (Marx et al., 1998; Kevy & Jacobson, 2001; Weibrich & Kleis, 2002; Schmitz & Hollinger, 2001; Marx, 2001). Collagen production has been shown to decrease the wrinkles. This procedure is not a surgical face lift and helps restore youthful skin. Improvement of skin texture and tone can be noticeable within three weeks and continues to be improved for the next 8 months (Bailin, P.L. & Bailin, M.D., 1988). In this thesis, PBSC was selected as the innovative cell-based therapies to be evaluated for its efficacy and safety for the treatment of crow's feet wrinkles.

Peripheral blood stem cells (PBSCs) are hematopoietic stem cells that circulate in the blood. They have the plasticity potential to differentiate into osteoblasts, chondroblasts, adipocytes, skeletal muscles, skin cells, etc (Abkowitz, 2002; Zavan et al., 2010; Waese & Kandel, 2008; Oh & Choo, 2011). It is most likely that this type of stem cells can be effective in decreasing wrinkles as well. The PBSCs extraction is considered to be non-invasive. In addition, much higher number in the circulation can be achieved by administering certain cytokines such as granulocyte colony-stimulating factor (G-CSF) or when hematopoietic recovery occurs following chemotherapy (Sorapop Kiatpongsan, Yuen Tannirandorn. & Pramuan Virutamasen, 2006)

In this thesis, Filgrastim (Human granulocyte colony stimulating factor (Hu-G-CSF) was used to stimulate bone marrow production. The peripheral blood stem cells extracted were injected to crow's feet wrinkles of the healthy volunteers of both sexes in a systemic manner. The efficacy and safety of PBSCs in treatment of crow's feet wrinkles were evaluated comparing before and after results of the same subjects.

1.2 Reason for Conduction Human Subjects

1.2.1 Because crow's feet wrinkles appear in human

1.2.2 Safety used in human

1.2.2.1 Use of Filgrastim in stem cell mobilization has already been approved by the U.S. and Thai FDAs.

1.2.2.2 Subject medical status indicates Filgrastim use.

1.3 Research Objective

To study the effectiveness of peripheral blood stem cell therapy on crow's feet wrinkles.

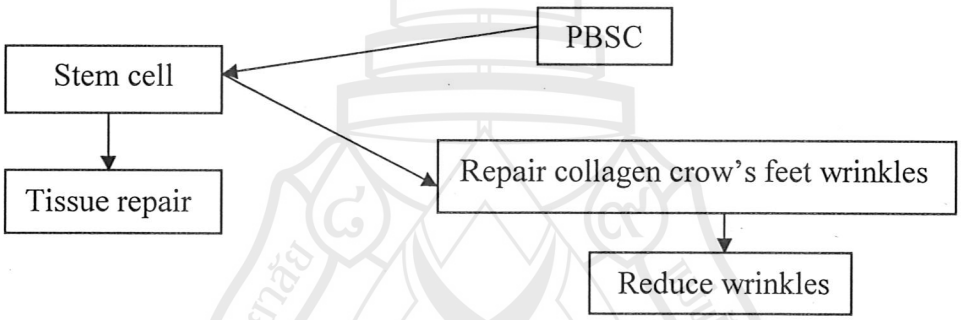
1.4 Research Question

Can peripheral blood stem cell therapy reduce crow's feet wrinkles?

1.5 Research Hypothesis

Peripheral blood stem cell therapy can reduce crow's feet wrinkles.

1.6 Conceptual Frame Work



Figures 1.1 Conceptual Frame Work

1.7 Scope of Research

This study is an open study to evaluate the efficacy and safety of PBSC for the treatment of crow's feet wrinkles. The 25 subjects were recruitment from healthy volunteer groups of both sexes with ages ranging between 35-60 years old, using inclusion and exclusion criteria. Written consent was given before participating in the study. PBSCs were isolated by centrifugation and injected to crow's feet wrinkles both eyes, all subjects in a systematic manner.

The crow's feet wrinkles were assessed by Rao-Goldman 5-point visual scoring scale and VisioScan® VC98 before and after treatment (wk0, wk2, wk4, wk6). Patient's satisfaction questionnaires were evaluated at the end of study.

CHAPTER 2

LITERATURE REVIEW

2.1 The Concept of Stem Cells and Their Role in Aging

2.1.1 The Concept of Stem Cells

The earliest stages of human cellular differentiation occur during the transition from of morula to blastocyst stage of preimplantation development, they give in two lineages, one is trophectoderm (TE), that become to syncytiotrophoblast and cytotrophoblast of the primitive endoderm and the placenta, the second is inner cell mass, that generates the embryo proper and extraembryonic tissues (Adjaye et al., 2005; Gardner & Beddington, 1988). The inner cell mass is the source of embryonic stem (ES) cells, that have ability to self-renew, go through numerous cycles of cell division and maintaining the undifferentiated state. ES cells are pluripotent, they can differentiate into cells representing for three primary germ layers ectoderm, mesoderm and endoderm (Thomson et al., 1998).Unlike ES cells, which can generate all cell types and are pluripotent, adult stem cells are more restricted, but still have other characteristics of ES cells such as long life, self-renewal, high proliferative potential and can give rise to several other cell types (multipotent) (Sharpless & Depinho, 2007).

2.1.2 Extrinsic and Intrinsic Signals

The self renewing stem cell might grow old as a result of extrinsic (external) factor eg. changes in their supporting niches and intrinsic (internal) factor eg. DNA damage. Extrinsic and intrinsic signals regulates the balance of self-renewal and differentiation in all stem cells (Moore & Lemischka, 2006).

2.1.3 The Role of Stem Cell in Aging

The decline of tissue regenerative potential is a hallmark of aging and may be due to age related changes in tissue-specific stem cells (Conboy, I. M., Conboy, M. J., Girma, Weissman, & Rando, 2005). Stem cell numbers and self-renewal capabilities do not necessarily decline with age, but their function eg. the ability to differentiated cells and produce progenitors cells declines with age (Sharpless & DePinho, 2007).

2.2 Aging Changes in Skin

Skin changes are among the most visible signs of aging eg. wrinkles, sagging skin, whitening or graying of hair. Benefit of skin are protects from the environment, control body temperature, fluid and electrolyte balance, contains nerve receptors to feel sensations such as pain, temp, touch and pressure. Skin divided into three main parts: 1. The outer part (epidermis) contains skin cells, pigment and proteins. 2. The middle part (dermis) contains nerves, blood vessels, oil glands and hair follicles. 3. The inner layer (subcutaneous) contains sweat glands, blood vessels, fat and some hair follicles. (Yaar & Gilchrest, 2008; พรทิพย์ ภูวบัณฑิตสิน, 2551)

Each layer contains connective tissue with collagen fibers to give support and elastin fibers to provide flexibility and strength. Skin changes are related to environmental factors, nutrition, genetic, other factors. The greatest single factor is sun exposure.

2.3 Causes of Aging Skin

There are two distinct types of aging. Aging caused by the genes is called intrinsic (internal) aging. The other type that caused by environmental factors such as exposure to the sun's rays is called extrinsic (external) aging. (Yaar & Gilchrest, 2008; Mehta & Fitzpatrick, 2007)

2.3.1 Intrinsic Aging

The natural aging process, is a continuous process that normally begins in mid 20yrs old. Within the skin, collagen production is slows, elastin and the substance that help skin to snap back into place, has a little bit less spring, dead skin cells do not peel as normal and turnover of new skin cells may decrease. The signs of intrinsic aging are:

2.3.1.1 Fine wrinkles
2.3.1.2 Dry skin
2.3.1.3 Thin and transparent skin
2.3.1.4 Bones shrink away from the skin due to bone loss, that causes sagging skin
2.3.1.5 Loss of underlying fat, leading to eye sockets, hollowed cheeks and loss of firmness on hands and neck
2.3.1.6 Inability to sweat sufficiently to cool the skin
2.3.1.7 Nail plate thins
2.3.1.8 Hair loss
2.3.1.9 Graying hair that eventually turns white
2.3.1.10 Unwanted hair

2.3.2 Extrinsic Aging

Extrinsic or external factors often act together with the normal aging process to premature aging skin. Most premature aging is caused by sun exposure. Other external factors are repetitive facial expressions, sleeping positions, smoking and gravity.

2.3.2.1 The Sun. Without protection from the sun's rays can cause skin changes eg. fine wrinkles, rough and leathery skin, loss of elasticity (elastosis), pigment changes such as liver spots freckles and age spots, a blotchy complexion, thickening of the skin, spider veins on face, actinic keratoses (thick wart-like, rough, reddish patches of skin) and skin cancer including basal cell epithelioma, squamous cell carcinoma and melanoma.

2.3.2.2 Facial Expressions. Repetitive facial movements actually lead to fine lines and wrinkles. Each time when use a facial muscle, a groove forms under the surface of the skin, the lines form with each facial expression. When skin ages and loses their elasticity, the skin stops springing back to its line-free state, and these grooves become permanently etched on the face as fine lines and wrinkles.

2.3.2.3 Sleeping Positions. Resting face on the pillow in the same way every night for years can leads to wrinkles (sleep lines), wrinkles become etched on the surface of the skin and no longer disappear when the head is not resting on the pillow.

2.3.2.4 Smoking. Cigarette smoking causes biochemical changes in the bodies that accelerate aging.

2.3.2.5 Gravity. Gravity constantly pulls on the bodies. Changes related to gravity become more pronounced with age. In people more than 50yrs old, the skin's elasticity declines and the effects of gravity become evident. Gravity causes the eyelids to fall, ears to elongate, tip of nose to droop, jowls to form and upper lip to disappear while lower lip becomes more pronounced.

2.4 Effect of Changes

2.4.1 With aging, the outer skin layer (epidermis) thins but the number of cell layers remains unchanged.

2.4.2 Number of pigment-containing cells (melanocytes) decreases but increase in size. Aging skin appears in clear (translucent), more pale and thinner. Large pigmented spots (age spots, liver spots or lentigo) may appear in sun-exposed areas.

2.4.3 Connective tissue change to reduce the skin's elasticity (elastosis), skin strength and is especially pronounced in sun-exposed areas (solar elastosis).

2.4.4 Blood vessels of dermis become more fragile leads to bleeding under the skin (senile purpura), bruising, cherry angiomas and similar conditions.

2.4.5 Sebaceous glands produce less oil, so skin is harder to keep moist resulting in dryness and itchiness.

2.4.6 Subcutaneous fat layer thins, decrease in normal insulation and padding, also reduces ability to maintain body temperature and increases risk of skin injury.

2.4.7 Sweat glands produce less sweat so it harder to keep cool and increased risk for becoming overheated or developing heat stroke.

2.4.8 Growths such as skin tags, warts, and other blemishes are more common in older people.

Aging skin increased risk for skin injury because skin is thinner, more fragile and protective subcutaneous fat layer is lost ability to sense pain, heat, cold, touch, pressure and vibration. Skin changes and loss of subcutaneous fat combined with a tendency to be less active, as well as some nutritional deficiencies and other illnesses contribute to pressure ulcers. Aging skin repairs itself slowly than younger skin. Wound healing may be up to 4 times slower also lead to pressure ulcers and infections. Lowered immunity, blood vessel changes, diabetes and similar factors also affect healing. (Yaar & Gilchrest, 2008; Mehta & Fitzpatrick, 2007)

2.5 Common Problems

More than 90% of all older people have some type of skin disorder. Skin disorders can be caused by many conditions including (1) Nutritional deficiencies (2) Stress (3) Heart disease (4) Blood vessel diseases eg. arterosclerosis (5) Liver disease (6) Diabetes (7) Obesity (8) Reactions to medications (9) Other problem: clothing, allergies to plants and other substances, indoor heating, exposures to industrial and household chemicals and Climate.

2.6 Wrinkles

A wrinkle is a furrow, fold, or crease in the skin, particularly of a type seen with increasing occurrence as a result of sun exposure, cigarette smoking; associated with degeneration of dermal elastic tissue. (Ian & Martin, 2006)

Wrinkles are an inevitable part of the natural aging process (Manaloto & Alster, 1999). When older the skin is drier, thinner, less elastic and reduced ability to protect itself from damage. Then skin has wrinkles, creases and lines. Most wrinkles tend to appear in the parts of the body which receive the most sun exposure including face, neck, tops of forearms and backs of hands.

