THE EFFECTIVENESS OF *LACTOBACILLUS* SERUM FOR REDUCTION OF WRINKLES

KYAW MIN THU

MASTER OF SCIENCE

IN

DERMATOLOGY

SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE

MAE FAH LUANG UNIVERSITY

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Kyaw Min Thu

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ABSTRACT

Lactobacillus is one of the lactic acid bacteria (LAB) species, which are gram-positive organisms and are acid-tolerant, plant derived, and strictly fermentative. These *Lactobacillus* probiotics are included in foods or dietary supplements. Although the GI tract is the primary target of *Lactobacillus*, it is found that skin might also be benefitted by *Lactobacillus*. *Lactobacillus* played an essential role in enhancing human cell immunity and maintaining a healthy surface barrier. It balances the systemic immune status, including the release of regulatory cytokines, which play an important role in skin homeostasis. It also promotes the anti-oxidant function by quenching the free radical and protects oxidative stress damage to the cells. It also inhibits elastase and collagenase activity and maintains elastin and collagen to result in delaying skin-aging process. So, it can be used in treatment of fine wrinkles and maintenance of skin elasticity and moisturization.

The purpose of this study is to determine the effectiveness of Lactobacillus serum for reduction of wrinkles. This research is an experimental study with 16 participants. The study population is healthy male and female between the age of 30-55 years old. They need to apply the Lactobacillus serum on both sides of the face

twice a day for 12 weeks. Follow-up appointments will be at 4th, 8th and 12th week. Wrinkles assessment is done by Rao-Goldman 5 point visual score. Wrinkles and elasticity of the skin will be measured by VISIA® Complexion Analysis System and Cutometer® MPA 580. To measure the improvement, photographs taken with VISIA® Complexion Analysis System will be evaluated by 3 dermatologists. The participants were assessed by physician and questionnaires to detect any adverse effects and participants' satisfaction. This study may also be used as a reference data for future researches and studies.

Results: The outcome of the mean change of cutometer scores at 1cm above the eyebrow within the mid-pupillary line and 1cm below the eyelid withing the mid-pupillary line were significantly increased in different visit. Therefore, Lactobacillus can promote the elasticity of the skin. Wrinkle evaluation score by VISIA was also statistically increased and Rao-Goldman 5-point visual scale was significantly decreased in follow-up visits. This study also achieved high participants' satisfactory score and no adverse effect was detected.

Conclusion: The daily topical application of Lactobacillus serum is safe and effective for reduction of wrinkles. Therefore, Lactobacillus can be an alternative treatment for anti-aging and facial wrinkle reduction.

Keywords: Lactobacillus, Probiotics, Wrinkles, Elasticity, Cutometer

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CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays, having flawless, glowing skin without wrinkles, fine lines, and dark spots is becoming the undeniable beauty goal for almost everyone around the world. Therefore, facial rejuvenation turns into one of the cosmetic methods used for developing or revitalizing healthy and youthful skin appearance. For more details, it is all about restoring the appearance of youthful skin by using different facial rejuvenation methods for eye brow lift, aging spot removal, eye bag removal, and wrinkle reduction.

The skin aging process has an influence on endogenous so called intrinsic factors such as genetics, hormonal and metabolic actions; and exogenous so called extrinsic factors like UV rays, smoking, alcohol drinking, chemicals, toxins, air pollution and ionizing radiation. The above accumulative agents result in morphological, structural, and biochemical transformations causing degenerative conditions and skin aging characterized by fine lines and wrinkles, skin texture changes, asymmetrical skin tone, skin pigmentation, and losing in skin elasticity [1].

Wrinkles are mainly occurred with over-action of facial muscles caused by different kinds of facial expressions and loss of collagen and elastin, together with loss of water due to free radicals and expression of matrix metalloproteinases (MMPs). The major action of collagen is keeping the strength of the skin, connective tissues and also bone. Free radicals possess a damaging effect to cell membranes, lipids, proteins and DNA resulting in collagen breakdown and impairment of new collagen synthesis, and are also involved in the progressing of photo aging, inflammation and cancer. Reactive oxygen species (ROS) is caused by the absorption of UVB rays on the skin, causing melasma, freckles, spots and wrinkles that are

clearly seen with the facial expressions [2]. Free radicals may be attacked by exogenous antioxidants. Antioxidants are the ones that help to protect the skin from oxidative effects of free radicals and environmental factors like UV rays and air pollution, and to promote the synthesis of skin collagen for skin elasticity. [3]

Currently, there are several treatment methods for aging skin like topical application, chemical peeling, micro-dermabrasion, filler injection and botulinum toxin injection, laser treatment, and cosmetic surgery [4]. Topical application is non-expensive, safe and effective method. So, it becomes the first priority to choose for people.

Lactobacillus is one of the lactic acid bacteria (LAB) species, gram-positive creatures and are acid-tolerant, and strictly fermentative. *Lactobacillus* are dominant on the skin homeostatis by balancing the systemic immune status. Moreover, the previous researches showed the effectiveness of *Lactobacillus* extract in promoting level of ceramides of young and old subjects hence lowering the dryness of skin, loss of tone, and water loss, opened now potential projects against the pathophysiological skin alteration, such as skin aging. Furthermore, *Lactobacillus* can restore the normal pH level of the skin and promote the synthesis of free fatty acids (FFAs). Likewise, it possesses the action to reduce the MMP expression and oxidative stress to relieve skin aging [5].

Topical application of *Lactobacillus* ferment can also provide the skin as a defensive armor by acting as a competitive inhibitor of binding sites, so that it can restrict migration of other potential pathogens [6]. It can also support the skin with antimicrobial activity and anti-inflammatory activity [7]. Some studies showed *Lactobacillus* ferment reduced the elastase activity and promoted collagen expression in the UVB-induced photoaging in vitro model, decreased the activity of collagenase, and increased the expression of moisture factor and antioxidant enzymes, so that it might be useful in the cosmetic industry because of its protective effects against UVB-induced photoaging [8]. Together with these actions, *Lactobacillus* can prevent skin aging from extrinsic factors by means of antioxidant, anti-inflammatory and anti-photo-aging.

The aim of this study is to discover that the antioxidant activity of *Lactobacillus* which could be useful in the treatment of skin hydration, facial wrinkles and skin elasticity, and to study the effectiveness of *Lactobacillus* serum for the treatment of facial wrinkles.

Figure 1.1 *Lactobacillus* bacteria

1.2 Research Question

Does *Lactobacillus* have a good effectiveness in reduction of wrinkle?

1.3 Objectives

1.3.1 General Objective

To study effectiveness of *Lactobacillus* ferment in reduction of wrinkles

1.3.2 Specific Objective

1.3.2.1 Primary outcome

To compare the effectiveness of *Lactobacillus* serum before and after the research

1.3.2.2 Secondary outcome

1. To observes the side effects of *Lactobacillus* ferment in this study
2. To evaluate the patients satisfaction for *Lactobacillus* serum

1.4 Hypothesis

Lactobacillus serum has an effectiveness and satisfaction in reduction of wrinkles.

1.5 Benefits

1.5.1 To use as an alternative method for reduction of wrinkles

1.5.2 To use as a reference data for other research

1.6 Conceptual Framework

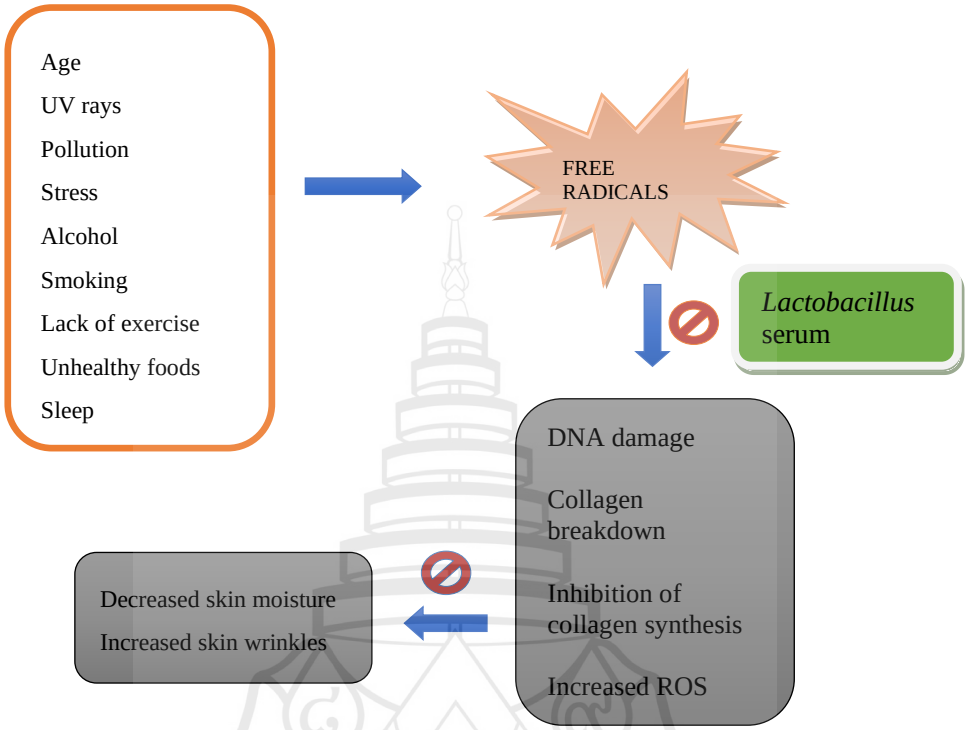


Figure 1.2 Conceptual framework of *Lactobacillus* on reduction of wrinkles

1.7 The Scope of the Research

This study is to assess the efficacy and satisfactory of *Lactobacillus* serum for the treatment of facial wrinkles, skin elasticity and skin moisturization. The sample size, male and female 16 volunteers were chosen with the age range of 30 to 55 years old using inclusion and exclusion criteria. *Lactobacillus* serum was applied at the whole face, twice a day (morning and night) for 12 consecutive weeks. All of the volunteers were explained completely about the ingredients, possible benefits and outcomes of serum and the ways to apply the serum. In addition, skin elasticity was measured by using Cutometer MPA 580. The VISIA complexion Analysis System was used for recording of score and number of skin wrinkle. All parameters were taken and recorded at 4th week, 8th week and 12th week respectively. Adverse effects

and patient's satisfactory score were assessed and documented throughout the research.

1.8 Definition

1.8.1 Effectiveness

Effectiveness is the capability which is determined by 3 factors: (i) fulfillness of the active product to meet the purpose of the research, (ii) patients' reorganization of the active product's benefits, (iii) significant changes in result parameters.

1.8.2 Satisfaction

Satisfaction is the measurement of positive feelings or happiness after experiencing a product or service or capabilities.

1.8.3 *Lactobacillus* Serum

Lactobacillus serum is a serum, which consists of *Lactobacillus* and other basic ingredients of the serum.

1.8.4 Face Wrinkles

Facial wrinkles are creases, folds, or ridges in the facial skin. They normally appear as natural when people get ages. They usually come out when the skin becomes thin and less elastic with ages. Wrinkles may also appear on the other parts of the body that receive most sun exposure, such as neck, back of hands, and arms.

1.8.5 VISIA Analysis System

The VISIA complexion analysis system is a device used to scan the skin of the face. It uses a multispectral imaging and analysis system to capture key visual information. It is used to measure the eight parameters of skin health and appearance, UV spots, photo-damage, pigmentation, pore size, porphyrins (bacteria proof), texture and wrinkles in the same environment.

1.8.6 Cutometer

The cutometer MPA 580 is a standard device to measure the elasticity and other biomechanical parameters of the skin. The measurement is based on suction.

1.8.7 Rao-Goldman 5 Points Visual Score

The Rao-Goldman 5 points visual score for facial aging is a five-point (1-5) scale assessment that was performed by investigator to evaluate wrinkles.

0 - no wrinkles

1 - just perceptible wrinkle

2 - shallow wrinkles

3 - moderately deep wrinkles

4 - deep with well-defined edges

5 - very deep with redundant folds

1.8.8 Patient's Satisfaction Score

Patient's satisfaction score is the score that are collected from patients based on their satisfaction of the specific process within the range of 0-4.