2.6.1 Main Factors that Cause Wrinkles

2.6.1.1 Exposure to UV (ultraviolet light) people who spend more time in h sun during their leisure hours are more likely to develop wrinkles earlier.

2.6.1.2 Facial expressions-people who repeatedly smile, squint or frown will develop fine lines and wrinkles earlier than other who do not do these facial expressions so often. Each time when use a facial muscle a groove forms under the surface of the skin. When younger the skin springs back, but as it gets older and loses

its flexibility springing back becomes harder and less frequent, resulting in more permanent grooves

2.6.1.3 Smoking due to reduced blood supply to the skin.

2.6.1.4 Jobs-people who work to more sunlight tend to become wrinkly earlier than others eg. farmers, sailors, golfers, fishermen, gardeners and tanning booth employees (UV exposure).

2.6.1.5 Light skin trend to have a higher level of sun damage.

2.6.1.6 Clothes.

2.6.1.7 Hair style.

2.6.1.8 Genetic factors.

2.6.2 The Treatment Options for Wrinkles

Getting rid of wrinkles or taking steps to postpone their development are nearly always optional measures, because wrinkles are not usually considered as medical conditions or diseases, they are not generally life-threatening or harmful to physical health. There are many treatment option for wrinkles.

2.6.2.1 Medications

1. Topical retinoids (Kligman, D & Kligman, A. M., 1998; Leyden et al., 1995; Weinstein et al., 1991; Weiss et al., 1988) help reduce fine wrinkles, skin roughness and some pigmentations. Topical renitoids are derived from vitamin A, it can make the skin more sensitive to burning from sunlight exposure so people needs to use it with sunscreen hat or cloth to protect the skin. Topical retinoids may sometimes cause dryness, redness, burning sensation, itching, and tingling sensation.

2. OTC (over the counter, no prescription required) wrinkle creams - the effectiveness of OTC wrinkle creams varies considerably and depends on what's the active ingredient. Results may be obtained from retinol, alpha hydroxy acids (AHAs), coenzyme Q10, antioxidants, kinetin and copper peptides.

2.6.2.2 Surgery and other medical procedures

1. Face lift (Bailin, P. L. & Bailin, M. D., 1988; Papadavid & Katsambas, 2003) is known as a rhytidectomy, a type of cosmetic surgery aimed at giving a more youthful appearance. A rhytidectomy usually involves removal of excess facial skin and fat, with or without tightening of underlying tissues. The skin of the patient's face and/or neck is redraped. Healing times may be lengthy and the patient will experience bruising and swelling for a few weeks after surgery.

2. Dermabrasion (Nelson et al., 1995) is a surgical procedure involving the controlled wearing away (abrasion) of the upper layers of skin with sandpaper or some other mechanical. Dermabrasion is used for the removal of fine wrinkles, scars, nevi (moles) and tattoos. Results are not immediate and may take several months. There will be some scabbing, swelling and redness, which generally go away after a couple of weeks. The pinkness may take several months to fade.

3. Microdermabrasion (Nelson et al., 1995) a powerful vacuum is used to spray microcrystals of aluminum oxide across the surface of the skin. The procedure removes the outermost layers of skin cells, part of the stratum corneum and stimulating cell growth in the underlying dermis. Microdermabrasion is help to give a smoother appearance and fresher to the skin, diminishing the appearance of wrinkles, lines, coarse skin,enlarged pores and sun damage. Patient may have a little redness in the areas of treated skin. Repeated treatments are required because results are temporary.

4. Laser, light source and radiofrequency (Goh & Khool, 2002; Tanzi & Alster, 2003) in wounding (ablative) laser resurfacing, the epidermis is destroyed by a laser beam, while the dermis is heated up, it's stimulates the development of new collagen fibers. When the wound heals, new smoother and tighter skin is formed. Ablative laser resurfacing can take several months to heal up. Non-ablative laser, pulsed light sources and radiofrequency devices do not damage the epidermis, the dermis is heated, triggering the formation of new collagen and elastin. The skin feels firmer and appears refreshed after several treatments, resulting in faster recovery times. Patient mat have redness, burn, post inflammatory pigmentation.

5. Chemical peel (Nelson et al., 1995; Kligman, D. & Kligman, A.M., 1998) a chemical solution is applied to wrinkly areas, causing dead skin to slough off and peel off. The regenerated skin is generally smoother and less wrinkled than the old skin.

6. Botox (Botulinum Toxin Type A) (Carruthers, A. & Carruthers, J., 1997; Guerrissi & Sarkissian, 1997) blocks the chemical signals that cause muscles to contract. It is injected in small doses into targeted muscles. If the muscles can no longer tighten the skin flattens, giving a less wrinkled and smoother appearance. Botox is effective for lines between the eyes (frown lines), on the forehead and crow's feet. As treatments generally last about three to four months, repeated injections are required. Patient may have bruise, ptosis.

7. Soft tissue fillers include collagen, hyaluronic acid or fat (Bailin, P. L. & Bailin, M. D., 1988). They are injected into deeper face wrinkles, plumping and smoothing them out, giving the skin more volume. Patient may have swelling, redness and bruising in the treated areas for a short period. Good results are depend on several factors, how their depth and where the wrinkles.

There is no current treatment that can stop the ageing process.

2.6.3 Preventing Wrinkles and Lines

There is nothing can do to stop the aging process, eventually everybody will have wrinkles and lines, which become more prominent over time but there are many steps to slow the process include

2.6.3.1 The skin and sunlight the more skin is exposed to the sun the faster it will age. Protecting skin by sun screens, wearing hats and protective clothing will reduce sunlight damage and consequent developing of wrinkles.

2.6.3.2 Moisturizers not prevent the wrinkling process but may help temporarily make tiny lines and creases less evident.

2.6.3.3 Sleep people who get at least 7 hours good quality sleep each day will over the long-term enjoy better physical and mental health, as well as protecting the quality of their skin.

2.6.3.4 Smoking accelerates the aging of skin.

2.7 Platelet Rich Plasma (PRP)

Platelet rich plasma (PRP) has been used clinically since 1970s. Recent advances in methods for PRP preparation and use have made it possible for surgeons to take advantage of it's concentrated form of growth factors and cytokines that are naturally present in blood clots. (Ebisawa et al., 2008; Kakudo, 2008; Rutkowski, 2008; Anderson & Baker, 2003)

Platelet-rich plasma (PRP) is blood plasma that has been enriched with platelets. As a concentrated source of autologous platelets so it's limiting the potential for disease transmission. The composition of PRP varies from patient to patient and may vary with the device used to prepare it, the method and time of storage until it is used, and whether it is used or interacts with other biologics or materials. PRP contains (and releases through degranulation) several different growth factors and other cytokines that stimulate healing of bone and soft tissue.

Effective use of PRP is when an injury occurs, platelets release factors within the clot that stimulate the recruitment of mesenchymal cells to the site and promote their proliferation. These factors also reduce inflammation and stimulate angiogenesis.

The efficacy of certain growth factors in healing various injuries and the concentrations of these growth factors found within PRP are the theoretical basis for the use of PRP in tissue repair. The platelets collected in PRP are activated by the addition of thrombin and calcium chloride, which induces the release of these factors from alpha granules. The growth factors and other cytokines present in PRP include

1. epithelial growth factor (EGF)
2. platelet-derived growth factor (PDGF αα)
3. platelet-derived growth factor (PDGF αβ)
4. platelet-derived growth factor (PDGF ββ)
5. fibroblast growth factor acidic (aFGF or FGF-1)
6. fibroblast growth factor basic (bFGF or FGF-2)
7. vascular endothelial growth factor (VEGF)
8. insulin-like growth factor 1 (IGF-1)
9. insulin-like growth factor 2 (IGF-2)
10. transforming growth factor beta (TGF- β1)
11. transforming growth factor beta (TGF- β2)
12. connective tissue growth factor (CTGF)
13. interleukin-1β (IL-1β)
14. Interleukin-8 (IL-8)
15. keratinocyte growth factor (KGF or FGF-7)
16. granulocyte/macrophage colony-stimulating factor (GM-CSF or CSF)
17. tumor necrosis factor alpha (TNFα)

Another component in PRP are fibrin, fibronectin and vitronectin, protein in blood for cell adhesion molecules for connective tissue, osteoconduction, matrix for bone and epithelial migration

TGF-ß1, IGF-1, and PDGF stimulate proliferation of mesenchymal cells; TGF-ß1 in particular stimulates extracellular matrix production, including collagen. These factors are important for stabilizing tissue during the initial phases of tissue repair, but they can also lead to fibrous connective tissue and scar formation. VEGF and FGF-2 are

important for stimulating new blood vessel formation to bring nutrients and progenitor cells to the site. Additional factors are needed for stabilization of the neovasculature.

2.7.1 Preparation

There are, at present, two methods of PRP preparation approved by the U.S. Food and Drug Administration. Both processes involve the collection of whole blood that is anticoagulate with citrate dextrose before undergoing two stages of centrifugation designed to separate the PRP aliquot from platelet-poor plasma and red blood cells. In humans, the typical baseline blood platelet count is approximately 200,000 per μL; therapeutic PRP concentrates the platelets by roughly five-fold. There is however broad variability in the production of PRP by various concentrating equipment and techniques.

2.7.2 Clinical Applications

In humans, PRP has been investigated and used as clinical tool for several types of medical treatments, including nerve injury, tendinitis, cardiac muscle injury, bone repair and regeneration, plastic surgery, oral surgery and aesthetic. PRP has also received attention in the popular media as a result of its use in treating sports injuries in professional athletes.

2.8 Peripheral Blood Stem Cells (PBSCs)

PBSCs are hematopoietic stem cells (HSCs) that are circulating in the blood. HSCs circulate at low levels in the blood all the time but can increase in number when a person with certain cytokines (substances that stimulate the bone marrow) such as granulocyte colony-stimulating factor (G-CSF) or when hematopoietic recovery occurs following chemotherapy. PBSC transplants tend to engraft faster than bone marrow transplants because they have a greater proportion of mature progenitors. Current clinical applications of stem cells/progenitor CD34+ cells consist of both allogenic (in bone marrow aplasias, storage diseases, hematopoietic malignancies, immunodeficiencies, hemoglobinopathies) and autologous (in acute leukemias, lymphomas, myelomas, germ cell tumors, breast and ovarian cancer, autoimmune diseases) transplantation. Another are in the process of replacing and revolutionizing treatments in heart infarcts, heart insufficiency, diabetic and atherosclerotic ulcers and their increase is exponential. They have the plasticity potential to differentiate into osteoblasts, chondroblasts, adipocytes, skeletal muscles, skin cells, etc. (Waese & Kandel, 2008). Suitable sources of human non embryonic hematopoietic stem cells are umbilical cord blood, bone marrow and mobilized peripheral blood. (Abkowitz, 2002; Zavan et al., 2010; Waese & Kandel, 2008; Oh & Choo, 2011)

2.9 Filgrastim

Filgrastim is a human granulocyte colony-stimulating factor (Hu-G-CSF), produced by recombinant DNA technology. Filgrastim has been selected as the name for recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF). Filgrastim is a 175 amino acid protein, produced by *Escherichia coli* (*E coli*) bacteria into which has been inserted the human granulocyte colony-stimulating factor gene. Filgrastim has a molecular weight of 18,800 daltons. The protein has an amino acid sequence that is identical to the natural sequence predicted from human DNA sequence analysis, except for the addition of an N-terminal methionine necessary for expression in *E coli*. (Lacy, Armstrong, Goldman & Lance, 2006)

2.9.1 Clinical Pharmacology

Colony-stimulating factors are glycoproteins which act on hematopoietic cells by binding to specific cell surface receptors and stimulating proliferation, differentiation commitment, and some end-cell functional activation. Endogenous G-CSF is a lineage specific colony-stimulating factor which is produced by monocytes, endothelial cells and fibroblasts. G-CSF regulates the production of neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens). G-CSF is not species specific and has been shown to have minimal direct in vivo or in vitro effects on the production of hematopoietic cell types other than the neutrophil lineage.

2.9.2 Indication and Usage

2.9.2.1 Patients undergoing peripheral blood progenitor cell collection and therapy

Filgrastim is indicated for the mobilization of hematopoietic progenitor cells into the peripheral blood for collection by leukapheresis. Mobilization allows for the collection of increased numbers of progenitor cells capable of engraftment compared with collection by leukapheresis without mobilization or bone marrow harvest. After myeloablative chemotherapy, the transplantation of an increased number of progenitor cells can lead to more rapid engraftment, the result in a decreased need for supportive care.

2.9.2.2 Cancer patients receiving myelosuppressive chemotherapy

2.9.2.3 Patients with acute myeloid leukemia receiving induction or consolidation chemotherapy

2.9.2.4 Cancer patients receiving bone marrow transplant

2.9.2.5 Patients with severe chronic neutropenia

2.9.3 Dosage and Administration

For patients undergoing peripheral blood progenitor cell collection and therapy. Dose of filgrastim for the mobilization of PBPC is 10 μg/kg/day sc, either as a bolus or a continuous infusion, given for at least 4 days before the first leukapheresis procedure and

continued until the last leukapheresis. Neutrophil counts should be monitored after 4 days of Filgrastim, and Filgrastim dose modification should be considered for those patients who develop a WBC count > 100,000/mm3.In all clinical trials of Filgrastim for the mobilization of PBPC, Filgrastim was also administered after reinfusion of the collected cells.

Filgrastim should be stored at 2° to 8°C (36° to 46°F) and avoid shaking.

2.9.4 Contraindications

Filgrastim is contraindicated in patients with known hypersensitivity to E coli-derived proteins, Filgrastim or any component of the product.

2.9.5 Precaution

Allergic reactions: allergic-type reactions occurring on initial or subsequent treatment have been reported in < 1 in 4000 patients treated with Filgrastim., most often skin (rash, urticaria, facial edema), respiratory (wheezing, dyspnea), and cardiovascular (hypotension, tachycardia). Some reactions occurred on initial exposure, within the first 30 minutes after administration and appeared to occur more frequently in patients receiving intravenous.

2.9.6 Adverse Reactions

Patient is well tolerated with Filgrastim, 44% musculoskeletal pain, 7% headache.

2.9.7 VisioScan® VC 98

VisioScan® VC 98 is a special UV-A light video camera with high resolution has been developed especially to study the skin surface directly. The images show the structure of the skin and the level of dryness very impressively, and also can be used to study degree of wrinkling.

In contrary to the conventional color video cameras, the VisioScan® VC 98 is shown in figure (1), consists of a special between video sensor chip with very high resolution, an objective and an UVA-light source in a small, easy to handle, plastic casing (Khazaka, 2005). Two special halogenide lights, arranged on opposite sides, illuminate the skin uniformly. The spectrum of the light, its intensity and the way it is arranged is chosen so that only the stratum corneum without reflections from deeper layers is monitored. This special light excludes almost all undesired light reflections on the skin, thus bringing out a very sharp, non glossy image of the skin and hair. The measuring area is 6*8 mm. the image of the skin is taken by a build in CCD-camera. The connection of the VisioScan® VC 98 to the PC is done via an image digitalization unit which configures the image in 256 gray levels pixel by pixel, where 0 is black and 255 is white. The special Surface Evaluation of Living Skin (SELS)-program offers a lot of possibilities and calculations of surface parameters which has been utilized by many previous researches on aging skin (Alain, 2005)

In this thesis, use few measurements of the skin surface that can be calculated by VisioScan® VC 98, and related to the objectives of this study. These measurements include: SELS parameters, the SELS calculations consist of following parameters: Roughness "Ser" calculates the portion of dark pixels. The smaller this value, the less rough the skin, Smoothness "SEsm" is proportional to width and form of the wrinkles.

A treatment with moisturizing or anti-aging products should let SEsm go up, and lastly Wrinkles, "Sew" is proportional to number and width of the wrinkles. The more wrinkles, the higher this value.

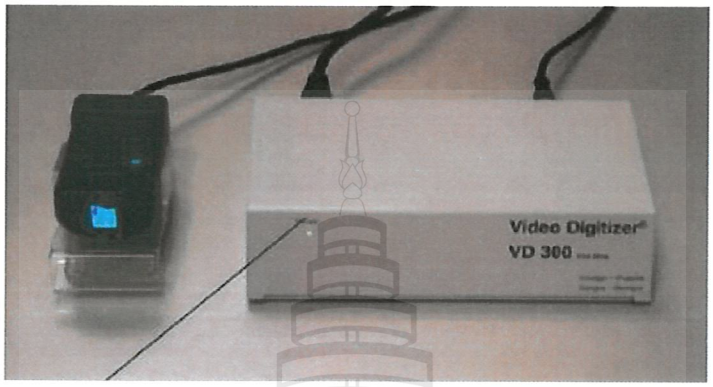


Figure 4.1 VisioScan® VC 98

2.10 Rao-Goldman 5-Point Visual Scoring Scale

2.10.1 Wrinkle absent
2.10.2 Shallow but visible
2.10.3 Moderately deep
2.10.4 Deep with well-defined edges
2.10.5 Very deep with redundant folds

CHAPTER 3

METHODOLOGY

3.1 Population

The subjects were healthy volunteers of both sexes between 35-60 years of age, whose CBC, liver function test (AST, ALT) and kidney function test (BUN, Cr) were of normal values. They had to give their written consent before participating in the study.

3.2 Sample Size Calculation

Sample size was defined by the following formula

$$n_0 = \frac{Z_{\infty}^2 PQ}{d^2}$$

Set confident interval at 95% α = 0.05
(P = 0.3, Q = 0.7, PQ = 0.21)
d = 20%
n = 15 subjects

Set allowance for 30% drop out rate: Sample size was 20 subjects.

3.3 Research Design

Prospective, Clinical Trial.
Measurement: wrinkles score.

3.4 Sampling

Volunteers were randomly recruited from normal working people in Bangkok. After volunteers were clearly explained the purpose of the study, they were checked for inclusion and exclusion criteria and then signed an informed consent.

3.4.1 Inclusion Criteria

3.4.1.1 Male or female

3.4.1.2 Ages 35-60 years old

3.4.1.3 Normal CBC, Liver function test (AST, ALT) and kidney function test (Bun, Cr)

3.4.2 Exclusion Criteria

3.4.2.1 Known allergy to E coli-derived proteins, Filgrastim or any component of the product

3.4.2.2 Periorbital skin lesions or active infection

3.4.2.3 Personal or family history of skin disease

3.4.2.4 Known systemic disease that can potentially have cutaneous manifestation

3.4.2.5 History of chronic systemic illness or immunocompromisation

3.4.2.6 Malignancy

3.4.2.7 Pregnant or breast feeding

3.4.2.8 History of botox or filler in the last 6 months

3.4.2.9 Ablative or nonablative laser treatment in the last 6 months

3.4.2.10 Actively undergoing any anti-wrinkling treatments

3.5 Materials

3.5.1 Filgrastim (Hu-G-CSF)

3.5.2 Lidocaine 9.6% cream

3.5.3 Syringe 1mL, 3mL, 25mL

3.5.4 Needle no. 20, 30

3.5.5 Butterfly 21G needle

3.5.6 70% Alcohol

3.5.7 EDTA tube for CBC

3.5.8 Clot blood tube for BUN, Cr, AST, ALT

3.6 Research Tools

3.6.1 Tube for Medifriger
3.6.2 Medifriger (J.P SELECTA CENTRIFUGES)
3.6.3 VisioScan® VC 98
3.6.4 Rao Goldman 5 point visual scoring scale
3.6.5 Patient's satisfaction questionnaires
3.6.6 Doctor record form
3.6.7 Informed consent form
3.6.8 Patient record form

3.7 Research Methodology

3.7.1 Volunteers recruited into research using inclusion and exclusion criteria
3.7.2 Explained purpose and procedure to research volunteers
3.7.3 Signed informed consent and patient record form
3.7.4 Used Rao Goldman 5 point visual scoring scale for evaluating crow's feet wrinkles both lateral periorbital areas
3.7.5 Used VisioScan® VC 98 for evaluating crow's feet wrinkles both lateral periorbital areas measuring from lateral canthus horizontally outward for 1.5cm, three times and then average
3.7.6 Injected Filgrastim 10 μg/kg/day subcutaneous at the abdominal area for 4 consecutive days
3.7.7 On the 5th day 30 ml of blood were drawn from right median cubital vein, 10ml of blood were collected for laboratory evaluation including CBC, liver and kidney function tests, then 20 ml of blood were divide equally into two tubes. At the same time, applied lidocaine 9.6% cream on periorital area both eyes.
3.7.8 The blood samples collected were centrifused at a speed of 1000 RPM, 5 min for separate blood components. The red blood cells were move to the bottom of the tube, the plasma fraction was float as the top layer and the buffy coat which contains the majority of platelets was between the plasma and above the red blood cells.
3.7.9 Aspirated plasma and buffy coat from two tubes. Mixed it then divide equally into two tubes, centrifused at a speed of 3000 RPM, 10 min. Throw away the upper plasma, used the white bottom residual
3.7.10 Three points injection of 0.1 ml PBSC id at both eyes, the 1st 1.5 cm from the lateral cantus, the 2nd and 3rd were given 1cm above and below the first.
3.7.11 Volunteers were followed up at the 2nd, 4th and 6thweek using Rao Goldman 5 point visual scoring scale and VisioScan® VC98 to evaluate crow's feet wrinkles at each visit. Side effects and complications were also assessed. At the end of 6thweek, volunteers were required to complete a Patient's satisfaction questionnaires

3.8 Evaluation Protocol

3.8.1 All volunteers were evaluated by the same study physician

3.8.2 All before and after data collect used the same piece of equipment and same study physician

3.8.3 CBC, liver and kidney function tests were done on all subjects before and day 5th of study

3.9 Result Evaluation

1. Measurement of both crow's feet wrinkles by Rao Goldman 5 point visual scoring scale at every visit
2. Measurement of both crow's feet wrinkles by VisioScan® VC98 at every visit
3. Measurement of side effect and complication at every visit
4. Patient's satisfaction questionnaires at 6^{th} week

3.9.1 Criteria to Withdraw from the Study

3.9.1.1 Volunteers had the right to withdraw from the study at any time

3.9.1.2 Volunteers failed to complete the schedule follow up

3.9.1.3 Volunteers couldn't tolerate the medication or procedure

3.9.2 Data Collection

3.9.2.1 General and Personal data of volunteers

3.9.2.2 Crow's feet wrinkles score from Rao Goldman 5-point visual scoring scale

3.9.2.3 Crow's feet wrinkles score from VisioScan® VC98

3.9.2.4 Side effect and complication

3.9.2.5 Laboratory test evaluation

3.9.2.6 Patient's satisfaction questionnaires

3.10 Statistics Used for Data Analysis

Wilcoxon signed ranks test used to compare before and after treatment data collect due to Rao Goldman 5 point visual scoring scale and for patient's satisfaction questionnaires. Repeated measure ANOVA used for VisioScan® VC98. Compare Laboratory test before and after injection of GCSF used paired t-test and Wilcoxon signed rank test.

CHAPTER 4

RESULTS

4.1 General Data

Twenty five volunteers were recruited in this study. They comprised of 4 males and 21 females, having the average ages of 47±9.6 yr (min 35 yr, max 58 yr old) in male and 44.2±7.1 yr (min 35 yr, max 56 yr old) in female.

Table 4.1 General Data

	Male (4)		Female (21)		Total (25)	
	n	%	n	%	n	%
Age group						
35-39	1	25.0	7	33.3	8	32.0
40-44	-		4	19.0	4	16.0
45-49	1	25.0	4	19.0	5	20.0
50-54	1	25.0	4	19.0	5	20.0
55-60	1	25.0	2	9.5	3	12.0
Mean±SD (min-max)	47±9.6(35-58)		44.2±7.1(35-56)		44.6±7.4(35-58)	

4.2 Personal Data

In this study, there were 2 volunteers (8%) who had the history of drug allergy (1 aspirin and 1 amoxicillin), 1 volunteer (4%) had food allergy (shrimp), 4 volunteers (16%) had underlying diseases (1 thalassemia carrier, 1 hypothyroid (treated) and 2 allergy. Two volunteers (8%) used medications (1 for peptic ulcer and 1 for lowering cholesterol). Another two volunteers (8%) were smoking (5 and 10 cigarettes per day). Two volunteers (8%) had genetic disease (DM).