0 - no satisfaction

1 - little satisfaction

2 - average satisfaction

3 - more satisfaction

4 - most satisfaction

1.9 Variable in the Study

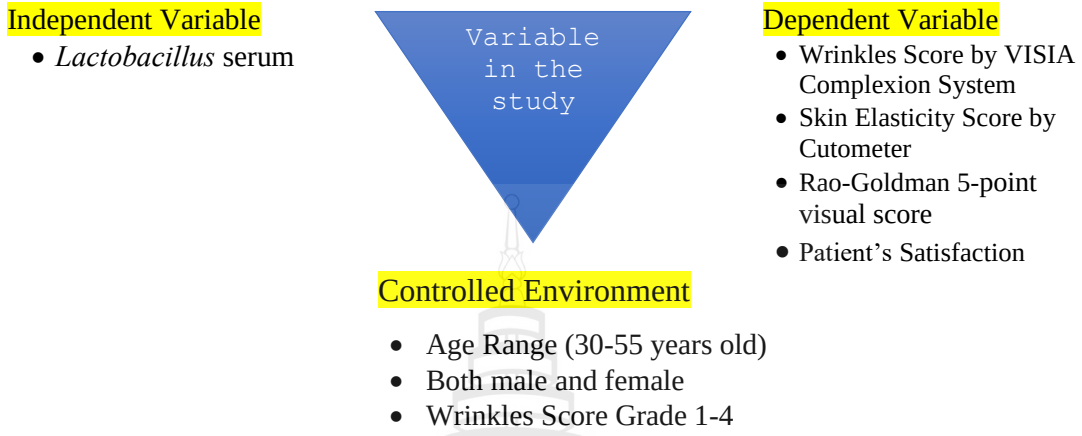


Figure 1.3 Variable in the study

CHAPTER 2

LITERATURE REVIEW

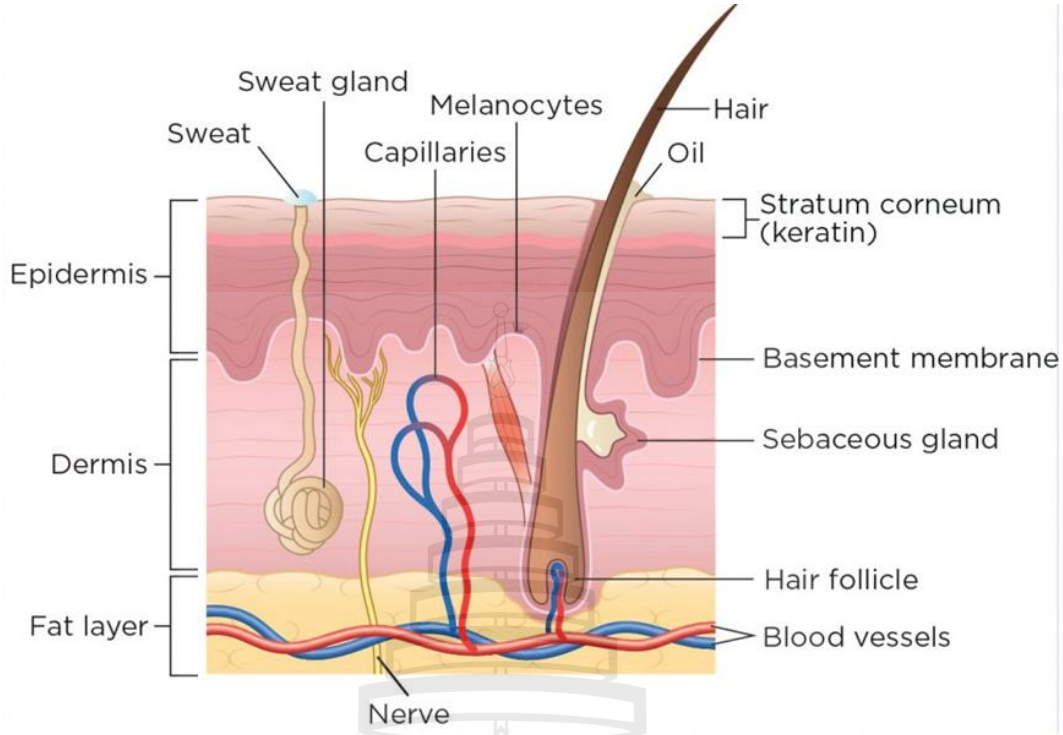
2.1 Structure and Function of Skin

Human skin is a large and complicated organ that weighs 15% of the total body weight of an adult. The functions of the skin are environmental protection, thermoregulation, fluid and electrolyte balance, sensations of pain, temperature, touch and pressure, repair and regeneration of wounds. It is made up of three layers:

2.1.1 Epidermis: The outermost layer consisting of dendritic cells, skin cells, pigments and proteins. It provides the primary barrier to protect the body from UV radiation, harmful chemicals and environmental pathogens, regulates the amount of water to keep skin moisture, and creates skin tone.

2.1.2 Dermis: Middle layer of the skin containing collagen, elastic tissue, and other extracellular components that contain vasculature, nerve endings, hair follicles, and glands. It serves as thermoregulation, sensation, elasticity and tensile strength.

2.1.3 Hypodermis (subcutaneous tissue): Inner layer of the skin that contains adipose tissue (fat cells), connective tissue, larger nerves and blood vessels. It functions as the main structural support for the skin, and also insulating the body from cold and supporting shock absorption. [9]



Source [10]

Figure 2.1 Structure of the skin

2.2 Skin Aging

Skin aging is a complex process that is influenced by intrinsic and extrinsic factors, and is strongly affected by the magnifying damage from the exposure to UV rays. Skin aging is featured by wrinkles, loose and pigmentary irregularities. These changes include both enzymatic degradation and a reduction of collagen synthesis, which causes wrinkles on the skin [11]. There are two main types of skin aging. They are intrinsic or internal aging which is caused by the genes, and extrinsic or external aging that is caused by environmental factors [12].

2.3 Causes of Aging Skin

2.3.1 Intrinsic Aging

It is also known as chronological or internal aging. Intrinsic aging is unavoidable physiologic changes of the skin that occur over time and are affected by genetic and hormonal factors. These changes include reduced collagen production, decreased lipid content, decreased blood flow, and the loss of rete ridges, degeneration of elastic fibers and dehydration of the skin. Clinical features of intrinsic aging are xerosis, less elasticity, wrinkles, thin skin, shrinkage of underlying fat that can balance the skin temperature, sagging and dry skin, decreased sweating that can make the skin cold, thin nail-plate, graying hair, hair loss and bone shrinkage from the skin. [13-14]

2.3.2 Extrinsic Aging

It is also known as environmental or external aging. Extrinsic aging can cause premature aging. It develops due to many environmental factors such as exposure to UV radiation, facial expressions, smoking, alcohol, air pollution, gravity and sleeping positions [15].

2.3.2.1 UV radiation

It is the most important factor in skin aging, mainly in premature aging. Not only UVB but also UVA induces photoaging. Photoaging is depended on the intent of UV exposure and types of the skin. It is more easily seen in Fitzpatrick skin type I and II. Over 80% of facial skin aging is due to prolonged exposure of UV radiation. UVB is the major factor for direct DNA damage and induces inflammation and immunosuppression, while UVA has an important role in photoaging. VU radiation promotes the production of collagen-degrading enzymes, matrix metalloproteinases (MMPs), and the xeroderma pigmentosum factor (XPF) that can induces the wrinkles [16].

2.3.2.2 Smoking

Smoking is one of the most common factors causing skin aging. Skin damage induced by smoking is related to oxidative stress, impaired collagen synthesis and activation of matrix metalloproteinases (MMPs). Smoking can promote skin

damage primarily by lowering the capillary blood flow to the skin and reduces the level of moisturization in the stratum corneum and vitamin A levels that is important for the reduction of collagen damaging. Premature skin aging is also induced by passive smoking exposure. Smoking reduces type I and type III collagens synthesis and can also cause elastosis and telangiectasis [17]

2.3.2.3 Repeated facial expression

Another extrinsic factor is facial expression especially in people with repeated expression of facial movements. These repeated facial movements can induce fine wrinkles. Every time when the facial muscles are used, groove may be occurred under the skin surface and the lines may form with facial expressions. Along with aging, the skin becomes lost in elasticity and cannot restore its rest position state. Furthermore, these grooves and lines become more dominant on the face and form wrinkles.

2.3.2.4 Air pollution

Pollution also plays an important role in skin aging. Air pollution is a contamination of chemical, physical or biological agent and contains two kinds of primary pollutants which are particulate matter (PM) and gases (O3, CO2, NO2). Exposure to air pollution comprising PM, NO2 and ground level ozone is related with the formation of pigmented spot and wrinkles in the face. PM induces oxidative stress in the skin that contributes to extrinsic skin aging. Repeated contact with ozone reduces the formation of antioxidants from the stratum corneum and promotes the arylhydrocarbon receptor (AhR) activation involved in PM-induced skin aging [18].

2.3.2.5 Sleeping posture

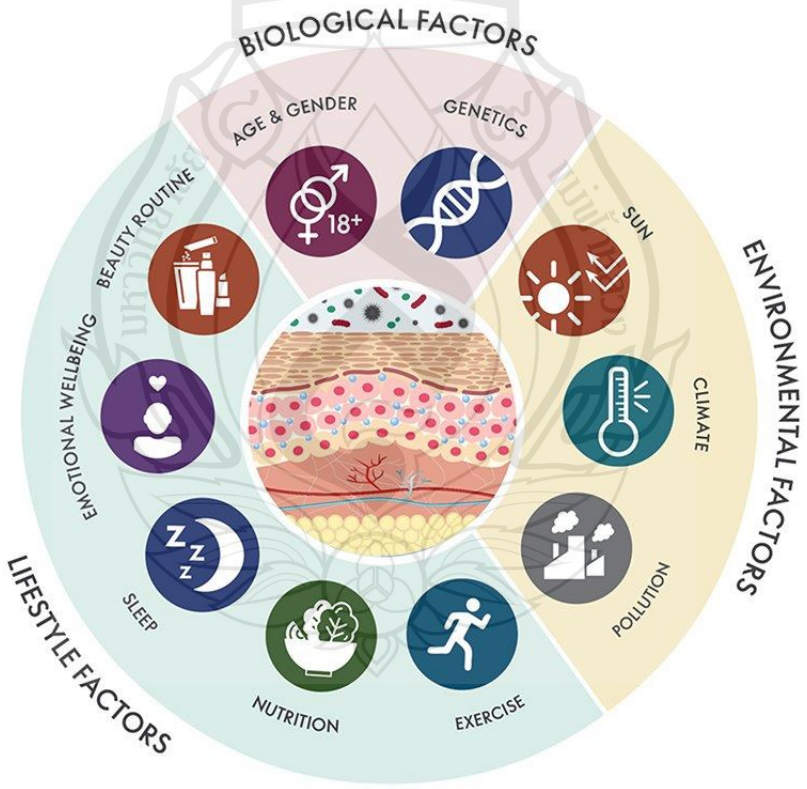
Sleeping posture can affect the wrinkle formation on the face, also known as sleep wrinkles. Facial distortion during sleep contributes to the appearance of aging with the progress of sleep wrinkles. Usually, due to compression, shearing, and pressure, sleeping on your side or lying down is more prone to sleep wrinkles. In supine position, only gravity acts on the face. Over time, the wrinkles become more prominent. In addition to repeated sleep patterns, the timing and intention of wrinkles can vary depending on the condition of the skin to deal with intrinsic and extrinsic influences [19].

2.3.2.6 Diet and nutrition

Both diet and nutrition may affect on the skin aging. Higher carbohydrate intake and excessive sugar intake are associated with premature aging. If there are fewer intakes of dietary foods rich in antioxidants such as vitamins, flavonoids and carotenoids, there will be more ROS formation and may result in skin aging [20].

2.3.2.7 Gravity

Gravity assists the stable pressure on the bodies to downward direction. Changes related to gravity become more significant with age. In the people above the age of 50 years, the elasticity of the skin becomes decline and the effect of gravity on the skin becomes more significant, Signs of skin aging due to gravity include droopy eyelids, elongated ears, drooping nose tip, jowls development, and upper lip thinning although the lower lip becomes more pronounced.



Source [21]

Figure 2.2 Causes of Skin Aging

2.4 Changes in Skin Aging

2.4.1 Epidermis, outer layer of the skin, becomes thin in skin aging while the number of skin layers is unchanged.

2.4.2 Melanocytes (pigment containing cells) is reduced in number but increased in size. This factor can make the skin thinner, translucent and pale. Large pigmented spots like liver spots or lentigo may be occurred in sun exposed areas.

2.4.3 The connective tissues of the skin will fall in elasticity and volume and may cause the solar elastosis in sun exposed areas.

2.4.4 In the dermis (inner layer of the skin), the blood vessels are likely to become weak and can lead to bleeding under the skin (senile purpura), bruising, cherry angiomas and other similar conditions.

2.4.5 The sebaceous glands become less production of sebum and the skin moisturization will be reduced resulting in dryness and itchiness.

2.4.6 The subcutaneous fat layer becomes thinner and its thermal regulating and barrier functions will decrease. Likewise, the ability to maintain body temperature will decrease and the risk of injury to the skin will increase.

2.4.7 Abnormal skin growths like skin tags, warts and other blemishes may be prominent in elderly people.

2.4.8 the reduction in sweat glands and their output in the skin with increasing age affects the whole body thermoregulation. It can make the problems in keeping the body cool and then can result in increased risk of heat stroke.

Older skin increases the risk of skin damage as the skin becomes thinner and more fragile. With the loss of the subcutaneous fat layer, the sensation of pain, touch, heat, vibration, pressure, cold and other abilities will become weaker. In addition, certain nutritional deficiencies and other illnesses can cause pressure ulcers. The rate of skin repair in old age is slower than the young skin. In addition, it causes bedsores and infections. Decreased immunity, changes in blood vessels, diabetes and impaired healing [20].

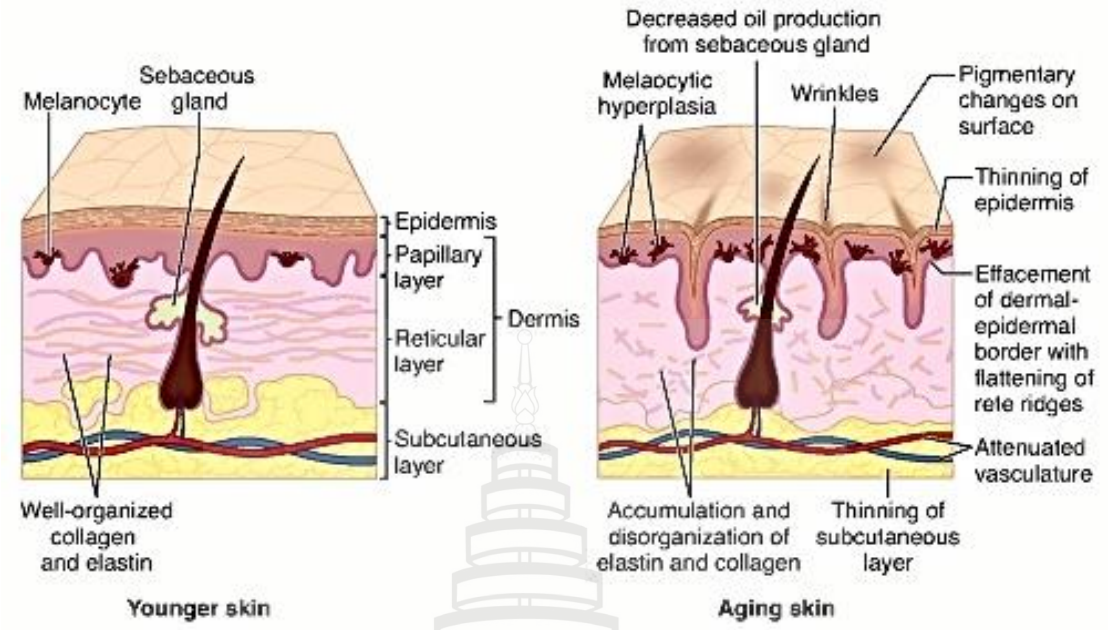


Figure 2.3 Changes of Skin Aging

2.5 Common Problems and Mechanism of Skin Aging

Normally, more than 90% of elderly people may suffer from skin diseases. These may be affected by many factors such as anxiety, nutritional deficiencies, vascular diseases such as arteriosclerosis, diabetes, obesity, heart disease, liver disease, drug reaction and other issues such as exposure to plants and other substances, clothing, household chemicals, climate and industry.

Different molecular mechanisms can cause skin aging, such as oxidative stress, DNA damage, telomere shortening, regulation of microRNA (miRNA), accumulation of advanced glycation end products, gene mutation and inflammation. Among them, the oxidative damage and inflammation to skin cells by the generation of ROS and free radicals contribute in the internal and external skin aging [22].

2.6 Wrinkles

Wrinkles, a natural process of aging, are most common on UV-exposed skin like face, hands, neck and forearms. Despite the fact that the genetics primarily regulate the structure and texture of the skin, sun exposure is the main cause of wrinkles, dominantly for people with light colored skin. Pollutants and smoking can also cause wrinkles. Wrinkles can be seen easily on the skin. If wrinkles have width and depth less than 1 mm, they can called are fine wrinkles.Wrinkles with width and depth having 1 mm or more, they are interpreted as coarse wrinkles [23].

2.7 Main Factors Distributing the Formation of Wrinkles

2.7.1 Facial expression is one of the extrinsic agings, especially for people with repeated facial movements. Frequent and repetitive facial movements can cause fine lines and wrinkles. When people use facial muscles, wrinkles will form under the surface of the skin along the lines of facial expressions. After many years, the skin loses its elasticity and will no longer return to its resting position. Over time, skin wrinkles on the face become more prominent in the form of fine lines and wrinkles.

2.7.2 UV radiation plays an important role in photoaging skin. Photoaging skin is identified by changing in dermal connective tissue. Levels of precursors and cross-links between type I and III collagens are decreased in photoaging. Photoaging is more common in skin type I and II while less common in skin type V and VI. UV radiation can damage keratinocytes, melanocytes, fibroblasts, and endothelial cells, resulting in wrinkles, loss of elasticity, pigment irregularities, telangiectasias, and the development of skin malignancy [16].

2.7.3 Smoking forces the skin to get premature aging by imbalance production of collagen and increasing tropoelastin and matrix metalloproteinases (MMP) production and results in degradation of collagen and elastic fibers. ROS are also involved in smoking induced skin aging. The appearance of wrinkles is exaggerated by direct contact of smoking, which reduces the levels of moisturization in the stratum corneum. Passive smoking may also induce premature skin aging and wrinkles [17].

2.7.4 Genetic factors are also associated with photoaging and wrinkle formation. Wrinkles in Asians tend to occur later and less severely than in Caucasians, and dry skin is more prominent in African American and Caucasian women. Male skin is more sensitive to environmental aggressions, in particular to exposure to ultraviolet rays.

2.8 Treatment Options for Wrinkles and Skin Aging

It is impossible to stop the aging process but it can be possible to prevent the rapid onset of wrinkles. In fact, wrinkles are not considered a disease or medical condition because they are not life threatening or harmful to health. But nowadays, treatments for wrinkles are very popular among people because of cosmetic purpose. There are many treatments for wrinkles.

2.8.1 Topical Applications

2.8.1.1 Retinoids is one of the effective topical wrinkle treatments. Topical retinoids help to reduce fine lines, skin pigmentation, and promote skin texture and appearance. Retinoid is a group of compounds derived from vitamin A. Retinoids are common active ingredients in many dermatological medications and cosmetic products due to their positive effect on the skin and its appearance. Although it is a good topical anti-wrinkle medication, it can cause burning, itching, dryness, peeling, and increased sensitivity to the sun [24].

2.8.1.2 Applying topical antioxidants such as Vitamin C, Vitamin E, Coenzyme Q10, Alpha Lipoic Acid can also reduce the wrinkles by preventing free radical formation, helping collagen systhesis, and keeping the skin moisturization [25].

2.8.2 Invasive and Non-invasive Procedures

This includes face lifting, thread lifting, dermabrasion, microdermabrasion, chemical peeling, laser resurfacing, botox injections, and fillers.

2.8.2.1 Facelift, so called rhytidectomy, is one type of cosmetic surgery. It provides a younger or flawless appearance. It generally removes excess skin and fat

from the face, with or without strain on the underlying tissue. However, it can take a long time for healing and swelling and bruising can be occurred for a few weeks after surgery [26].

2.8.2.2 Dermabrasion, a surgical procedure, is used to remove scars, fine wrinkles, tattoos, and nevi (moles). It is a minimally invasive, low risk technique for skin resurfacing. However, the results may not be seen immediately and can be long for several months. There will be scabs, redness and swelling, which will normally disappear after two weeks [27].

2.8.2.3 Microdermabrasion is the minimally invasive procedure used to renew overall skin tone and texture. It can improve the appearance of photo damage, wrinkles, age spots, acne scars and other skin related conditions. It is a new technique that provides many benefits of dermabrasion, chemical peeling and laser resurfacing. It can support the skin to look fresh and smooth, and also help in reducing wrinkles, fine lines, rough skin, enlarged pores and sun damage. The patient may have a small redness in the treated skin areas. But repeated treatments are necessary due to temporary results [28].

2.8.2.4 Chemical peel is a procedure that uses chemical ingredients to improve skin texture and to treat facial wrinkles. Peeling solutions are trichloroacetic acid, salicylic acid, and phenol. Chemical peeling support the youthful and smooth skin and increased collagen synthesis so that the skin becomes tightened and fading wrinkles can be resulted [29].

2.8.2.5 Laser, light source and radio frequency are laser (ablative) rejuvenating techniques. When the dermis got high heat, the epidermis is eroded by laser. From this process, it accelerates the synthesis of new collagen fibers. Also, when the wound got healing, the softer and smoother new skin will be developed. Though the ablative laser may take some months for healing, the non-ablative laser pulses, light sources and radio frequency machines are not harmful to the epidermis and then the dermis is heated and triggers the process of new collagen and elastin synthesis. Subjects can feel tight and have renewed skin after receiving several treatments. Nevertheless, subjects may experience burns, redness, and post inflammatory skin pigmentation [30].

2.8.2.6 Botox (botulinum toxin type A) blocks chemical signals and causes the muscle contraction. It is usually injected into the targeted muscles with small dose, resulting in a smoother, less wrinkled appearance. Botox is effective for the lines between the eyes (frown lines), on the forehead, and crow's feet. Repeated injections are needed because treatments normally last three to four months. The patient may have side effects of bruising and ptosis [31].

2.8.2.7 Filler is used to support the facial volume and facial lifting to restore the younger look. It uses mainly hyaluronic acid (HA) filler type with varying degree of cross-linking. Usually, they are injected into the wrinkles of the face, filling and smoothing them and promoting skin volume. The effect can last 3-6 months and up to a year in high HA linked fillers. The patient may have swelling, bruising and redness in the treated areas for a short time. In addition, good results depend on several factors, the location of the wrinkles, their depth and the skill of the doctor. However, there is no complete current treatment that can stop the aging process [32].

2.8.3 Preventive Methods for Wrinkles and Aging

There is no way to prohibit the process of aging. When people get old, signs of aging like wrinkles will be prominent more and more. However, the wrinkles and aging could be slow down by some methods.

2.8.3.1 Avoid the sunlight

2.8.3.2 Maintain the skin moisture

2.8.3.3 Sleep enough time

2.8.3.4 Avoid smoking

2.8.3.5 Take balance and good nutritious diets

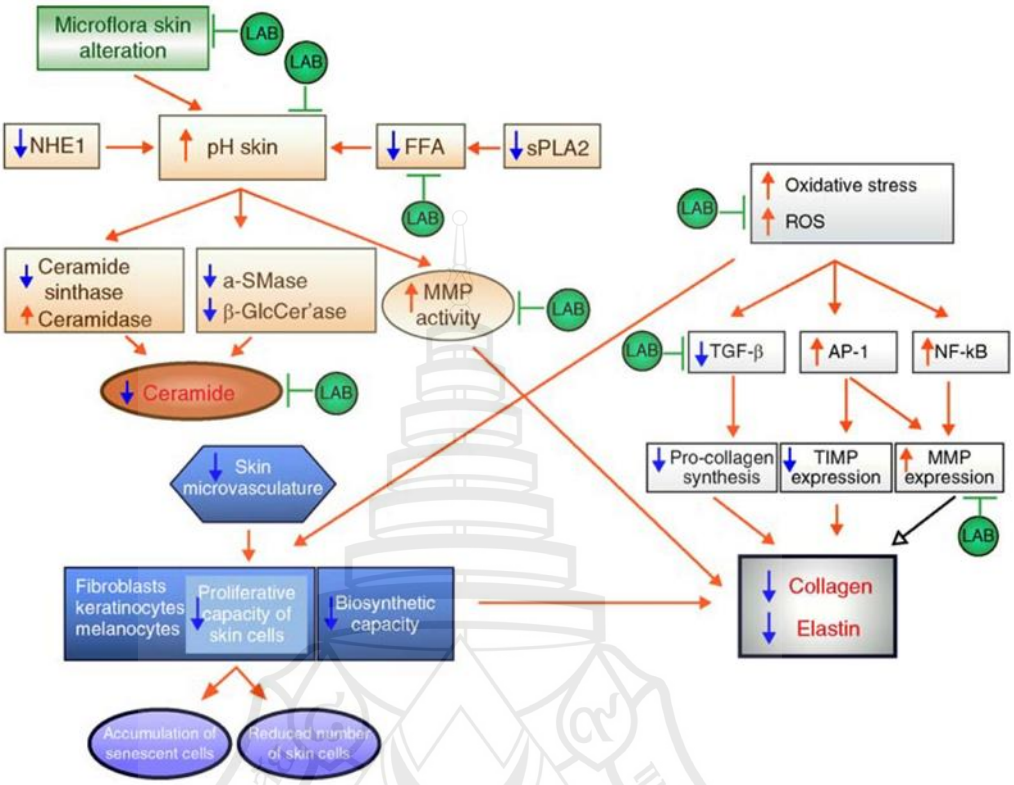
2.9 Information about *Lactobacillus*

2.9.1 General Information

Lactobacillus is one of the lactic acid bacteria (LAB) species, which are gram-positive organisms and are acid-tolerant, and strictly fermentative. It is the main end outcome of sugar fermentation. These Lactobacillus probiotics are also contained in foods and dietary supplements. Although the GI tract is the primary target of Lactobacillus, it is found that other conditions not concerned with the gut micro biota might also be affected by Lactobacillus. The genus Lactobacillus has more than 200 species that are characterized by a phylogenetic and metabolic diversity that exceeds that of a typical bacterial family. Recent phylogenetic analyses revealed that lactobacilli could be subdivided into at least 24 phylogenetic groups. Members of the genus Lactobacillus are non-spore forming, non-motile and rod-shaped coccobacilli. The optimal growth temperature of Lactobacillus is mostly between 30 and 40∘C, even though the overall growth temperature can vary between 2 and 53∘C; the pH for growth ranges between 3 and 8. Generally they endure oxygen but also grow well under anaerobic conditions. They produce lactic acid as the major fermentation product from sugars [5].