No subjects were pregnant or on breast feeding. There were no subjects suffering from skin diseases or had the family history of serious skin diseases. Summary of personal data are show in table 2.

Table 4.2 Personal Data

Data	n	%
Drug allergy		
No	23	92.0
Yes	2[a]	8.0
Food allergy		
No	24	96.0
Yes	1[b]	4.0
Underlying disease		
No	21	84.0
Yes	4[c]	16.0
Medication use		
No	23	92.0
Yes	2[d]	8.0
Smoking		
No	23	92.0
Yes	2[e]	8.0
Pregnancy/breast feeding (female only)		
No	21	100.0
Yes	-	
Family history of skin disease		
No	25	100.0
Yes	-	
Genetic disease		
No	23	92.0
Yes	2[f]	8.0

Note. a = 1 aspirin and 1 amoxicillin, b = shrimp, c = 1 thalassemia carrier, 1 thyroid (treat) and 2 allergy, d = 1 peptic ulcer and 1 lowering cholesterol, e = 5 and 10 cigarettes per day, f = DM

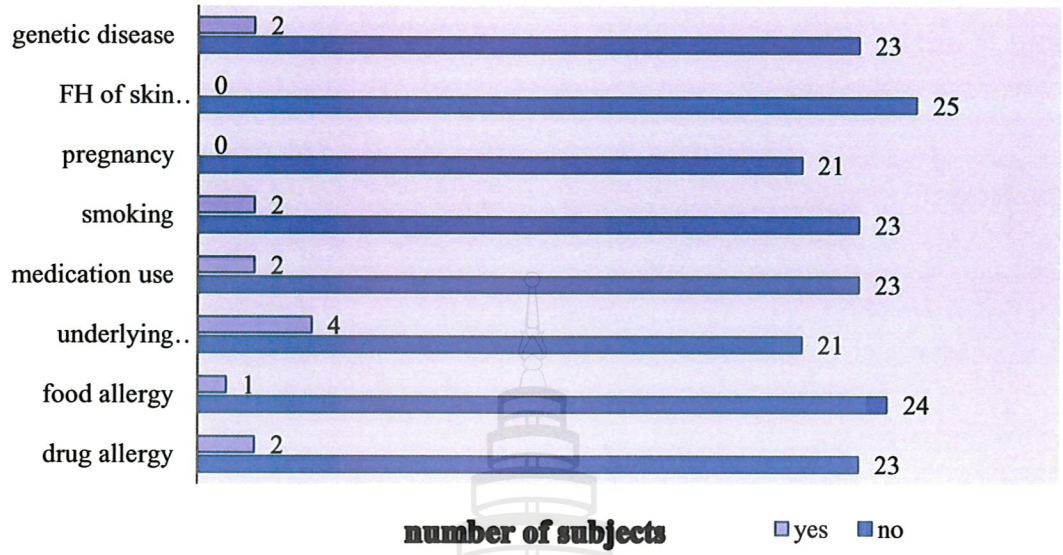


Figure 4.1 Personal Data

4.3 Factor Affecting Crow's Feet Wrinkles

From 25 volunteers, there were 4 volunteers (16%) who had the history of previous exposure to ultraviolet light (mean 3.8±1 hr/day, range 3 to 5 hr/day), There were 21 volunteers (84%) regularly avoiding direct sun exposure by wearing hat, using umbrella, etc.. Twenty two volunteers (88%) used sun screen (SPF mean 49.8±13, range 30-100); while 22 volunteers (88%) used moisturizers. Seventeen volunteers (68%) usually frowned frequently or did certain facial expression. There were 18 volunteers (72%) reporting to have good quality of sleep (mean 7.4±1.4 hr/day, range 5 to 12 hr/day).

Table 4.3 Factor Affecting Crow's Feet Wrinkles

Factors	n	%
1. Exposure to ultraviolet light		
No	21	84.0
Yes	4	16.0
Hour mean±SD (min-max)	3.8±1.0(3-5)	
2. Prevent from ultraviolet light		
No	4	16.0
Yes	21	84.0

Table 4.3 (Continue)

Factors	n	%
3. Sun screen		
No	3	12.0
Yes	22	88.0
SPF mean±SD (min-max)	49.8±13.0(30-100)	
4. Moisturizer		
No	3	12.0
Yes	22	88.0
5. Facial expression		
No	8	32.0
Yes	17	68.0
6. Good quality of sleep		
No	7	28.0
Yes	18	72.0
Hour mean±SD (min-max)	7.4±1.4(5-12)	

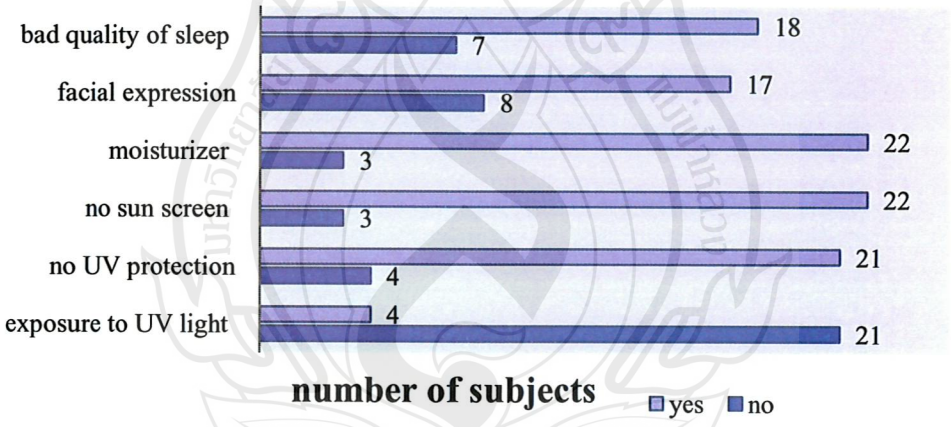


Figure 4.2 Factor Affecting Crow's Feet Wrinkles

4.4 Crow's Feet Wrinkle Treatment Data

From 25 volunteers, there were 21 volunteers (84%) who used over the counter wrinkles treatment cream, 13 volunteers (52%) received botox injection, more than 6 months up to two years prior to the study. Three volunteers (12%) underwent laser therapy more than 6 month ago. Three volunteers (12%) received filler injection, more than 1 year before entering into the study. Three volunteers (12%) had facial

surgery more than 2 years ago. All subjects did not receive any active crow's feet wrinkles treatment, periorbital ablation. They were also free of any infections.

Table 4.4 Crow's Feet Wrinkles Treatment Data

Treatment	n	%
1. Over the counter wrinkles treatment cream		
No	4	16.0
Yes	21	84.0
2. Botox		
No	12	48.0
Yes	13	52.0
last botox use		
> 6 mo	6	46.2
> 1 yr	5	38.5
> 2 yrs	2	15.4
3. Laser		
No	22	88.0
Yes	3	12.0
last laser use		
> 6 mo	3	100.0
4. Filler		
No	22	88.0
Yes	3	12.0
last filler use		
> 1 yr	2	66.7
> 2 yrs	1	33.3
5. Facial surgery		
No	22	88.0
Yes	3	12.0
last facial surgery		
> 2 yrs	3	100.0
6. Active crow's feet wrinkles treatment		
No	25	100.0
Yes	-	
7. Periorbital ablation or/and infection		
No	25	100.0
Yes	-	

4.5 RAO Goldman 5 Point Visual Scoring Scale

Both right and left crow's feet wrinkles were not significantly improved at wk2. The increase in degree of moderately deep and shallow but visible were in wk4 and wk6.

Table 4.5 RAO Goldman 5 Point Visual Scoring Scale

	Wrinkle absent	**Shallow**	**Mod**	**Deep**	**Very deep**
	n(%)	**n(%)**	**n(%)**	**n(%)**	**n(%)**
Right crow's feet					
wk 0	-	2(8.0)	11(44.0)	12(48.0)	-
wk 2	-	2(8.0)	11(44.0)	12(48.0)	-
wk 4	-	4(16.0)	18(72.0)	3(12.0)	-
wk 6	-	7(28.0)	16(64.0)	2(8.0)	-
Left crow's feet					
wk 0	-	2(8.0)	11(44.0)	12(48.0)	-
wk 2	-	2(8.0)	11(44.0)	12(48.0)	-
wk 4	-	3(12.0)	16(64.0)	6(24.0)	-
wk 6	-	6(24.0)	18(72.0)	1(4.0)	-

Note. mod = moderately

Statistic analyses by McNemar test indicated that right crow's feet wrinkles were significantly improved of changed between wko-wk4, wk0-wk6, wk2-wk4 and wk2-wk6, p-value = 0.004, 0.001, 0.004 and 0.001 consecutively. Left crow's feet wrinkles were significantly improved of changed between wko-wk4, wk0-wk6, wk2-wk4, wk2-wk6 and wk4-wk6, p-value = 0.03, 0.001, 0.03, 0.001 and 0.018 consecutively.

Table 4.6 Change of RAO Goldman 5 Point Visual Scoring Scale between wk0 and wk2, Right Eye

week 0	week 2			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	2(8.0)	-	-	-
Moderately	-	11(44.0)	-	
Deep	-	-	12(48.0)	

Note. McNemar test

Table 4.7 Change of RAO Goldman 5 Point Visual Scoring Scale between wk0 and wk4, Right Eye

week 0	week 4			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	2(8.0)	-	-	*
Moderately	2(8.0)	9(36.0)	-	
Deep	-	9(36.0)	3(12.0)	

Note. McNemar test, * p-value=0.004

Table 4.8 Change of RAO Goldman 5 Point Visual Scoring Scale between wk0 and wk6, Right Eye

week 0	week 6			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	2(8.0)	-	-	*
Moderately	5(20.0)	6(24.0)	-	
Deep	-	10(40.0)	2(8.0)	

Note. McNemar test, * p-value=0.001

Table 4.9 Change of RAO Goldman 5 Point Visual Scoring Scale between wk2 and wk4, Right Eye

week 2	week 4			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	2(8.0)	-	-	*
Moderately	2(8.0)	9(36.0)	-	
Deep	-	9(36.0)	3(12.0)	

Note. McNemar test, * p-value=0.004

Table 4.10 Change of RAO Goldman 5 Point Visual Scoring Scale between wk2 and wk6, Right Eye

week 2	week 6			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	2(8.0)	-	-	*
Moderately	5(20.0)	6(24.0)	-	
Deep	-	10(40.0)	2(8.0)	

Note. McNemar test, * p-value=0.001

Table 4.11 Change of RAO Goldman 5 Point Visual Scoring Scale between wk4 and wk6, Right Eye

week 4	week 6			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	4(16.0)	-	-	-
Moderately	3(12.0)	15(60.0)	-	
Deep	-	1(4.0)	2(8.0)	

Note. McNemar test

Table 4.12 Change of RAO Goldman 5 Point Visual Scoring Scale between wk0 and wk2, Left Eye

week 0	week 2			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	2(8.0)	-	-	-
Moderately	-	11(44.0)	-	
Deep	-	-	12(48.0)	

Note. McNemar test

Table 4.13 Change of RAO Goldman 5 Point Visual Scoring Scale between wk0 and wk4, Left Eye

week 0	week 4			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	2(8.0)	-	-	*
Moderately	1(4.0)	10(40.0)	-	
Deep	-	6(24.0)	6(24.0)	

Note. McNemar test, * p-value=0.03

Table 4.14 Change of RAO Goldman 5 Point Visual Scoring Scale between wk0 and wk6, Left Eye

week 0	week 6			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	2(8.0)	-	-	*
Moderately	4(16.0)	7(28.0)	-	
Deep	-	11(44.0)	1(4.0)	

Note. McNemar test, * p-value=0.001

Table 4.15 Change of RAO Goldman 5 Point Visual Scoring Scale between wk2 and wk4, Left Eye

week 2	week 4			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	2(8.0)	-	-	*
Moderately	1(4.0)	10(40.0)	-	
Deep	-	6(24.0)	6(24.0)	

Note. McNemar test, * p-value=0.003

Table 4.16 Change of RAO Goldman 5 Point Visual Scoring Scale between wk2 and wk6, Left Eye

week 2	week 6			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	2(8.0)	-	-	*
Moderately	4(16.0)	7(28.0)	-	
Deep	-	11(44.0)	1(4.0)	

Note. McNemar test, * p-value=0.001

Table 4.17 Change of RAO Goldman 5 Point Visual Scoring Scale between wk4 and wk6, Left Eye

week 4	week 6			p-value
	Shallow n(%)	Moderately n(%)	Deep n(%)	
Shallow	3(12.0)	-	-	*
Moderately	3(12.0)	13(52.0)	-	
Deep	-	5(20.0)	1(4.0)	

Note. McNemar test, * p-value=0.018

4.6 Compared RAO Goldman 5 Point Visual Scoring Scale at Each Time

Statistic analyses by Wilcoxon signed ranks test comparing Rao goldman 5 point visual scoring scale with wk 0 data indicated significant decrease at wk 4 and 6. Both right and left crow's feet wrinkles in wk0 and wk2 means ± SD were 3.4±0.6, p-value=1. Right crow's feet wrinkles in wk4 and wk6 means ± SD were 3.0±0.5 and 2.8±0.6, p-value=0.001 and <0.001 consecutively. Left crow's feet wrinkles in wk4 and wk6 means ± SD were 3.1±0.6 and 2.8±0.5, p-value=0.008 and <0.001 respectively.

Table 4.18 Compared RAO Goldman 5 Point Visual Scoring Scale at Each Time

	Mean±SD	**p-value**
Right crow's feet		
wk 0	3.4±0.6	
wk 2	3.4±0.6	
wk 4	3.0±0.5	*
wk 6	2.8±0.6	**
Left crow's feet		
wk 0	3.4±0.6	
wk 2	3.4±0.6	
wk 4	3.1±0.6	***
wk 6	2.8±0.5	**

Note. 1. Wilcoxon Signed Ranks Test compared with wk0
2. * p-value = 0.001, ** p-value < 0.001, *** p-value = 0.008

The results from RAO Goldman 5 point visual scoring scale indicated that there were significant decrease in crow's feet wrinkles in both eyes at wk4 and wk6, p-value <0.05.

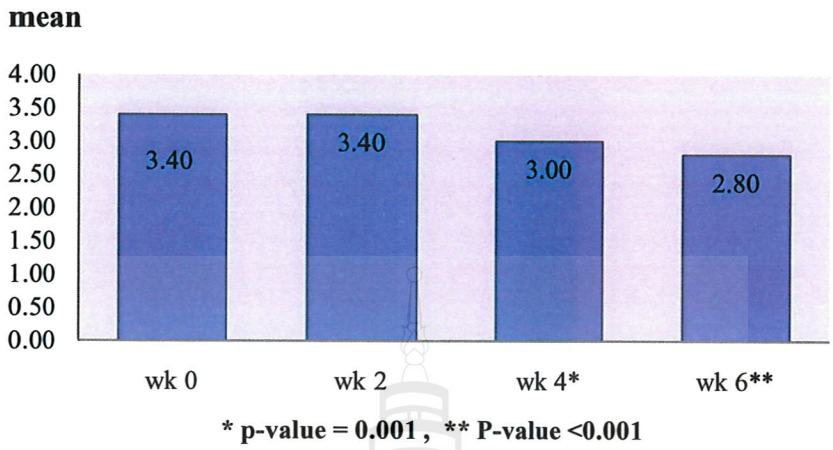


Figure 4.3 Compared RAO Goldman 5 Point Visual Scoring Scale at Each Time (Right)

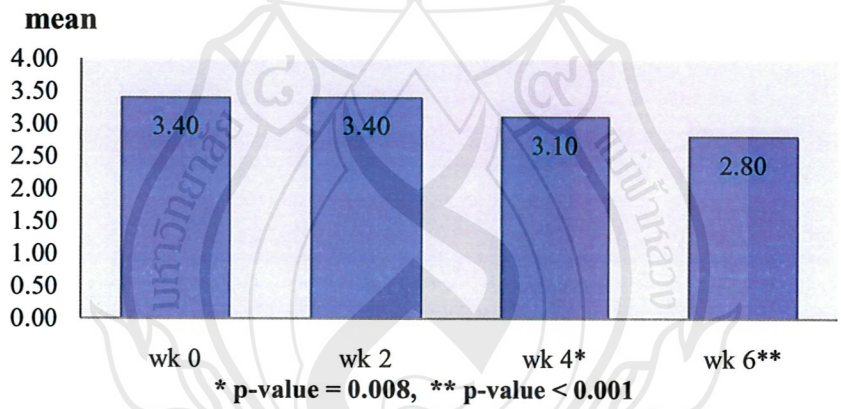


Figure 4.4 Compared RAO Goldman 5 Point Visual Scoring Scale at Each Time (Left)

4.7 VisioScan® VC98 Evaluation

Statistic analysis by paired t-test show significant improvement both right and left crow's feet wrinkles in every follow up (wk2, wk4, wk6). p-value <0.001.

Table 4.19 VisioScan® VC98 Evaluation, Right Crow's Feet Wrinkles

week	Mean±SD	week	Mean±SD	t	df	p-value
wk0	38.4±5.4	wk2	35.8±5.5	10.009	24	*
		wk4	32.1±4.9	14.686	24	*
		wk6	29.3±4.9	12.708	24	*
wk2	35.8±5.5	wk4	32.1±4.9	9.752	24	*
		wk6	29.3±4.9	9.000	24	*
wk4	32.1±4.9	wk6	29.3±4.9	4.050	24	*

Note. Paired t-test, *p-value < 0.001

Table 4.20 VisioScan® VC98 Evaluation, Left Crow's Feet Wrinkles

week	Mean±SD	week	Mean±SD	t	df	p-value
wk0	38.9±5.3	wk2	36.0±5.1	8.580	24	*
		wk4	32.0±4.7	14.458	24	*
		wk6	29.3±4.7	14.214	24	*
wk2	36.0±5.1	wk4	32.0±4.7	9.185	24	*
		wk6	29.3±4.7	10.551	24	*
wk4	32.0±4.7	wk6	29.3±4.7	5.836	24	*

Note. Paired t-test, *p-value < 0.001

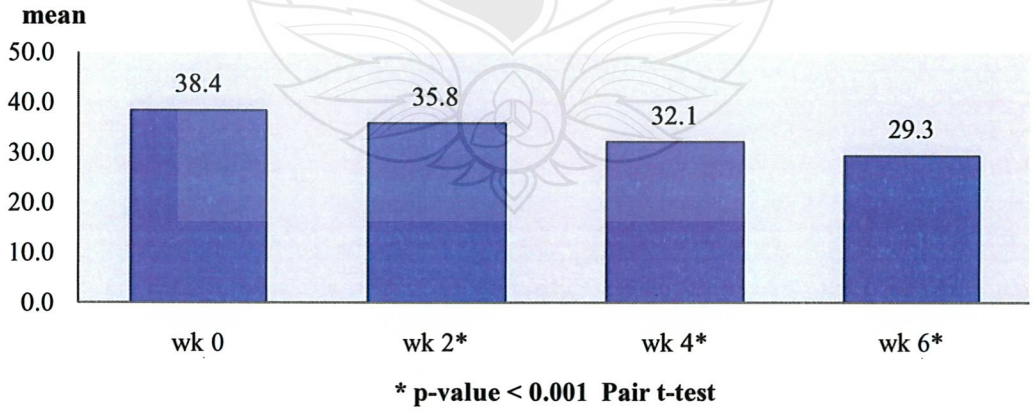


Figure 4.5 Right Crow's Feet Wrinkles (VisioScan® VC98 Evaluation)

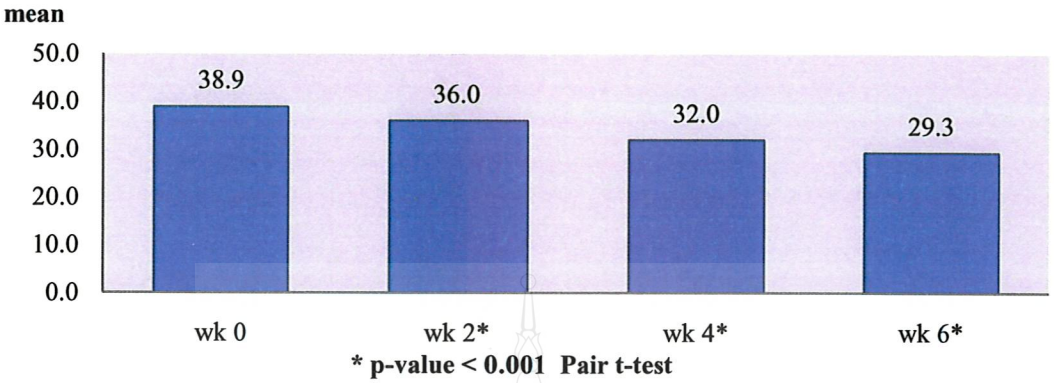


Figure 4.6 Left Crow's Feet Wrinkles (VisioScan® VC98 Evaluation)

4.8 Side Effect and Complication

Statistic analysis by wilcoxon signed ranks test revealed that only 11 volunteers had side effects and complications in right crow's feet wrinkles and 10 volunteers had side effects and complications in left crow's feet wrinkles. All of the adverse events were found at wk0 (right at the end of the procedure). Side effects and complications included pin point bleeding found in 2 volunteers (8%) in right crow's feet wrinkles, redness was found in 5 volunteers (20%) and 6 volunteers (24%) in right and left crow's feet wrinkles. Three volunteers (12%) were noticed to have slight edema in both crow's feet wrinkles. Ecchymosis was found in 1 volunteer (4%) in both crow's feet wrinkles. Redness was most commonly observed.

There were no significant differences observed between the adverse events observed in right and left crow's feet wrinkles, p-value = 1.0.

Table 4.21 Side Effect and Complication

Side effect and complication	Right crow's feet				Left crow's feet			
	wk0	wk2	wk4	wk6	wk0	wk2	wk4	wk6
0	14(56.0)	-	-	-	15(60.0)	-	-	-
1	2(8.0)	-	-	-	-	-	-	-
2	5(20.0)	-	-	-	6(24.0)	-	-	-
3	3(12.0)	-	-	-	3(12.0)	-	-	-
4	1(4.0)	-	-	-	1(4.0)	-	-	-

Note. 1. p-value = 1.000 (Wilcoxon Signed Ranks test)
2. 0 = no, 1 = pin point bleeding, 2 = redness, 3 = edema, 4 = ecchymosis

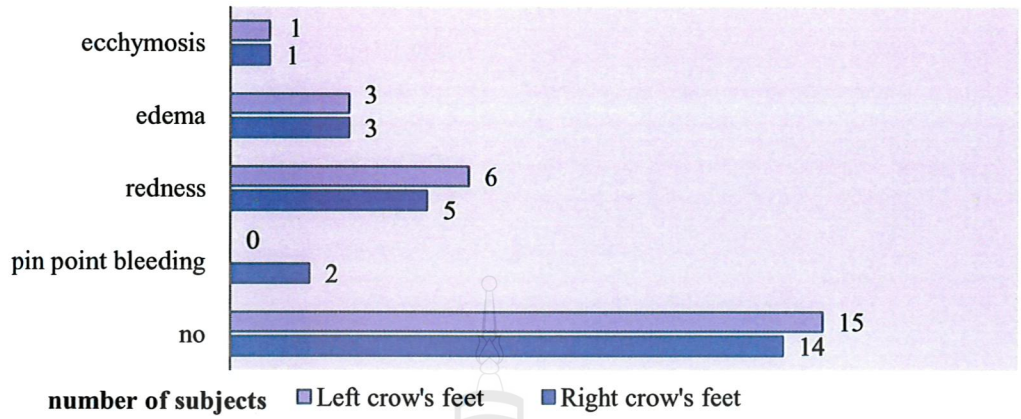


Figure 4.7 Side Effect and Complication in wk0 (Right at the End of the Procedure)

4.9 Laboratory Test Evaluation

There were significant increase in WBC, creatinine, AST but significant decrease in platelet in laboratory data as determined by paired t-test and Wilcoxon signed rank test.