Lactobacillus played an essential role in enhancing human cell immunity and maintaining a healthy surface barrier [33]. It balances the systemic immune status, including the release of regulatory cytokines, which play as a main chapter in skin homeostasis. Thus, the use of the effects of Lactobacillus on the skin is becoming one of the topics of interest today [5]. Lactobacillus is applicable to the skin where there is a normal micro flora.

2.9.2 Function of Lactobacillus on Skin Aging



Source [5]

Figure 2.4 Skin aging process and the sites of action of *Lactobacillus*

2.9.2.1 Micro flora Alteration

The normal micro flora of the skin contains a certain number of microbial types, mostly gram-positive species. The physiological situations like hydration, pH, O2 and growth media are the main factors determining the restriced number of microbial species which migrate to human skin. The skin microflora prevents the skin against premature aging, inflammation and dehydration, and also participates in the competitive exclusion of pathogens and restore the acidic nature of the skin providing with antimicrobial activities. Moreover, it also inhibits the adhesion of pathogens and supports the resistance to colonization [5].

2.9.2.2 Maintenance of skin pH

The normal pH of the skin is acidic in the range of 4.2 to 5.6. The acidic environment inhibits bacterial colonization and contributes a moisture barrier through the absorption of moisture by amino acids, salts and other substances in the acid mantle and further regulates the activity of many enzymes in the stratum corneum. Increase in the pH of the stratum corneum that leads to abnormalities in the structure of the extracellular lipid membranes and a decrease in the permeability of the skin barrier function. Topical application of a Lactobacillus preparation to aging skin helps keep the skin's pH close to the physiological acidic pH and helps repair the function of abnormal skin enzymes during aging. Acidic skin pH also supports generating free fatty acids which is important for skin barrier function [5].

2.9.2.3 Increased Ceramides Composition in skin

Ceramides are important in the barrier function and water-holding function of the healthy stratum corneum. A decrease in ceramides in the SC will lead to water loss and barrier dysfunction in the epidermis. Ceramides result from the hydrolysis of cerebrosides and sphingomyelins by β-glucocerebrosidase and SMase, respectively. The presence of high levels of neutral sphingomyelinase activity in Lactobacillus was responsible for increasing the ceramide levels of the stratum corneum and provides better skin barrier function and maintains flexibility of the stratum corneum. Topical application of Lactobacillus, an exogenous source of SMase capable of hydrolyzing skin SM and consequently producing ceramides, thereby reducing dry skin, improving loss of tone, fullness and water loss, thus delaying the process skin aging [5, 34]

2.9.2.4 Decreased Matrix Metalloproteinase

Matrix metalloproteinase is an enzyme that breaks down collagen in the skin by stimulating the transcription factor, an activating protein (AP-1). Collagen is one of the major components of human skin, which support the skin with the strength and elasticity. Dermal fibroblasts produce precursor molecules called pro collagen, which are converted to collagen. In aging skin, there is a combination of maximizing collagen breakdown and minimizing synthesis of new collagen, resulting in an overall decline in the collagen levels of the dermis and the formation of wrinkles. Lactobacillus ferment could decrease the activity of MMPs related to skin aging and improve skin laxity by increasing collagen synthesis [5].

2.9.2.5 Neutralizing Reactive Oxygen Species

Reduction of antioxidants components and generation of reactive oxygen species (ROS) and free radicals are important causes in skin aging. Antioxidants help to neutralize the toxic oxide substances result from body metabolism. ROS cause damage to lipids, proteins and DNA and effect on cellular senescene. Free radicals, products from body metabolism, damage to connective tissues, components of the dermis, particularly collagen. Lactobacillus generates exopolysaccharides (EPS) and superoxide dismutase (SOD) that may create a barrier from peroxide free radicals. The antioxidant property of Lactobacillus may support to redeem the balance between the formation of free radicals and antioxidants. This revived normal balance in the skin brings a chance to slow the photoaging process [5].

2.9.2.6 Reducing skin inflammation

The activity of the immune system falls with ages. The failure of the immune system results in cell-mediated as well humoral immunity results an increase in proinflammatory cytokines and ROS production. Immune regulation by Lactobacillus ferment is medicated through balancing control of pro-inflammatory and anti-inflammatory cytokines. Probiotics give anti-inflammatory properties by decreasing nitric oxide synthase activity [34].

2.10 Information about Prebiotics

2.10.1 General Information

Prebiotics are non-digestible, selectively fermented dietary fibers. Prebiotic research has largely targeted on two main types of carbohydrates; lactulose and galactooligosaccharides (GOS), which are served as food to increase the growth of health-promoting bacteria in the gastrointestinal tract. It promotes the growth of one or more bacteria in the GI tract and then supports health benefits to the host. The dietary intake of prebiotics decreases the contribution and duration of infectious disease, reduce inflammation and symptoms correlated with inflammatory bowel disease, use barrier effects to obstruct the cancer and boost the bioavailability and uptake of minerals, including calcium magnesium and iron. Similarly to the gut micro

flora, the skin's micro biota plays a valuable role. Topical applications of prebiotics in anti-aging might prohibit the formation of fragmented elastin fibers, search and consume free radicals, and trigger dermal microcirculation [35].

2.10.2 Functions of Prebiotics

2.10.2.1 Rebalance the composition of skin's micro flora

Improving the composition of the skin microflora, decreases the development of pathogenic species and preserves or supports the growth of helpful bacteria at the same time. Colonization of human skin with helpful bacteria provides advantageous effects in human skin that extend beyond the prevention of overgrowth pathogenic bacteria in human skin. Presence of prebiotics can alter the function of skin barrier and the development of innate immune responses in the skin. Such a useful connection between the skin and its micro flora may contribute to the treatment of skin diseases with defects in barrier function and innate immunity such as atopic eczema [35].

2.10.2.2 Protection of Transepidermal Water Loss

Galacto-oligosaccharides (GOS), a substance in prebiotics, inhibited transepidermal water loss (TEWL) and increased the water holding capacity. It also reported to upregulate the CD44 gene, which is closely related to the maintenance of hyaluronic acid homeostasis. GOS also demonstrated the moisturizing effect on skin and suppressed the production of inflammatory cytokines [36].

2.10.2.3 Reduce Matrix-Metalloproteinase Enzymes

Wrinkle formation and skin dehydration are related to the collagen deficiency. The breakdown of collagen is moderated by matrix metalloproteinase (MMP) and by the tissue inhibitor of metalloproteinase (TIMP). MMP ex.pression is caused by ROS generated upon UV exposure, and after its expression, collagen and elastic fibers (elastin) that are the major parts of ECM, are downgraded and lead to the formation of wrinkle and skin dehydration. Prebiotics thereby decrease skin wrinkles formation by degrading MMP synthesis [36].

2.10.2.4 Supporting Lactobacillus in the Skin

Prebiotics serve as food sources for the Lactobacillus and other good bacteria in the skin and can provide their prolonged survival. There are the synergistic

effects between prebiotics and probiotics that provide additional benefits to consumers [34].

2.11 Conclusions

When people get aged, the skin receives the prolonged duration of exposure to UV radiation and then oxidative stress becomes increased resulting in aging and wrinkles. Topical application of Lactobacillus with prebiotics serum could be used in the treatment of wrinkles due to the benefits such as microflora skin alteration, restoring the acidic skin pH, inhibiting the MMPs, promoting ceramides, and deleting the oxidative stress. Moreover, it is safe to use, effective and no known side effects on human skin.

CHAPTER 3

STUDY METHODOLOGY

3.1 Study Design

Clinical controlled experimental study

3.2 Study Population

Male and female healthy volunteers, aged between 30 to 55 years with their written consent before participating in this research.

3.3 Study Location

Mae Fah Luang University Hospital, Bangkok

3.4 Sample Size Determination

In order to calculate the sample size, the reference data was taken from the double-blinded study between *Spirulina plantensis* serum and placebo standard serum for the treatment of facial wrinkles. The sample size is calculated by two means dependent. Using Cutometer for skin elasticity, the results for *Spirulina plantensis* serum showed 0.76±0.08 at baseline and 0.88±0.06 at 12^{th} week follow-up. In standard base serum, the results showed 0.76±0.07 at baseline and 0.83±0.07 at 12^{th} week follow-up.

Different change from baseline of *Spirulina plantensis* extract (μ_1) = 0.88-0.76 = 0.12

Different change from baseline of placebo (μ_2) = 0.83-0.76 = 0.07

Comparing two sample means dependent, $n = \frac{(Z_{\alpha/2} - Z_{\beta})^2 \times \sigma^2}{d^2}$

Mean difference (d) = 0.12-0.07 = 0.05

Set α = 0.05 (two-tailed) $Z_{0.025}$ = 1.96

Set β = 0.20 $Z_{0.20}$ = 0.84

S_1 = 0.06 (*Spirulina plantensis*)

S_2 = 0.07 (placebo)

$n = \frac{(Z_{\alpha/2} - Z_{\beta})^2 \times \sigma^2}{d^2}$ $S_p^2 = \frac{(n_1-1)S_1^2+(n_2-1)S_2^2}{n_1+n_2-2}$

$= \frac{(1.96+0.84)^2 \times 0.00425}{0.05^2}$ = 0.00425

=13.3 ≈ 13

Where n = sample size

S = σ = variance

$S_p^2 = \sigma^2$ = pooled variance

d = mean difference

The expected dropout rate is 20%, so 16 volunteers (n=16) would be enrolled.

3.5 Inclusion Criteria

3.5.1 Male and female participants with age of 30 to 55 years old

3.5.2 Participants who accept to apply *Lactobacillus* serum on their faces

3.5.3 Participants who accept to avoid other skin care products including sunscreen during the study

3.5.4 Participants who accept to avoid botulinum and filler injection during the study

3.5.5 Participants who accept to avoid facial treatment including laser treatment during the study

3.5.6 Participants who are willing to sign and give informed consent to participate in the study and come to hospital for 3 continuous months follow up

3.6 Exclusion Criteria

3.6.1 Any condition that is not suitable for application

3.6.2 Subjects who had allergy to *Lactobacillus* or any ingredients of serum

3.6.3 Previous botulinum or filler injection within 6 months

3.6.4 Previous cosmetic surgery or laser treatment within 6 months

3.6.5 Women with pregnant or breastfeeding

3.6.6 Subjects with recent alcohol or drug abuse

3.7 Discontinuation Criteria

3.7.1 When volunteer cannot use *Lactobacillus* serum continuously

3.7.2 When volunteer had severe allergy or adverse effects

3.7.3 When volunteer cannot participate anymore in the study

3.7.4 When volunteer had other medical problems

3.8 Equipments

3.8.1 Cutometer MPA 580

Figure 3.1 Cutometer MPA 580

The Cutometer is a measurement device for the measurement of skin elasticity, pliablility and hydration. it is used to evaluate the efficacy of the skin treatments like anti-aging and anti-cellulite products. The technology of the Cutometer is based on the suction method using negative pressure and can measure the elasticity of the upper layer of the skin. With this principle, the machine can provide the quantitative measure of the skin elasticity and skin aging. (www.courage-khazaka.de)