Table 4.22 Laboratory Test Evaluation

	Day 0		Day 5		p-value
	Mean±SD	Median(MinMax)	Mean±SD	Median (Min-Max)	
WBC	5957.4 ±1673.5	5600 (1070-8650)	36952.4 ±13658.3	37560 (4580-59750)	*
Plt	274400 ±58182.2	271000 (185000-463000)	256040 ±49236.2	255000 (186000-390000)	**
Cr	0.8±0.2	0.8(0.52-1.1)	0.9±0.1	0.9(0.6-1.2)	***
AST	21.2±6.0	20(12-39)	26.3±15.8	22(15-95)	****
BUN	10.8±3.1	10.5(6.2-18.4)	9.8±3.1	8.9(5.8-19.2)	
Hct	37.6±4.1	37.1(26.3-45.9)	37.4±3.0	37(31.4-43.2)	
ALT	19.2±10.0	15(10-45)	22.6±24.8	17(10-128)	

Note. 1. Paired t-test, Wilcoxon Signed Ranks test
2. *p-value <0.001, ** p-value = 0.031, *** p-value = 0.019, **** p-value = 0.001
3. Day 0 = before inject Filgrastim, Day 5 = after inject Filgrastim for 4 consecutive days
4. WBC = white blood cell, Hct = hematocrit, Plt = platelet, BUN = blood urea nitrogen, Cr = creatinine, AST = aspartate transaminase, ALT = Alanine aminotransferase
5. Normal range: WBC = 4000-11000, Hct = 35-45, Plt = 150000-400000, BUN = 7.9-20.1,
6. Cr = 0.6-1.1, AST = 0-31, ALT = 0-34

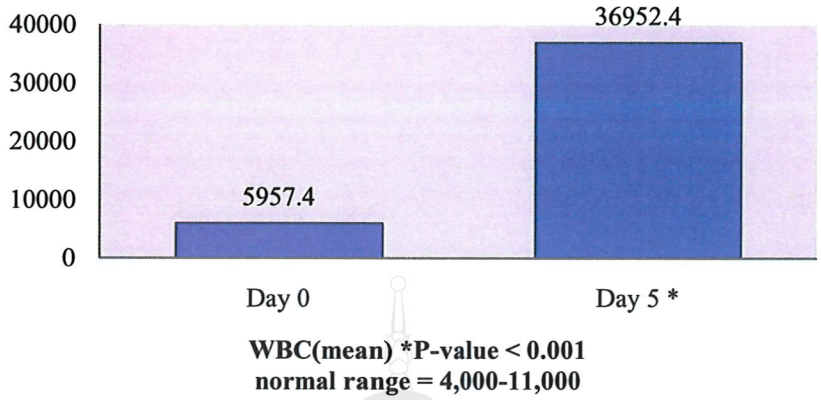


Figure 4.8 Laboratory Test Evaluation (WBC)

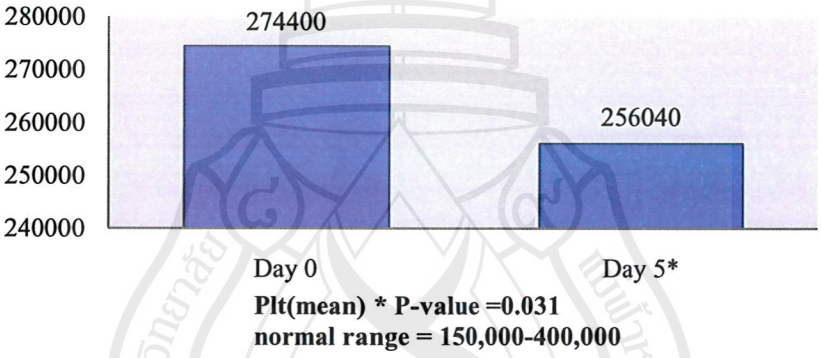


Figure 4.9 Laboratory Test Evaluation (Plt)

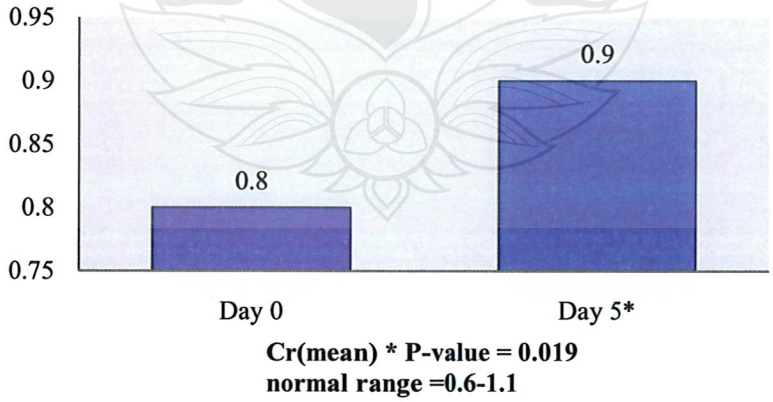


Figure 4.10 Laboratory Test Evaluation (Cr)

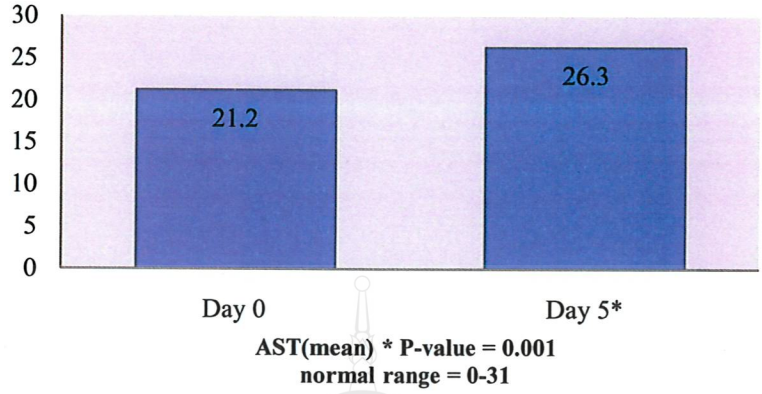


Figure 4.11 Laboratory Test Evaluation (AST)

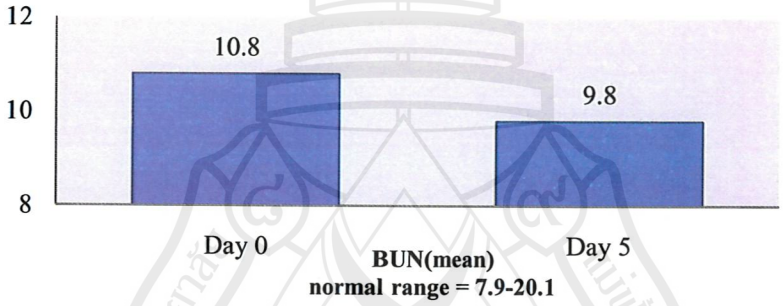


Figure 4.12 Laboratory Test Evaluation (BUN)

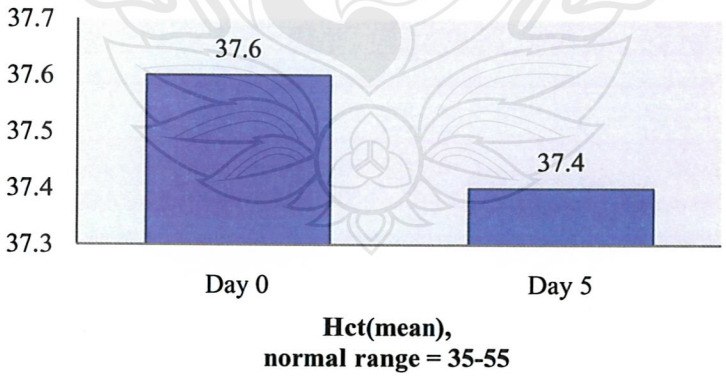


Figure 4.13 Laboratory Test Evaluation (Hct)

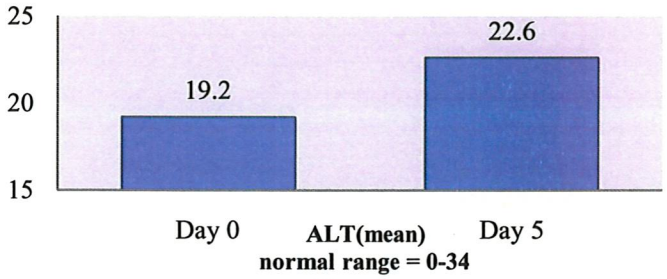


Figure 4.14 Laboratory Test Evaluation (ALT)

4.10 Patient's Satisfaction Questionnaires

Statistic analysis by Wilcoxon signed ranks test of changes in right crow's feet wrinkles showed that there were 11 volunteers (44%), 11 volunteers (44%) and 3 volunteers (12%) who had very good, good and moderate satisfaction consecutively, having mean ± SD of 2.3±0.7 (moderately less to significantly less), % improve =76.66% . In left crow's feet wrinkles, there were 10 volunteers (40%), 10 volunteers (40%), 4 volunteers (16%) and 1 volunteer (4%) who had very good, good, moderate and unchanged satisfaction consecutively, having means ± SD of 2.2±0.9 (moderately less to significantly less), % improve =73.33%. There were no significant differences in right and left crow's feet wrinkles, p-value=0.102.

Table 4.23 Patient's Satisfaction Questionnaires

	worst = -1 n(%)	unchanged = 0 n(%)	moderate = 1 n(%)	good = 2 n(%)	very good = 3 n(%)
Right crow's feet	-	-	3(12.0)	11(44.0)	11(44.0)
Left crow's feet	-	1(4.0)	4(16.0)	10(40.0)	10(40.0)

Note. Wilcoxon Signed Ranks Test

Table 4.24 Percent Improve of Patient's Satisfaction Questionnaires

	Mean±SD	% improve
Right crow's feet	2.3±0.7	76.66%
Left crow's feet	2.2±0.9	73.33%

Note. p-value 0.102

CHAPTER 5

DISCUSSION, CONCLUSION AND SUGGESTIONS

Fetal mesenchymal stem cells from amniotic fluid have similar characteristics and differentiation capacity to the pluripotent bone marrow derived mesenchymal stem cells (You et al., 2012). They had excellent wound healing effect in the skin fibroblast, excellent wound closure and skin regeneration effects in the wounded mouse skin, another benefit in clinical study show excellent effects of improving whitening, skin damages, skin regeneration, skin lifting and wrinkles (You et al., 2012). PBSCs have similar characteristics and differentiation capacity to the pluripotent bone marrow derived mesenchymal stem cells too, so they could improve crow's feet wrinkles.

The results of this study clearly indicated that PBSCs were effective in decreasing crow's feet wrinkles. All parameters measured revealed similar results. The anti wrinkle effects took place starting from 2 weeks with more improvement observed at 6 weeks. All subjects reported to have satisfaction with the treatments received.

No serious adverse effects were observed in all tested subjects. The redness and signs of skin irritation noted were judged to be due to injection reactions. Due to the autologous nature of the treatment, allergic reactions were unlikely to occur. The increase in white blood cell counts (WBCs) on day 5^{th} of the study was expected to be induced by GCSF administration. In fact, the increase in granulocytes was correlated with stem cell mobilization. The level of WBCs were known to come down to baseline levels within 4 day after stopping GCSF administration. The decline in platelet counts observed in some patients were also associated with filgastrim injection. However, there should not be any clinical significance regarding the slight decline in platelets. Other changes in laboratory data such as creatinine and AST were also of no clinical significance, even though the changes were shown to be statistically significant.

There seemed not to be significant response among the subjects of different age and sex. However, the number of subjects were too small to be able to accurately assess any differences between age and sex parameters. The improvement in skin texture and wrinkle appearance found in this study was definitely due to the effects of PBSC therapy. However, the mechanisms explaining how stem cells can lead to skin tissue regeneration were still unclear.

Peripheral blood stem cell mobilization was the procedure utilized in this study in order to reassure that sufficient levels of stem cells could be obtained. Filgastrim (Hu-G-CSF) has been accepted as the main agent currently available aiming to increase the number of stem cells in the blood circulation. This drug has been long employed to increase WBC counts in neutropenic patients. The colony stimulating factors are glycoproteins that specifically interact with the hematopoietic cell by binding to specific cell surface receptors and stimulating proliferation, differentiation and activation of some cell lines like some neutrophil granulocytes (Lacy et al., 2006)

Filgrastim administration resulted in a dose-dependent increase in circulation neutrophil counts over the dose range of 1 to 70 μg/kg/day. This increase in neutrophil counts was observed whether filgrastim was administered intravenously or subcutaneous (by daily treatment or continuous infusion). With discontinuation of filgrastim therapy, neutrophil counts returned to baseline, in most cases within four days.

The use of Filgastrim in healthy individuals to promote stem cell mobilization has not been well documented in the scientific literature. The safety profile of this agent was demonstrated in this study. No subjects had any serious adverse effects. The only side effect reported by the subjects was mild pain. This adverse event did not require any pain medications. All subjects had the average WBC counts of 36,952 cells/cu.mm, which indicated that adequate stem cell mobilization had been achieved.

The specific mechanisms of action involved in stem cell therapy are suggested to be due to two main principles (Jackson & Majka, 2001; Kawada & Fujita, 2004)

1. Stem cells reach the site of injury via local or systemic administration (homing). These cells can then integrate into the site of injury, replacing damaged tissues, and facilitated improved function of the organs or tissues (engraftment).

2. Stem cells have the capacity to release soluble factors such as cytokines, chemokines, and growth factors which act in a paracrine or endocrine manner (paracrine effects). These factors facilitate self-healing of the organ or tissue. They may also secrete factors which facilitate angiogenesis, anti-inflammation, and anti-apoptosis.

The PBSCs can exert a positive effect on skin tissue observed in this study by involving the paracrine effect, forming more blood vessels and tissue regeneration. However, the latter effect can be more significantly evident in skin tissue having tissue injury. Therefore, any damage induced by injection or surgical intervention can promote the skin regeneration more efficiently. In Korea, dermatologists and plastic surgeons prefer to use invasive procedures to cause tissue damage before stem cell administration.

In this study, facial rejuvenation with PBSCs is shown to be a promising, easy-to-use technique and can favorably improve all small skin wrinkles, as well as in skin texture and elasticity. There should be further study to compare the efficacy and safety between PRP and PBSC in facial rejuvenation. In addition, long term follow up should also be performed in order to determine the long lasting effect of PBSC.

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APPENDICES

APPENDIX A

INFORMATION SHEET

เอกสารคำอธิบายโครงการวิจัยแก่ผู้เข้าร่วมโครงการ (Information Sheet)

เรียน ผู้เข้าร่วมวิจัยทุกท่าน

ท่านเป็นผู้ได้รับเชิญจากแพทย์ให้เข้าร่วมการศึกษาวิจัยประสิทธิผลของการใช้เซลล์ต้นกำเนิดจากเม็ดโลหิต ในการรักษาริ้วรอยบริเวณหางตา ก่อนที่ท่านตกลงเข้าร่วมการศึกษาดังกล่าว ขอเรียนให้ท่านทราบถึงเหตุผลและรายละเอียด ของการศึกษาวิจัยครั้งนี้

ชื่อโครงการ: ประสิทธิผลของการใช้เซลล์ต้นกำเนิดจากเม็ดโลหิต ในการรักษาริ้วรอยบริเวณหางตา

ผู้รับผิดชอบโครงการวิจัย

แพทย์หญิง วรมน ดุลยพัชร์
โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพมหานคร
38/11-13 อโศกเพลส สุขุมวิท 21 ถ.อโศกมนตรี เขตวัฒนา คลองเตย กรุงเทพ 10110
เบอร์ที่ทำงาน : 02-6642295
เบอร์มือถือ : 081-637-9499

วัตถุประสงค์การวิจัย

เพื่อศึกษาผลของการรักษาริ้วรอยบริเวณหางตา โดยใช้เซลล์ต้นกำเนิดจากเม็ดโลหิต

วิธีการวิจัย

ฉีดเซลล์ต้นกำเนิดจากเม็ดโลหิต ที่บริเวณหางตาทั้งสองข้าง

ความเป็นมาของโครงการ

การเกิดมีริ้วรอยเหี่ยวย่นของผิวหนัง เป็นสภาวะหนึ่งที่แสดงถึงการเสื่อมสภาพของผิวหนัง ซึ่งบ่งชี้ถึงภาวะความชรา เมื่อมีริ้วรอยเหี่ยวย่นเกิดขึ้นบนผิวหนัง โดยเฉพาะบริเวณรอบดวงตา ซึ่งเป็นส่วนที่เห็นได้ชัด จึงสามารถส่งผลกระทบต่อบุคคลผู้นั้นและผู้พบเห็นได้โดยชัดเจน

ในปัจจุบันการรักษาริ้วรอยรอบดวงตาทำได้หลายวิธี เช่น การทายาประเภทกรดวิตามินเอ การทาเอเอชเอ การลอกผิว การใช้เลเซอร์ การฉีดโบทอกซ์ (Botox) และการฉีดสารเติมเต็ม เป็นต้น ซึ่งจากวิธีการรักษาต่างๆที่กล่าวมา ยังไม่พบว่ามีการรักษาใดที่ได้ผลดีที่สุดและปลอดภัยในการรักษาริ้วรอยรอบดวงตา

สเต็มเซลล์ หรือเซลล์ต้นกำเนิด คือเซลล์ที่มีคุณสมบัติพิเศษ 3 อย่างคือ แบ่งเซลล์และเพิ่มจำนวนได้เป็นระยะเวลานาน ไม่มีความจำเพาะเจาะจง แต่สามารถเปลี่ยนแปลงไปเป็นเซลล์ที่มีความจำเพาะได้

ในงานวิจัยนี้ ใช้เซลล์ต้นกำเนิดจากเม็ดโลหิต ซึ่งมีข้อดีคือ สะดวก, จัดเก็บง่าย, ค่าใช้จ่ายไม่สูง และเป็นเซลล์ต้นกำเนิดที่มาจากผู้ร่วมวิจัยเอง จึงไม่ต้องกังวลเกี่ยวกับความเข้ากันไม่ได้ของเซลล์ต้นกำเนิด หรือโรคที่สามารถติดต่อได้ทางเม็ดโลหิต เช่น HIV หรือไวรัสตับอักเสบ เป็นต้น

นอกจากนี้เซลล์ต้นกำเนิดจากเม็ดโลหิต ยังมีความสามารถพิเศษในการเปลี่ยนแปลงเซลล์ตัวเอง ให้กลายเป็นเซลล์กระดูกอ่อน, เซลล์ไขมัน, เซลล์กล้ามเนื้อ, เซลล์ผิวหนังและยังสามารถซ่อมแซมคอลลาเจนใต้ผิวหนัง ซึ่งจะทำให้คอลลาเจนบริเวณหางตา มีความแข็งแรงและความยืดหยุ่นมากขึ้น จึงทำให้ผู้วิจัยมีความสนใจที่จะศึกษาผลของการรักษาริ้วรอยบริเวณหางตา โดยใช้เซลล์ต้นกำเนิดจากเม็ดโลหิต

สถานที่ทำการวิจัย

1. โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพมหานคร
   38/11-13 อโศกเพลส สุขุมวิท 21 ถ.อโศกมนตรี เขตวัฒนา คลองเตย กรุงเทพ 10110
2. ฟีนิกซ์ คลินิก อาคารธนิยะ พลาซ่า ถ. สีลม กรุงเทพ 10500

ระยะเวลาในการทำวิจัย: 7 สัปดาห์

วิธีการวิจัย

1. ผู้เข้าร่วมโครงการวิจัยทุกคนจะถูกซักประวัติ,ทำแบบสอบถาม, ตรวจร่างกาย และตรวจเลือดหากเข้าตามเกณฑ์จะได้รับคำอธิบายเกี่ยวกับวิธีทดลอง, เซ็นใบยินยอมเข้าร่วมการทดลอง

2. ก่อนเริ่มการรักษา ผู้เข้าร่วมโครงการวิจัยจะได้รับการตรวจริ้วรอยบริเวณหางตาทั้งสองข้าง โดยเครื่องมือ Visioscan® VC 98 และ Rao Goldman 5 point visual scoring scale

3. ฉีดตัวยา Filgrastim 10 ไมโครกรัม /กิโลกรัม/วัน วันละครั้งเข้าชั้นไขมันใต้ผิวหนังบริเวณหน้าท้อง ต่อเนื่องเป็นเวลา 4 วัน เพื่อชักจูงให้เซลล์ต้นกำเนิดเม็ดเลือดเข้าสู่กระแสโลหิต

4. วันที่ 5 เจาะเลือดจากข้อพับแขนด้านขวา จำนวน 30 ซีซี โดยแบ่งเป็นสองส่วน, 10 ซีซี นำมาตรวจ CBC, BUN, Cr, AST, ALT และ 20ซีซี นำมาปั่นคัดแยกด้วยเครื่อง Centrifuse โดยจะคัดแยกเพื่อให้ได้เฉพาะส่วนที่มีเซลล์ต้นกำเนิดจากเม็ดโลหิต

5. ทายาชาที่บริเวณหางตาทั้งสองข้างเป็นเวลา 30 นาที ฉีด เซลล์ต้นกำเนิดจากเม็ดโลหิตเข้าใต้ผิวหนังที่บริเวณหางตาทั้งสองข้าง ข้างละ 3 จุด จุดละ 0.1 ซีซี โดยจุดแรก ห่างจากหางตาออกมา 1.5 ซม. จุดที่สองและสาม จะอยู่บนและใต้ต่อจุดแรก ในแนวดิ่ง 1 ซม.ตามลำดับ

6. ระหว่างการทดลองไม่อนุญาตให้ผู้เข้าร่วมโครงการวิจัยใช้วิธีการรักษาริ้วรอยบริเวณหางตาโดยวิธีอื่น

7. หลังจากเริ่มการทดลอง จะมีการนัดตรวจติดตามในสัปดาห์ที่ 2,4 และ 6 เพื่อประเมินผลการรักษา, ติดตามอาการและเฝ้าระวังอาการไม่พึงประสงค์

8. เมื่อครบ 6 สัปดาห์ จะมีการประเมินความพึงพอใจในการลดลงของริ้วรอย

ประโยชน์ของการวิจัย

1. เพื่อการศึกษาเพิ่มเติมและเป็นข้อมูลทางเลือกของผู้บริโภค เพื่อรักษาริ้วรอยบริเวณหางตา
2. เป็นข้อมูลพื้นฐานสำหรับการศึกษาวิจัยในอนาคต

ข้อพิจารณาด้านจริยธรรม

ก่อนดำเนินการวิจัย ผู้ศึกษาได้ส่งโครงร่างของการศึกษาวิจัยครั้งนี้ เพื่อขอรับการรับรองอนุญาตจากคณะกรรมการพิจารณาจริยธรรม มหาวิทยาลัยแม่ฟ้าหลวง และเมื่อได้รับการรับรองจึงได้ ดำเนินการเก็บข้อมูล เนื่องจากข้อมูลส่วนบุคคลเกี่ยวกับข้อมูลทั่วไปของผู้ป่วย การศึกษานี้ข้อมูลส่วนบุคคลจะไม่มีการเปิดเผย โดยผู้วิจัยจะรักษาความลับโดยไม่เปิดเผยต่อสาธารณะ การนำเสนอข้อมูลทั้งหมดจะเป็นภาพรวมไม่แสดงเป็นรายบุคคล หรือปรากฏชื่อในงานวิจัย ผู้เข้าร่วมงานวิจัยมีสิทธิที่จะปฏิเสธการตอบคำถามได้ทั้งหมดและสามารถถอนตัวได้เมื่อไม่อยากร่วมหรือรู้สึกไม่สบายใจในการมีส่วนร่วมในการวิจัยครั้งนี้ โดยไม่มีผลกระทบต่อการดูแลรักษาที่พึงได้รับตามปกติ ที่สำคัญงานวิจัยครั้งนี้ได้ดำเนินการภายใต้หลักจริยธรรมของเฮลซิงกิทุกประการ