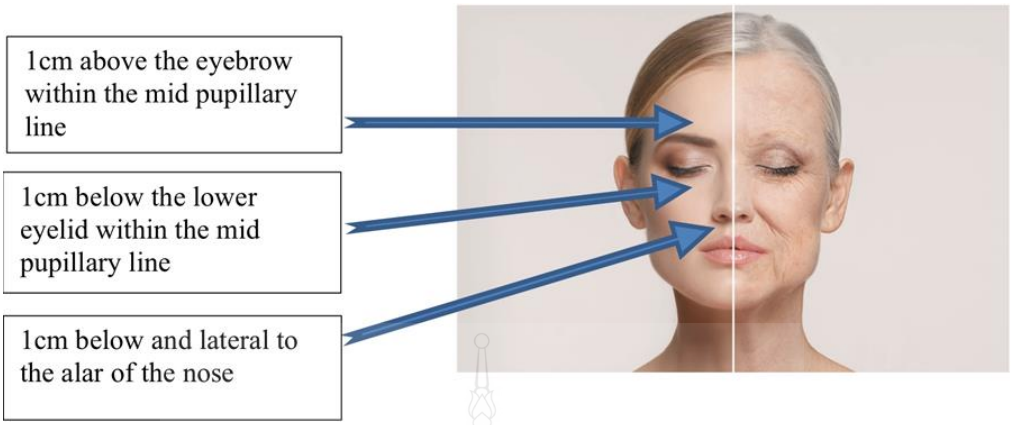


Figure 3.2 Landmark for cutometer measurement

3.8.2 The VISIA® Complexion Analysis System for Wrinkle Score

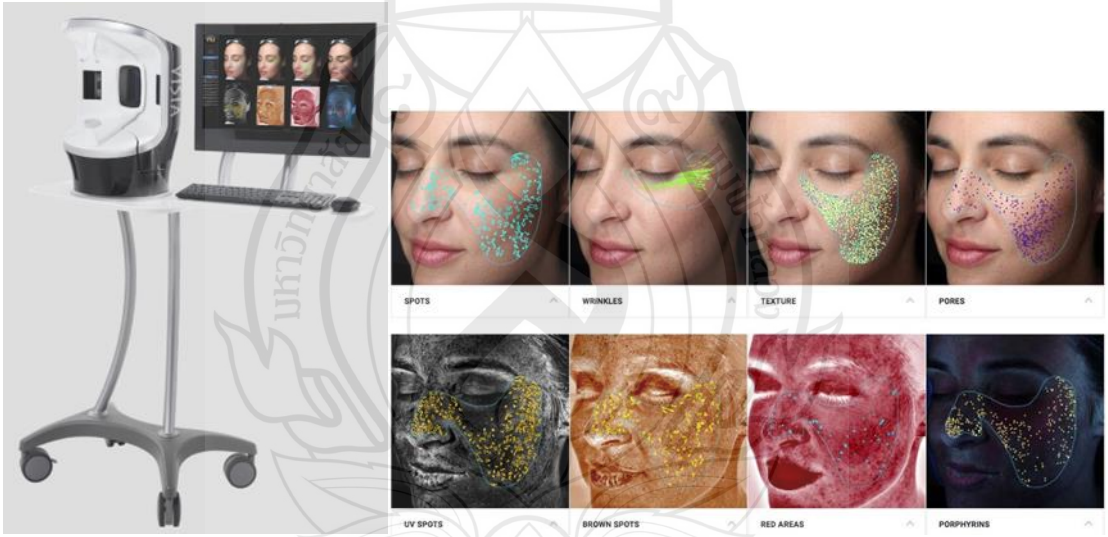


Figure 3.3 VISIA and parameters that can be measured by VISIA

VISIA Complexion Analysis System is the device for the facial skin scanning and captures key visual information by using multi-spectral imaging and analysis system. It is used to measure 8 parameters of skin's overall conditions such as pigmentation, dark spots, UV spots, photo damage area, inflammation, pores, wrinkles, skin texture, red areas and porphyrin (evidence of bacteria).

3.8.3 *Lactobacillus* Serum

Ingredients list of *Lactobacillus* serum

3.8.3.1 Propylene glycol

3.8.3.2 Xanthan gum

3.8.3.3 Glycerin

3.8.3.4 Hydroxyethyl acrylate

3.8.3.5 Sodium acryloyldimethyl taurate copolymer

3.8.3.6 *Lactobacillus* ferment

3.8.3.7 Glycerin and aqua and polymnia sinchifolia root juice

3.8.3.8 Phenoxyethanol

3.8.3.9 Triethylene glycol

3.9 Steps of Research

3.9.1 Selection of Volunteers

Volunteers with wrinkles were chosen under the inclusion and exclusion criteria.

3.9.2 Explanation about Research

Volunteers were explained about the aims and steps of research, and possible side effects of the treatments.

3.9.3 Informed Consent

Volunteers signed the informed consent form for participation in the study.

3.9.4 History Taking

General information such as age, sex, address, contact number, occupation and previous medical history were collected.

3.9.5 Patch Test

Lactobacillus serum was applied on the volunteers' arms under waterproof patch test and waited for one day (24 hours). The subject had to keep away from excessive sweating activities, prolong exposure to daylight during the test and

monitored any effects until 48 and 96 hours. Any reaction that was found at the area of patch test was noted according to the international contact dermatitis research group system as follow:

+? = any doubtful reaction: only mild redness

+ = weak positive reaction: skin redness and the skin become slightly thickened

++ = strong positive reaction: red swollen skin with individual small water blister

+++ = extreme positive reaction: intense redness and swelling with coalesced spreading reaction or large blisters

The red skin will improve after the patch is removed.

NT = not tested

IR = irritant reaction

Participants with positive patch test result of ++ and above were excluded for the research.

3.9.6 Photo Taking and Measurement

The researcher measured skin elasticity by using Cutometer MPA 580 and measured facial wrinkle score by using the VISIA complexion analysis system. The photographs were taken under the favorable condition.

Favorable condition: switch off the lighting and take the photo with 12-megapixel resolution camera with automatic white balance correction and automatic focus.

Facial position: Right 37, Left 37, Center 0, multi-spectral imaging (cross polarized flash and UV lighting, standard daylight fluorescent lighting).

2 drops (about 1cc) of *Lactobacillus* serum is applied to each side of the volunteer's face in the morning and at night. The data had to be collected at the baseline and every follow-up. After applying serum for 4 weeks continuously, the volunteers had to come for follow-up and photos and measurements were recorded. Skin elasticity and skin moisturization were measured with cutometer MPA 580 and facial wrinkle score was measured by VISIA. After that, the data were collected and calculated for every visit.

3.9.7 Instructions to Apply Serum

Clean the face with mild cleansing foam in the morning and at night as the following steps:

1. wash the face with clean water and mild cleansing foam following a gentle rub the face by fingers.

2. Afterwards, splash the face with water again until the face is totally out of foam.

3. Use the clean and dry tissue or towels to wipe the face.

Lactobacillus serum will be used twice a day by the following steps:

1. After washing and drying the face, the hand is also cleaned with a mild, wet alcohol-free tissue.

2. Next, the serum is put by 2 drops (1 cc) on each side of the face and applied using the palm of the hand.

3. Apply the serum on the face until it is fully absorbed into the face skin.

4. All of the above steps have to be redone in the morning and at night.

5. Explain the volunteers thoroughly not to apply any other serum on the face.

6. The volunteers have to be checked regularly every 4 weeks during the study period and the progress and adverse effects will be noted in each visit.

Figure 3.4 Proper steps to apply serum on the face

3.10 Data Collection and Analysis

Skin elasticity data was recorded with Cutometer MPA 580. Data were taken at 1 cm above the eyebrow within the mid-papillary line, 1 cm below the lower eyelid within mid-papillary line, and 1 cm below and lateral to the alar of the nose. Facial wrinkle score evaluations were recorded using photographs taken with the VISIA.

3.10.1 General data were noted: age, sex, occupation, and past history associated with skin aging and previous skincare treatment history.

3.10.2 Skin elasticity score from Cutometer MPA 580.

3.10.3 Facial assessment of wrinkle score from the VISIA complexion analysis system.

3.10.4 Rao-Goldman 5-points visual score by dermatologist

Score 1 = Wrinkle absent

Score 2 = Shallow but visible

Score 3 = Moderate wrinkle

Score 4 = Deep wrinkle with well-defined edges

Score 5 = Very deep wrinkles

3.10.5 Access patient satisfaction score in 0th and 12th week.

Score 0 = No satisfaction

Score 1 = Little satisfaction

Score 2 = Average satisfaction

Score 3 = More satisfaction

Score 4 = Most satisfaction

3.10.6 The side effects were noted if present.

3.11 Statistic

Microsoft Excel 2010 recorded the medical record data and the results of the volunteers in this investigation.

3.11.1 The general data were calculated with descriptive statistics

Percentage of frequencies, mean, modes, ranges and SD standard deviations

3.11.2 Compare skin elasticity by Cutometer score before and after the study

3.11.2.1 Normal distribution: repeated measure ANOVA test

3.11.2.2 No normal distribution: Wilcoxon sign rank test

*CI 95% (p-value 0.05%)

3.11.3 Compare the mean change of skin elasticity by Cutometer, change of wrinkle score by VISIA and Rao Goldman 5 point visual score at 0th, 4th, 8th and 12th weeks

3.11.3.1 Normal distribution: repeated ANOVA test

3.11.3.2 No normal distribution: Friedman's test

3.11.4 Descriptive statistics were used to analyze the satisfactory score of the volunteers between both sides at 12th week and to analyze side effects.

*CI 95% (p-value 0.05%)n this investigation.

CHAPTER 4

RESULTS

4.1 Participants' Demographic Characteristics

General demographic data of 16 participants were recorded as shown in table 4.1 for the study. 14 participants completed the study and 2 participants dropped out the study for personal cases at 8th week. Their age range is 30-55 years.

Table 4.1 Participants' demographic characteristics

Demographic	n=16
Gender	
• Male	9
• Female	7
Occupation	
• Student	5
• Employee	7
• Employer	2
Underlying disease	
• Yes	0
• No	16
History of facial treatment before study	
• Yes	0
• No	16

Table 4.1 (continued)

Demographic	n=16
History of medication	
• Yes	0
• No	16
Photosensitivity or drug induced photosensitivity	
• Yes	0
• No	16
Skin types	
• Dry	3
• Oily	5
• Combination	8

4.2 Cutometer Results

4.2.1 1 cm Above the Eyebrow Within the Mid Pupillary Line

Table 4.2 Statistical analysis of Cutometer at 1 cm above the eyebrow within the mid pupillary line landmark on follow-up 0th, 4th, 8th, and 12th week

	Overall (n=14)	Right side (n=14)	Left side (n=14)
Week 0	0.4782±0.1414	0.5159±0.2257	0.4404±0.1635
Week 4	0.6764±0.1074	0.7124±0.1687	0.6404±0.2251
Week 8	0.6151±0.1640	0.6453±0.1339	0.5848±0.2352
Week 12	0.6513±0.1051	0.5637±0.2515	0.7389±0.1892
P-value	<0.001*	0.074	0.004*

Note Data expressed as mean±SD, P-value determine by Repeated measure ANOVA and Multiple comparison determines by the Bonferroni method

* Statistically significant at the 0.05 level

According to the statistical analysis result from table 4.2, the mean of cutometer at 1 cm above the eyebrow within the mid pupillary line landmark overall on 0th week was 0.4782±0.1414, 4th week 0.6764±0.1074, 8th week 0.6151±0.1640, and 12th week 0.6513±0.1051. The mean of cutometer in each visit statistically significant increased at the level of 0.05 ($p<0.001$). Considering the split-faces of participants indicated that mean of cutometer at 1 cm above the eyebrow within the mid pupillary line landmark right side on 0th week was 0.5159±0.2257, 4th week 0.7124±0.1687, 8th week 0.6453±0.1339, and 12th week 0.5637±0.2515. The mean of cutometer in each visit not statistically significant difference ($p=0.074$). The mean of cutometer at 1 cm above the eyebrow within the mid pupillary line landmark left side on 0th week was 0.4404±0.1635, 4th week 0.6404±0.2251, 8th week 0.5848±0.2352, and 12th week 0.7389±0.1892. The mean of cutometer in each visit statistically significant increased at the level of 0.05 ($p=0.004$). Therefore, the multiple comparison analysis is shown in figure 4.1.

Overall	**Week 0** (**0.4782**)	**Week 8** (**0.6151**)	**Week 12** (**0.6513**)	**Week 4** (**0.6764**)
Right side	Week 0 (0.5159)	Week 12 (0.5637)	Week 8 (0.6453)	Week 4 (0.7124)
Left side	Week 0 (0.4404)	Week 8 (0.5848)	Week 4 (0.6404)	Week 12 (0.7389)

Figure 4.1 The line joining for multiple comparison of cutometer at 1 cm above the eyebrow within the mid pupillary line landmark between follow-up 0th, 4th, 8th and 12th week

According to the line joining for multiple comparison result from figure 4.1, It found that mean of cutometer at 1 cm above the eyebrow within the mid pupillary line landmark overall on 4th, and 12th week higher than 0th week statistically significant at the level of 0.05. (%change 28.27% and 26.56%, respectively). For left side faces, the mean of cutometer at 1 cm above the eyebrow within the mid pupillary line landmark on 12th week higher than 0th week statistically significant at the level of 0.05 (%change 40.47%). There were not statistically significant differences for all pair comparison on the right side faces.

4.2.2 1 cm Below the Lower Eyelid Within the Mid Pupillary Line

Table 4.3 Statistical analysis of Cutometer at 1 cm below the lower eyelid within the mid pupillary line landmark on follow-up 0th, 4th, 8th, and 12th week

	Overall (n=14)	**Right side (n=14)**	**Left side (n=14)**
Week 0	0.4498±0.1462	0.4089±0.2005	0.4908±0.1598
Week 4	0.5941±0.1060	0.5984±0.1215	0.5899±0.1376
Week 8	0.5801±0.084	0.5954±0.1036	0.5647±0.1151
Week 12	0.6540±0.2287	0.6222±0.1922	0.6858±0.4299
P-value	0.015*	0.003*	0.222

Note Data expressed as mean±SD, P-value determine by Repeated measure ANOVA and Multiple comparison determines by the Bonferroni method

* Statistically significant at the 0.05 level

According to the statistical analysis result from table 4.2, the mean of cutometer at 1 cm below the lower eyelid within the mid pupillary line landmark overall on 0th week was 0.4498±0.1462, 4th week 0.5941±0.1060, 8th week 0.5801±0.084, and 12th week 0.6540±0.2287. The mean of cutometer in each visit statistically significant increased at the level of 0.05 (p=0.015). Considering the split-faces of participants indicated that mean of cutometer at 1 cm below the lower eyelid

within the mid pupillary line landmark right side on 0th week was 0.4089±0.2005, 4th week 0.5984±0.1215, 8th week 0.5954±0.1036, and 12th week 0.6222±0.1922. The mean of cutometer in each visit statistically significant increased at the level of 0.05 (p=0.003). The mean of cutometer at 1 cm below the lower eyelid within the mid pupillary line left side landmark on 0th week was 0.4908±0.1598, 4th week 0.5899±0.1376, 8th week 0.5647±0.1151, and 12th week 0.6858±0.4299. The mean of cutometer in each visit not statistically significant difference (p=0.222). Therefore, the multiple comparison analysis is shown in figure 4.2.