ความเสี่ยงที่อาจจะเกิดขึ้นกับอาสาสมัคร

ผลข้างเคียงที่อาจพบได้ อาทิเช่น ผื่นแดง คัน บวม เป็นต้น

วิธีการป้องกันและแก้ไข กรณีเกิดปัญหากับอาสาสมัคร

1. หากเกิดผลข้างเคียงจากการรักษา ผู้เข้าร่วมวิจัยจะได้รับการรักษาโดยทันทีเพื่อควบคุมอาการข้างเคียงที่เกิดขึ้น และผู้เข้าร่วมวิจัยมีสิทธิ์ที่จะถอนตัวจากการศึกษา เวลาใดก็ได้

2. ผู้เข้าร่วมวิจัยสามารถติดต่อได้ตลอดเวลาหากมีข้อซักถามหรือเกิดปัญหาใด ๆ ขึ้นระหว่างการรักษาตามเบอร์โทรศัพท์ที่ผู้วิจัยให้ไว้

ค่าตอบแทน ชดเชยดูแลรักษาต่าง ๆ แก่อาสาสมัคร

ผู้เข้าร่วมโครงการวิจัย จะไม่ได้ค่าตอบแทนสำหรับการร่วมศึกษาครั้งนี้เมื่อสิ้นสุดโครงการ แต่ผู้เข้าร่วมโครงการวิจัย ไม่ต้องเสียค่าใช้จ่ายในการรักษาตลอดระยะเวลาทำการวิจัย หากเกิดอันตรายหรือผลข้างเคียงจากการรักษา ผู้เข้าร่วมโครงการวิจัย จะได้รับการดูแลรักษาทันทีโดยไม่ต้องเสียค่าใช้จ่าย

หากท่านมีข้อสงสัยเกี่ยวกับวิธีการศึกษาวิจัย แพทย์จะแจ้งให้ท่านทราบและยินดีตอบคำถามต่าง ๆ ที่ท่านสงสัยโดยละเอียด ประการสำคัญที่ท่านควรทราบคือ ผลการศึกษานี้ใช้สำหรับวัตถุประสงค์ทางวิชาการเท่านั้นโดยข้อมูลส่วนบุคคลต่าง ๆ จะถูกเก็บไว้ในคอมพิวเตอร์และไม่มีการเผยแพร่สู่สาธารณชน ขอรับรองว่าจะไม่มีการเปิดเผยชื่อของท่านตามกฎหมาย

ขอขอบคุณในความร่วมมือของท่านมา ณ ที่นี้

APPENDIX B

INFORMED CONSENT FORM

หนังสือให้ความยินยอมเข้าร่วมในโครงการวิจัย (Informed consent form)

เขียนที่.....

วันที่

.....

ข้าพเจ้า.....อายุ.....ปีอยู่บ้านเลขที่.....ถนน.....

หมู่ที่.....แขวง/ตำบล.....เขต/อำเภอ.....จังหวัด.....

ขอทำหนังสือนี้ให้ไว้ต่อหัวหน้าการวิจัยเพื่อเป็นหลักฐานแสดงว่า

ข้อ 1 ข้าพเจ้าได้รับทราบถึงโครงการวิจัย ของนางสาว วรมน ดุลยพัชร์ และคณะ เรื่องประสิทธิผล ของการใช้เซลล์ต้นกำเนิดจากเม็ดโลหิต ในการรักษาริ้วรอยบริเวณหางตา

ข้อ 2 ข้าพเจ้าได้รับการอธิบายเกี่ยวกับวัตถุประสงค์ วิธีการวิจัย ถึงประสิทธิภาพความปลอดภัย อาการหรืออันตราย ที่อาจเกิดขึ้นรวมทั้งประโยชน์ที่จะได้รับจากการวิจัยโดยละเอียดแล้ว

ข้อ 3 ข้าพเจ้าได้รับรองจากผู้วิจัยว่าจะเก็บข้อมูลส่วนตัวของผู้ถูกทำวิจัยเป็นความลับ จะเปิดเผยเฉพาะผลสรุป เท่านั้น

ข้อ 4 ข้าพเจ้าได้รับทราบจากผู้วิจัยแล้วว่าหากมีอันตรายใดอันเกิดขึ้นจากการวิจัยดังกล่าว ผู้ถูกทำการวิจัยจะได้รับ การรักษาพยาบาลโดยไม่มีค่าใช้จ่าย

ข้อ 5 ข้าพเจ้าได้รับทราบว่า มีสิทธิในการบอกเลิกการร่วมโครงการวิจัยนี้ และไม่มีผลกระทบต่อการดูแลรักษาที่จะพึงได้รับต่อไป

ข้อ 6 ผู้ดำเนินการวิจัยได้อธิบายเกี่ยวกับรายละเอียดต่างๆ ประโยชน์ที่จะได้รับจากการวิจัย รวมทั้งความเสี่ยงที่อาจเกิดขึ้นให้ได้รับทราบ

ข้อ 7 ข้าพเจ้ายินดีเข้าร่วมการวิจัยนี้ ตามเงื่อนไขและวิธีการที่กำหนดและชี้แจงแล้วโดยผู้ดำเนินการวิจัย โดยไม่มีข้อเรียกร้องอื่นใด

ข้าพเจ้าได้อ่านและเข้าใจข้อความในหนังสือนี้ทั้งหมดแล้วเห็นว่าถูกต้องตามเจตนาของข้าพเจ้า

จึงได้ลงลายมือชื่อไว้เป็นสำคัญพร้อมหัวหน้าโครงการวิจัยและต่อหน้าพยาน

ลงชื่อ.....	ลงชื่อ.....
(.....)	(.....)
ผู้ยินยอม	หัวหน้าโครงการวิจัย
ลงชื่อ.....	ลงชื่อ.....
(.....) พยาน	(.....) พยาน

APPENDIX C

PATIENT RECORD FORM

แบบบันทึกข้อมูลโครงการวิจัย (Patient record form)

เรื่อง ประสิทธิผลของการใช้เซลล์ต้นกำเนิดจากเม็ดโลหิต ในการรักษาริ้วรอยบริเวณหางตา

เลขที่แบบบันทึกข้อมูล.....

วันที่.....

ข้อมูลส่วนตัว

ชื่อ..... สกุล.....

วัน/เดือน/ปี เกิด..... อายุ..... เพศ ☐ ชาย ☐ หญิง

ที่อยู่ เลขที่.....หมู่บ้าน.....หมู่/ซอย.....อาคาร.....ชั้น.....

ถนน.....แขวง/ตำบล.....เขต/อำเภอ.....

จังหวัด.....เบอร์โทรศัพท์บ้าน.....เบอร์โทรศัพท์มือถือ.....

ประวัติส่วนตัว

ประวัติแพ้ยา	☐ ไม่มี	☐ มี (โปรดระบุ) .....
ประวัติแพ้อาหาร	☐ ไม่มี	☐ มี (โปรดระบุ) .....
ประวัติโรคประจำตัว	☐ ไม่มี	☐ มี (โปรดระบุ) .....
ยาที่รับประทานเป็นประจำ	☐ ไม่มี	☐ มี (โปรดระบุ) .....
สูบบุหรี่	☐ ไม่สูบ	☐ สูบ .....มวน/วัน
ตั้งครรภ์/ให้นมบุตร(เฉพาะเพศหญิง)	☐ ไม่ใช่	☐ ใช่

ประวัติครอบครัว

โรคผิวหนังในครอบครัว	☐ไม่มี	☐มี (โปรดระบุ) .....
โรคทางพันธุกรรม	☐ไม่มี	☐มี (โปรดระบุ) .....

ประวัติทั่วไปเกี่ยวกับริ้วรอย

1. อาชีพ/กิจวัตรประจำวันของท่าน สัมผัสแสงแดดเป็นประจำ

   ☐ไม่ใช่ ☐ใช่ .....ชม./วัน

2. ท่านมักจะใช้อุปกรณ์ในการป้องกันแสงแดด

   ☐ไม่ใช้ ☐ใช้ ☐ แว่นกันแดด ☐หมวก ☐ร่ม ☐อื่น ๆ

3. ท่านใช้สารกันแดดเป็นประจำ

   ☐ไม่ใช้ ☐ใช้ SPF.....

4. ท่านใช้ครีมบำรุงผิวเป็นประจำ

   ☐ไม่ใช้ ☐ใช้

5. ท่านมักจะแสดงสีหน้า โดยเฉพาะบริเวณหางตา

   ☐ไม่ใช่ ☐ใช่

6. การนอนหลับ

   ☐ไม่สนิท ☐สนิท

   นอนวันละประมาณ.....ชม.

ประวัติการรักษาริ้วรอยบริเวณหางตา

1. ท่านเคยทายาหรือครีมบำรุงบริเวณหางตาหรือไม่ ?

   ☐ไม่เคย ☐เคย

2. ท่านเคยใช้ Botox เพื่อการรักษาริ้วรอยบริเวณหางตาหรือไม่ ?

   ☐ไม่เคย ☐เคย (โปรดระบุวันที่ฉีดครั้งสุดท้าย) .....

3. ท่านเคยใช้เลเซอร์ เพื่อการรักษาริ้วรอยบริเวณหางตาหรือไม่ ?

   ☐ไม่เคย ☐เคย (โปรดระบุชนิดของเลเซอร์และวันที่ฉีดครั้งสุดท้าย) .....

4. ท่านเคยฉีดสารเติมเต็ม (Filler) เพื่อการรักษาริ้วรอยบริเวณหางตาหรือไม่ ?

   ☐ไม่เคย ☐เคย (โปรดระบุวันที่ฉีดครั้งสุดท้าย) .....

5. ท่านเคยผ่าตัด เพื่อการรักษาริ้วรอยบริเวณหางตาหรือไม่ ?

   ☐ไม่เคย ☐เคย (โปรดระบุวันที่ผ่าตัดครั้งสุดท้าย) .....

6. ปัจจุบัน ท่านกำลังรักษาริ้วรอยบริเวณหางตาหรือไม่ ?

   ☐ไม่ใช่ ☐ใช่ (โปรดระบุชนิด) .....

7. ปัจจุบัน ท่านมีแผลหรือการติดเชื้อที่บริเวณหางตาหรือไม่ ?

   ☐ไม่ใช่ ☐ใช่

APPENDIX D

DOCTOR RECORD FORM

ชื่อ..... สกุล.....

1. Rao Goldman 5 point visual scoring scale
   1) Wrinkle absent
   2) Shallow but visible
   3) Moderately deep
   4) Deep with well-defined edges
   5) Very deep with redundant folds

Area	Rao-Goldman 5-point visual scoringscale			
	Week 0	Week 2	Week 4	Week 6
Right crow's feet				
Left crow's feet				

2. VisioScan® VC98

Area	Visio Scan® VC98			
	Week 0	Week 2	Week 4	Week 6
Right crow's feet				
Left crow's feet				

APPENDIX E

PATIENT'S SATISFACTION QUESTIONNAIRES

แบบฟอร์มประเมินความพึงพอใจในการลดลงของริ้วรอย บริเวณหางตาโดยผู้เข้าร่วมวิจัย (สัปดาห์ที่ 6) (Patient's satisfaction questionnaires)

บริเวณผิวหนังที่ทำการประเมิน	การเปลี่ยนแปลงของริ้วรอย				
	ริ้วรอยดีขึ้นมาก	ริ้วรอยดีขึ้นปานกลาง	ริ้วรอยดีขึ้นเล็กน้อย	ริ้วรอยไม่เปลี่ยนแปลง	ริ้วรอยมากขึ้น
ผิวหนังบริเวณหางตาทางด้านขวา					
ผิวหนังบริเวณหางตาทางด้านซ้าย					

CURRICULUM VITAE

CURRICULUM VITAE

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EDUCATIONAL BACKGROUND	
2005	Doctor of Medicine Rangsit University
WORK EXPERIENCE	
2010-Present	Doctor Mae Fah Luang University Hospital, Bangkok
2006-2010	Doctor Aesthetic Clinic, Bangkok