Overall	**Week 0** (**0.4498**)	**Week 8** (**0.5801**)	**Week 4** (**0.5941**)	**Week 12** (**0.6540**)
Right side	Week 0 (0.4086)	Week 4 (0.5984)	Week 8 (0.5954)	Week 12 (0.6222)
Left side	Week 0 (0.4908)	Week 8 (0.5647)	Week 4 (0.5899)	Week 12 (0.6858)

Figure 4.2 The line joining for multiple comparison of cutometer at 1 cm below the lower eyelid within the mid pupillary line landmark between follow-up 0th, 4th, 8th and 12th week

According to the line joining for multiple comparison result from figure 4.2, It found that mean of cutometer at 1 cm below the lower eyelid within the mid pupillary line landmark overall on 12th week higher than 0th week statistically significant at the level of 0.05. (%change 31.19%). For right side faces, the mean of cutometer at 1 cm below the lower eyelid within the mid pupillary line landmark on 8th and 12th week higher than 0th week statistically significant at the level of 0.05 (%change 31.41% and 34.23%, respectively). There were not statistically significant differences for all pair comparison on the left side faces.

4.2.3 1 cm Below and Lateral to the Alar of the Nose

Table 4.4 Statistical analysis of Cutometer at 1 cm below and lateral to the alar of the nose landmark on follow-up 0th, 4th, 8th, and 12th week

	Overall (n=14)	**Right side (n=14)**	**Left side (n=14)**
Week 0	0.5582±0.1247	0.5879±0.1318	0.5286±0.1573
Week 4	0.6429±0.1273	0.6332±0.1619	0.6526±0.1462
Week 8	0.6637±0.1077	0.6390±0.0985	0.6885±0.1295
Week 12	0.6845±0.1929	0.6883±0.2035	0.6807±0.2032
P-value	0.062	0.336	0.060

Note Data expressed as mean±SD, P-value determine by Repeated measure ANOVA and Multiple comparison determines by the Bonferroni method

* Statistically significant at the 0.05 level

According to the statistical analysis result from table 4.3, the mean of cutometer at 1 cm below and lateral to the alar of the nose landmark overall on 0th week was 0.5582±0.1247, 4th week 0.6429±0.1273, 8th week 0.6637±0.1077, and 12th week 0.6845±0.1929. The mean of cutometer in each visit not statistically significant difference (p=0.062). Considering the split-faces of participants indicated that mean of cutometer at 1 cm below and lateral to the alar of the nose landmark right side on 0th week was 0.5879±0.1318, 4th week 0.6332±0.1619, 8th week 0.6390±0.0985, and 12th week 0.6883±0.2035. The mean of cutometer in each visit not statistically significant difference (p=0.336). The mean of cutometer at 1 cm below and lateral to the alar of the nose landmark left side on 0th week was 0.5286±0.1573, 4th week 0.6526±0.1462, 8th week 0.6885±0.1295, and 12th week 0.6807±0.2032. The mean of cutometer in each visit not statistically significant difference (p=0.060). Therefore, the result of multiple comparison analysis revealed that not statistically significant differences for all pair comparison on the overall, right, and left side faces as shown in figure 4.3.

Overall	**Week 0 (0.5582)**	**Week 4 (0.6429)**	**Week 8 (0.6637)**	**Week 12 (0.6845)**
Right side	Week 0 (0.5879)	Week 4 (0.6332)	Week 8 (0.6390)	Week 12 (0.6883)
Left side	Week 0 (0.5286)	Week 4 (0.6526)	Week 12 (0.6807)	Week 8 (0.6885)

Figure 4.3 The line joining for multiple comparison of cutometer at 1 cm below and lateral to the alar of the nose landmark between follow-up 0th, 4th, 8th and 12th week

4.3 Facial Assessment Wrinkle Score by VISIA

Table 4.5 Statistical analysis of Facial Assessment Wrinkle score on follow-up 0th, 4th, 8th, and 12th week

Improvement	**0th week, (n=14)**	**4th week, (n=14)**	**8th week, (n=14)**	**12th week, (n=14)**
no changes (0)	14 (100.0)	2 (14.3)	0 (0.0)	0 (0.0)
1-25% fair improvement (1)	0 (0.0)	8 (57.1)	6 (42.9)	0 (0.0)
>25-50% moderate improvement (2)	0 (0.0)	4 (28.6)	8 (57.1)	10 (71.4)
>50-75% good improvement (3)	0 (0.0)	0 (0.0)	0 (0.0)	4 (28.6)

Note Data expressed by number (%)

According to the statistical analysis results from table 4.4, most of facial assessment wrinkle score on 4th week were fair improvement (57.1%), followed by moderate improvement (28.6%) and, no changes (14.3%), respectively. The most of facial assessment wrinkle score on 8th week were moderate improvement (57.1%)

and fair improvement (42.9%), respectively. The most of facial assessment wrinkle score on 12th week were moderate improvement (71.4%) and good improvement (28.6%), respectively.

4.4 Rao-Goldman 5-points Visual Score by Dermatologists

Table 4.6 Statistical analysis of Rao-Goldman 5-points visual score on follow-up 0th, 4th, 8th, and 12th week

	0^{th} week, (n=14)	**4^{th} week, (n=14)**	**8^{th} week, (n=14)**	**12^{th} week, (n=14)**
Wrinkle absent	0 (0.0)	0 (0.0)	0 (0.0)	4 (28.6)
Shallow but visible	0 (0.0)	4 (28.6)	11 (78.6)	10 (71.4)
Moderate deep	9 (64.3)	9 (64.3)	3 (21.4)	0 (0.0)
Deep wrinkle with well defined edges	5 (35.7)	1 (7.1)	0 (0.0)	0 (0.0)

Note Data expressed by number (%)

According to the statistical analysis results from table 4.5, most of Rao-Goldman 5-points visual score on 0th week were moderate deep (64.3%) and deep wrinkle with well defined edges (35.7%), respectively. The most of Rao-Goldman 5-points visual score on 4th week were moderate deep (64.3%), followed by shallow but visible (28.6%) and deep wrinkle with well defined edges (7.1%), respectively. The most of Rao-Goldman 5-points visual score on 8th week were shallow but visible (78.6%) and moderate deep (21.4%), respectively. The most of Rao-Goldman 5-points visual score on follow-up 12th week were shallow but visible (71.4%) and wrinkle absent (28.6%), respectively.

4.5 Patients' Satisfactory Score

Table 4.7 Statistical analysis of patient satisfaction score on follow-up 12th week

Patients' satisfactory	**(n=14)**
Most satisfaction (4)	5
More satisfaction (3)	7
Average satisfaction (2)	2

Note Data expressed by number

According to the patients' satisfactory score on 12th week result from table 4.10, The majority of the subjects rated most satisfaction (5 subjects) and more satisfaction (7 subjects) and average satisfaction (2 subjects), respectively.

4.6 Adverse Effects

There was no adverse effect in all participants during and after the study with *Lactobacillus* serum.

CHAPTER 5

DISCUSSION, CONCLUSION AND SUGGESTIONS

5.1 Discussion

Wrinkles are creases, folds or ridges in the skin which naturally appear as people get older. Along with the aging, the skin becomes thinner, drier and less elastic, which means the ability to protect itself form damage is decreased. Thinning of the skin leads to wrinkles, creases and lines on the skin. There are various methods for rejuvenation that people are trying nowadays such as topical serum, cream, chemical peel, botox, filler, threads, microdermabrasion, ablative and non-ablative laser, and cosmetic surgeries. Each method has pros and cons. As for the daily basic skin care, topical usage of rejuvenating serum or cream is recommended. Topical usage of rejuvenating serum has pros such as low cost, little or no side effects and can be used as a daily skin care routine.

Lactobacillus is one of the lactic acid bacteria (LAB) species, which are gram-positive organisms and are acid-tolerant, and strictly fermentative. *Lactobacillus* played an essential role in enhancing human cell immunity and maintaining a healthy surface barrier. It balances the systemic immune status, including the release of regulatory cytokines, which play an important role in skin homeostasis. Thus, the use of the effects of *Lactobacillus* on the skin is becoming one of the topics of interest today. *Lactobacillus* is applicable to the skin where there is a normal micro flora. *Lactobacillus* can prevent skin aging from extrinsic factors by means of antioxidant and anti-inflammatory.

Prebiotics are non-digestible, selectively fermented dietary fibers. They serve as food to increase the growth of good microorganisms like *Lactobacillus*. Prebiotics function as rebalance the composition of skin’s micro flora, protection of TEWL, reduction of MMPs and promoting *Lactobacillus* in the skin. So, there are the

synergistic effects between prebiotics and probiotics that provide additional benefits to the consumers.

This was about *Lactobacillus* serum for the reduction of facial wrinkles with clinical controlled experimental study. In this study, there were 16 volunteers of 9 males and 7 females selected with inclusion and exclusion criteria and 14 out of 16 volunteers came regular follow up and completed the research. To analyze the demographic data, descriptive statistical analysis was used. This was carried out in healthy male and female subjects aged between 30-55 years old. All the participants had no history of underlying disease, hypersensitivity and personal medication. All participants did not receive any facial treatment before the study. All participants have no history of treatment before the study. 8 of the participants had combination skin and 5 had oily skin and 3 had dry skin.

In this study, wrinkle evaluation score by VISIA, Rao-Goldman 5-point visual scale, cutometer score, patients' satisfactory score and adverse effects were measured to evaluate the efficacy of *Lactobacillus* serum. For statistical analysis of wrinkle evaluation score by VISIA, Rao-Goldman 5-point visual scale, cutometer score, Friedman test was used to analyze the data and post hoc test was used to compare with different visits. Descriptive analysis was used for patients' satisfactory score and adverse effects. The outcomes indicated that there was a statistically significant reduction in facial wrinkles when compared to baseline visit. According to the collected data, following is the outcome of the efficacy of *Lactobacillus* serum.

Improvement was measured with wrinkle evaluation score by VISIA. According to the statistical analysis results, most of facial assessment wrinkle score on 4th week were fair improvement (57.1%), followed by moderate improvement (28.6%) and, no changes (14.3%), respectively. The most of facial assessment wrinkle score on 8th week were moderate improvement (57.1%) and fair improvement (42.9%), respectively. The most of facial assessment wrinkle score on 12th week were moderate improvement (71.4%) and good improvement (28.6%), respectively. The results were indicating that the improvement was increased in each follow-up.

Rao-Goldman 5-point visual scale was also used to evaluate the wrinkles. According to the statistical analysis results, most of Rao-Goldman 5-points visual score on 0th week were moderate deep (64.3%) and deep wrinkle with well defined

edges (35.7%), respectively. The most of Rao-Goldman 5-points visual score on 4th week were moderate deep (64.3%), followed by shallow but visible (28.6%) and deep wrinkle with well defined edges (7.1%), respectively. The most of Rao-Goldman 5-points visual score on 8th week were shallow but visible (78.6%) and moderate deep (21.4%), respectively. The most of Rao-Goldman 5-points visual score on follow-up 12th week were shallow but visible (71.4%) and wrinkle absent (28.6%), respectively.

According to the patients' satisfactory score on 12th week result, The majority of the subjects rated most satisfaction (5 subjects) and more satisfaction (7 subjects) and average satisfaction (2 subjects), respectively.

According to the statistical analysis result, the mean of cutometer at 1 cm above the eyebrow within the mid pupillary line landmark overall on 0th week was 0.4782±0.1414, 4th week 0.6764±0.1074, 8th week 0.6151±0.1640, and 12th week 0.6513±0.1051. The mean of cutometer in each visit statistically significant increased at the level of 0.05 ($p<0.001$).

According to the statistical analysis result, the mean of cutometer at 1 cm below the lower eyelid within the mid pupillary line landmark overall on 0th week was 0.4498±0.1462, 4th week 0.5941±0.1060, 8th week 0.5801±0.084, and 12th week 0.6540±0.2287. The mean of cutometer in each visit statistically significant increased at the level of 0.05 ($p=0.015$).

According to the statistical analysis result, the mean of cutometer at 1 cm below and lateral to the alar of the nose landmark overall on 0th week was 0.5582±0.1247, 4th week 0.6429±0.1273, 8th week 0.6637±0.1077, and 12th week 0.6845±0.1929. The mean of cutometer in each visit not statistically significant difference ($p=0.062$).

The study period is 12 weeks (3 months). There might be stronger effects and higher results if it is studied for more than 3 months. Nevertheless, the overall parameters of the study indicated that *Lactobacillus* serum is effective on reducing facial wrinkles. Any adverse effects such as erythema, skin irritation and hyper or hypopigmentation were not detected. For better results and efficacy, it should be used longer in further studies.

5.2 Conclusion

In this study, *Lactobacillus* serum can more significantly reduce facial wrinkles by promoting the skin elasticity starting from 8th week and 12th week. The serum is easily absorbed into the skin, easy to apply and has no side effects. The serum can reduce overall wrinkles of moderate and deep wrinkles. The study also received high score of patient's satisfaction and there was no adverse effect found. In conclusion, the daily topical application of *Lactobacillus* serum is safe and effective for wrinkle. Therefore, *Lactobacillus* can be an alternative treatment for anti-aging and facial wrinkle reduction.

5.3 Limitations

Some lifestyle habits such as smoking, drinking alcohol, eating junk foods, prolong sun exposure time and lack of sleeping can effect the wrinkles and the researcher cannot control these habits completely.

5.4 Suggestions

5.4.1 This study may be used as an alternative anti-aging treatment for wrinkles reduction and the collected data may be used in comparation with other topical anti-aging treatment.

5.4.2 The study may be useful as a database for further research about skin rejuvenation and elasticity of the skin.

5.4.3 The cosmetic use of *Lactobacillus* serum can be advantageous to other aesthetic conditions. So, it can be used as a reference for further researches and studied about the efficacy and safety for other cosmetic application.

5.4.4 It is possible to research about the efficacy and safety of *Lactobacillus* for clinical conditions such as acne and infection.

REFERENCES

REFERENCES

[1] Kim, J., Lask, G., & Nelson, A. (2010). *Comprehensive aesthetic rejuvenation*. CRC Press.

[2] Chuarienthong, P., Lourith, N., & Leelapornpisid, P. (2010). Clinical efficacy comparison of anti-wrinkle cosmetics containing herbal flavonoids. *Int J Cosmet Sci, 32*(2), 99-106.

[3] Manosroi, A., Jantrawut, P., Akihisa, T., Manosroi, W., & Manosroi, J. (2011). In vitro and in vivo skin anti-aging evaluation of gel containing niosomes loaded with a semi-purified fraction containing gallic acid from Terminalia chebula galls. *Pharm Biol, 49*(11), 1190-1203.

[4] Zerbinati, N., Riva, F., Paulli, M., Parodi, P. C., & Calligaro, A. (2016). Skin treatment with pulsed monochromatic UVA1 355 device and computerized morphometric snalysis of histochemically identified langerhans cells. *Biomed Res Int, 2016,* Article 3415136. https://doi.org/10.1155/2016/3415136

[5] Cinque, B., Palumbo, P., Torre, C. L., Melchiorre, E., Corridoni, D., Miconi, G., . . . Giuliani, M. (2016). Probiotics in Aging Skin. In M. Farage, K. Miller, & H. Maibach (Eds.), *Textbook of Aging Skin*. Springer.

[6] Lee, G. R., Maarouf, M., Hendricks, A. J., Lee, D. E., & Shi, V. Y. (2019). Topical probiotics: The unknowns behind their rising popularity. *Dermatol Online J, 25*(5), 1-3.

[7] Bowe, W. P., & Logan, A. C. (2011). Acne vulgaris, probiotics and the gut-brain-skin axis-back to the future?. *Gut Pathog, 3*(1), 1-11. https://doi.org/10.1186/17574749-3-1

[8] Kang, Y. M., Hong, C. H., Kang, S. H., Seo, D. S., Kim, S. O., Lee, H. Y., . . . An, H. J. (2020). Anti-photoaging effect of plant extract fermented with Lactobacillus buchneri on CCD-986sk fibroblasts and HaCaT keratinocytes. *J Funct Biomater, 11*(1). 1-14. https://doi.org/10.3390/jfb11010003

[9] Walters, K. A., & Roberts, M. S. (2002). The structure and function of skin. In A. W. Kenneth (Ed.,). *Dermatological and Transdermal Formulations* (pp. 19-58). CRC Press.

[10] Lawton, S. (2019) *Skin 1: The structure and functions of the skin*. Nursing Times. https://www.nursingtimes.net/clinical-archive/dermatology/skin-1-the-structure-and-functions-of-the-skin-25-11-2019/

[11] Kang, S., Fisher, G. J., & Voorhees, J. J. (2001). Photoaging: Pathogenesis, prevention, and treatment. *Clin Geriatr Med, 17*(4), 643-659.

[12] Parsons, P. A. (1996). The limit to human longevity: An approach through a stress theory of aging. *Mech Ageing Dev, 87*(3), 211-218.

[13] Hashizume, H. (2004). Skin aging and dry skin. *J Dermatol, 31*(8), 603-609.

[14] Makrantonaki, E., Zouboulis, C. C., & Williamn J. (2007). Cunliffe scientific awards: Characteristics and pathomechanisms of endogenously aged skin. *Dermatology, 214*(4), 352-360.

[15] Sjerobabski-Masnec. I., & Situm, M. (2010). Skin aging. *Acta Clinica Croatica, 49*, 515-518.

[16] Puizina-Ivic, N. (2008). Skin aging. *Acta Dermatovenerol Alp Pannonica Adriat, 17*(2), 47-54.

[17] Ortiz, A., & Grando, S. A. (2012). Smoking and the skin. *International Journal of Dermatology, 51*(3), 250-262. https://doi.org/10.1111/j.1365-4632.2011.05205.x

[18] Krutmann, J., Bouloc, A., Sore, G., Bernard, B. A., & Passeron, T. (2017). The skin aging exposome. *Journal of Dermatological Science, 85*(3), 152-161. https://doi.org/10.1016/j.jdermsci.2016.09.

[19] Anson, G., Kane, M. A., & Lambros, V. (2016). Sleep wrinkles: Facial aging and facial distortion during sleep. *Aesthetic Surgery Journal, 36*(8), 931-940. https://doi.org/10.1093/asj/sjw074

[20] Yaar, M., & Gilchrest, B. A. (1999). Aging of skin. In I. M. Freedberg, A. Eisen, K. Wolff, K. Austen, L. Goldmsith, . . . T. Fitzpatrick, (Eds.,). *Fitzpatrick's dermatology in general medicine* (pp. 1697-1706). McGraw-Hill Global Education Holdings

[21] Khmaladze, I., Leonardi, M., Fabre, S., Messaraa, C., & Mavon, A. (2020). The skin interactome: A holistic "Genome-Microbiome-Exposome" approach to understand and modulate skin health and aging. *Clin Cosmet Investig Dermatol, 13*, 1021-1040. https://doi.org/10.2147/CCID.S239367

[22] 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

[23] Manríquez, J. J., Cataldo, K., Vera-Kellet, C., & Harz-Fresno, I. (2014). Wrinkles. *BMJ Clin Evid, 22*(2014), 1-47.

[24] Mukherjee, S., Date, A., Patravale, V., Korting, H. C., Roeder, A., & Weindl, G. (2006). Retinoids in the treatment of skin aging: An overview of clinical efficacy and safety. *Clin Interv Aging, 1*(4), 327-348. https://doi.org/10.2147/ciia.2006.1.4.327.

[25] Ganceviciene, R., Liakou, A. I., Theodoridis, A., Makrantonaki, E., & Zouboulis, C. C. (2012). Skin anti-aging strategies. *Dermato-endocrinology, 4*(3), 308-319.

[26] Warren, R. J., Aston, S. J., & Mendelson, B. C. (2011). Face lift. *Plastic and Reconstructive Surgery*, *128*(6), 747-764.

[27] Kim, E. K., Hovsepian, R. V., Mathew, P., & Paul, M. D. (2011). Dermabrasion. *Clinics in Plastic Surgery*, *38*(3), 391-395. https://doi.org/10.1016/j.cps.2011.05.001

[28] Bernard, R. W., Beran, S. J., & Rusin, L. (2000). Microdermabrasion in clinical practice. *Clinics in Plastic Surgery*, *27*(4), 571-577.

[29] Starkman, S. J., & Mangat, D. S. (2020). Chemical Peel (Deep, Medium, Light). *Facial Plastic Surgery Clinics of North America*, *28*(1), 45-57.

[30] Goh, C. L., & Khoo, L. (2002). Laser skin resurfacing treatment outcome of facial scars and wrinkles in Asians with skin type III/IV with the Unipulse CO2 laser system. *Singapore Med J, 43*(1), 28-32.

[31] Kattimani, V., Tiwari, R. V. C., Gufran, K., Wasan, B., Shilpa, P. H., . . . Khader, A. A. (2019). Botulinum toxin application in facial esthetics and recent treatment indications (2013-2018). *J Int Soc Prev Community Dent, 9*(2), 99-105.

[32] Arsiwala, S. Z. (2015). Current trends in facial rejuvenation with fillers. *Journal of Cutaneous and Aesthetic Surgery, 8*(3), 125-126.

[33] Patil, E., Rawtal, B., & Sakharwade, S. (2020). Magical approach of probiotics in cosmetic. *World Journal of Pharmaceutical Research, 9*(11), 404-416.

[34] Lolou, V., & Panayiotidis, M. I. (2019). Functional role of probiotics and prebiotics on skin health and disease. *Fermentation, 5*(2), 1-17. https://doi.org/10.3390/fermentation5020041

[35] Jung, E. Y., Kwon, J. I., Hong, Y. H., & Suh, H. J. (2017). Evaluation of anti-wrinkle effects of duoligo, composed of lactulose and galactooligosaccharides. *Prev Nutr Food Sci, 22*(4), 381-384. https://doi.org/10.3746/pnf.2017.22.4.381

[36] Krutmann, J. (2009). Pre- and probiotics for human skin. *J Dermatol Sci, 54*(1), 1-5. https://doi.org/10.1016/j.jdermsci.2009.01.002

APPENDICES

APPENDIX A

INFORMED CONSENT FORM

ชื่อโครงการวิจัย: การศึกษาประสิทธิภาพของแลคโตบาซิลลัสเซรั่มในการลดริ้วรอยบนใบหน้า /
The Effectiveness of Lactobacillus Serum for Reduction of Wrinkles

ข้าพเจ้า นาย/นาง/นางสาว..... ที่อยู่.....
ได้อ่านรายละเอียดจากเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัยวิจัย
ฉบับวันที่.....

ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัยและสำเนาเอกสารแสดงเจตนายินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนามและลงวันที่ ทั้งนี้ก่อนที่จะลงนาม ข้าพเจ้าได้รับการอธิบายโดยละเอียดจากผู้วิจัยถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้น ประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น

ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัย โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้ารับทราบจากผู้วิจัยว่า หากเกิดอันตรายใด ๆ จากการวิจัย ข้าพเจ้าจะได้รับการรักษาพยาบาล ตามที่ระบุในเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย

ข้าพเจ้ามีสิทธิที่จะถอนตัวออกจากโครงการวิจัยเมื่อใดก็ได้ การถอนตัวนี้ไม่มีผลต่อการรักษาพยาบาลและสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ การรายงานหรือสรุปผลการวิจัยจะไม่ระบุชื่อนามสกุลของข้าพเจ้า การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่าง ๆ ที่เกี่ยวข้อง จะกระทำด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความสมัครใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

..... ลงนามผู้เข้าร่วมในโครงการวิจัย

(.....) ชื่อ-สกุล ผู้เข้าร่วมในโครงการวิจัย (ตัวบรรจง)

วันที่ .....เดือน.....พ.ศ.....

ข้าพเจ้าได้อธิบายโดยละเอียดถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้นประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น ให้ผู้เข้าร่วมในโครงการวิจัยได้ทราบและมีความเข้าใจดีแล้ว พร้อมทั้งลงนามในเอกสารแสดงเจตนายินยอมด้วยความสมัครใจ

..... ลงนามผู้วิจัย

(Kyaw Min Thu) ชื่อ-สกุล ผู้วิจัย (ตัวบรรจง)

..... ลงนามพยาน

(.....) ชื่อ-สกุล พยาน (ตัวบรรจง)

วันที่ .....เดือน.....พ.ศ.....

APPENDIX B

RESEARCH PROFILE (CONFIDENTIAL)

ID .....

General Information (Only official)

1. Date

2. Sex: Male …… Female ……

a. Pregnancy or lactation? 1. Yes…… 2. No ……

3. Age …… years

4. Occupation

……1. Government officer

……2. Employer

……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 of 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 Oral Retinoic Acid (within 12 months)

o Filler injection (Periorbital area: within 12 months)

o Botulinum Toxin Injection (Periorbital area: within 6 months)

o Microdermabrasion

o Intense Pulse Light therapy

o Other treatment ……...

11. Average time exposure to the sunlight during 10 am- 4 pm ……..….min

12. Skin types

o Dry

o Oily

o Combination

APPENDIX C

RESEARCH RECORD: REAEARCHER'S PART

CASE RECORD FORM, CRF

1. Cutometer® MPA 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. Facial Assessment Wrinkle Score by Dermatologist

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

3. Rao-Goldman 5-Points Visual Score evaluated by Dermatologist

Side	Baseline	Week 4	Week 8	Week 12
Left				
Right				

Score

1 = Wrinkle absent

2 = Shallow but visible

3 = Moderately deep

4 = Deep wrinkle with well-defined edges

5 = Very deep with redundant folds

4. Patients' Satisfactory Score

Satisfactory evaluation by Volunteers on 12th week (Please tick the circle)

o Score 0 = no satisfaction

o Score 1 = little satisfaction

o Score 2 = average satisfaction

o Score 3 = more satisfaction

o Score 4 = most satisfaction

5. Side Effect Record (For Researcher's Evaluation)

O Pruritus O Erythema

Side effect (if has) …….in week……..

Treatment ……

Result ……

APPENDIX D

CLINICAL EVALUATION

Table D1 Cutometer score at 1 cm above the eyebrow within the mid pupillary line for Lactobacillus serum 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	0.5616	0.5248	0.6021	0.6128	0.6809	0.6821	0.8135	0.8107
2	0.5276	0.5786	0.5305	0.5677	0.6203	0.6122	0.7008	0.6846
3	0.5873	0.5484	0.6211	0.6342	0.6950	0.6983	0.8051	0.8574
4	0.6119	0.6227	0.7238	0.7576	0.7463	0.7593	0.8073	0.8448
5	0.5677	0.5822	0.6030	0.6194	0.6635	0.6990	0.7372	0.7276
6	0.5557	0.5764	0.6528	0.6207	0.7637	0.7012	0.8342	0.8745
7	0.4615	0.4205	0.5054	0.5769	0.6327	0.6269	0.7219	0.7394
8	0.5689	0.5836	0.6242	0.6119	0.6313	0.6731	0.7003	0.7303
9	0.5322	0.5195	0.6827	0.6953	0.7025	0.7968	0.8256	0.8605
10	0.6885	0.6773	0.7108	0.7027	0.8228	0.8035	0.8517	0.8226
11	0.5434	0.5732	0.6608	0.6761	0.7227	0.7407	0.8473	0.7815
12	0.5916	0.5889	0.6329	0.6829	0.7337	0.7681	0.8734	0.8383
13	0.5193	0.5294	0.6043	0.6566	0.7047	0.7402	0.7462	0.8071
14	0.5909	0.5556	0.6410	0.6640	0.7043	0.7029	0.8333	0.9086

Table D2 Cutometer score at 1 cm below the lower eyelid within the mid pupillary line for Lactobacillus serum 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	0.6121	0.5730	0.7063	0.7041	0.7450	0.8045	0.8564	0.8310
2	0.5161	0.5368	0.5947	0.5732	0.6574	0.6817	0.7115	0.7017
3	0.5514	0.5404	0.6264	0.5660	0.6890	0.6340	0.7719	0.7386
4	0.5000	0.5643	0.6687	0.6068	0.7049	0.7418	0.8564	0.8708
5	0.4650	0.4748	0.6048	0.6134	0.7042	0.7746	0.7737	0.7859
6	0.5048	0.5633	0.6032	0.5902	0.7302	0.7722	0.8021	0.8456
7	0.5309	0.5346	0.6184	0.6452	0.6667	0.6667	0.7104	0.7402
8	0.4098	0.4245	0.5029	0.5923	0.7919	0.7436	0.8540	0.8121
9	0.6025	0.6430	0.7623	0.7692	0.8363	0.8585	0.9245	0.8648
10	0.7772	0.7601	0.8853	0.8645	0.9116	0.8201	0.9643	0.8320
11	0.4115	0.4067	0.5732	0.5154	0.6344	0.6398	0.7273	0.7151
12	0.6290	0.5892	0.6692	0.6802	0.7893	0.7670	0.8860	0.7970
13	0.4333	0.5000	0.6308	0.6405	0.7357	0.7095	0.8659	0.8724
14	0.5182	0.5029	0.5634	0.6189	0.6303	0.6403	0.7660	0.7292

Table D3 Cutometer score at 1 cm below and lateral to the alar of the nose for Lactobacillus serum 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	0.6121	0.5730	0.7063	0.7041	0.7450	0.8045	0.8564	0.8310
2	0.5161	0.5368	0.5947	0.5732	0.6574	0.6817	0.7115	0.7017
3	0.5514	0.5404	0.6264	0.5660	0.6890	0.6340	0.7719	0.7386

Table D3 (Continue)

No	Week 0		Week 4		Week 8		Week 12	
	Right	Left	Right	Left	Right	Left	Right	Left
4	0.5000	0.5643	0.6687	0.6068	0.7049	0.7418	0.8564	0.8708
5	0.4650	0.4748	0.6048	0.6134	0.7042	0.7746	0.7737	0.7859
6	0.5048	0.5633	0.6032	0.5902	0.7302	0.7722	0.8021	0.8456
7	0.5309	0.5346	0.6184	0.6452	0.6667	0.6667	0.7104	0.7402
8	0.4098	0.4245	0.5029	0.5923	0.7919	0.7436	0.8540	0.8121
9	0.6025	0.6430	0.7623	0.7692	0.8363	0.8585	0.9245	0.8648
10	0.7772	0.7601	0.8853	0.8645	0.9116	0.8201	0.9643	0.8320
11	0.4115	0.4067	0.5732	0.5154	0.6344	0.6398	0.7273	0.7151
12	0.6290	0.5892	0.6692	0.6802	0.7893	0.7670	0.8860	0.7970
13	0.4333	0.5000	0.6308	0.6405	0.7357	0.7095	0.8659	0.8724
14	0.5182	0.5029	0.5634	0.6189	0.6303	0.6403	0.7660	0.7292

Table D4 Wrinkle evaluation of Lactobacillus serum by VISIA

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

Table D4 (Continue)

No	Week 0	Week 4	Week 8	Week 12
9	-	1	2	3
10	-	1	2	2
11	-	0	1	2
12	-	2	2	3
13	-	1	2	2
14	-	2	1	2

Table D5 Dermatologists' evaluation score for Lactobacillus serum 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	4	4	4	3	3	3	2	2	2	2	2	2
2	3	3	3	2	2	2	2	2	2	1	1	1
3	3	3	3	3	3	3	2	2	3	1	1	2
4	4	4	4	3	3	3	3	2	2	2	2	2
5	3	3	3	3	3	3	2	2	2	2	2	2
6	3	3	3	3	3	2	2	2	2	1	1	1
7	4	4	3	4	4	3	2	3	2	2	2	2
8	3	3	3	2	2	3	2	2	2	2	2	2
9	3	3	3	2	2	3	2	2	2	1	1	1
10	3	3	3	3	3	2	3	2	2	2	2	2
11	3	3	4	3	3	3	3	2	2	2	2	2
12	4	4	3	3	3	3	3	3	2	2	2	2
13	4	4	4	3	3	3	3	3	2	1	1	1
14	3	3	3	2	3	3	2	3	3	2	2	2

Table D6 Patients' satisfactory score on 12th week for *Lactobacillus* serum

No	Satisfaction score
1	4
2	3
3	3
4	3
5	2
6	4
7	4
8	2
9	4
10	3
11	3
12	4
13	3
14	3

APPENDIX E

MATERIAL

Figure E1 Packaging of *Lactobacillus* serum

APPENDIX F

GRAPH

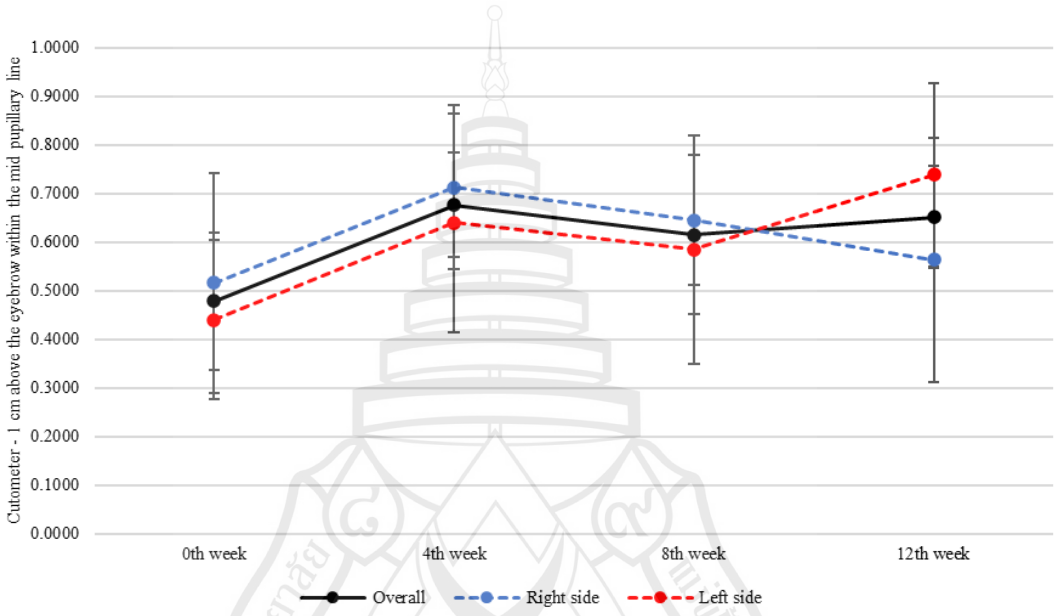


Figure F1 Line graph showing mean of cutometer at 1 cm above the eyebrow within the mid pupillary line landmark in each visit

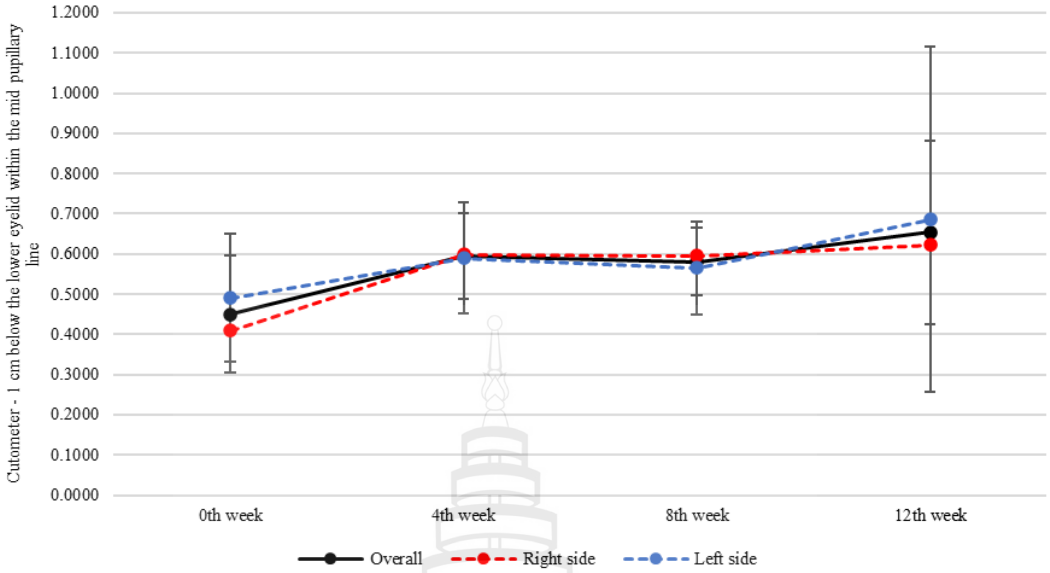


Figure F2 Line graph showing mean of cutometer at 1 cm below the lower eyelid within the mid pupillary line landmark in each visit

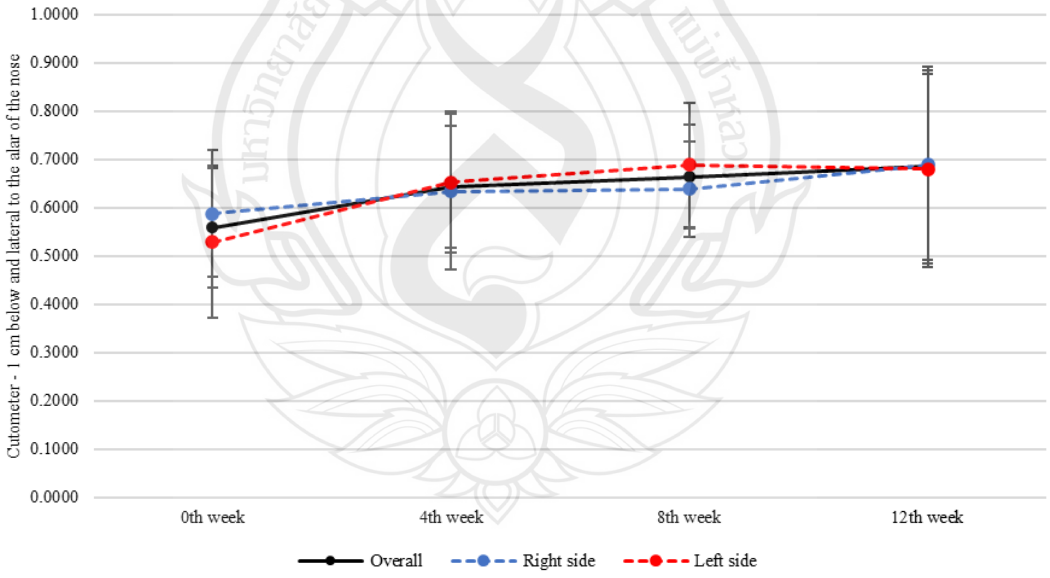


Figure F3 Line graph showing mean of cutometer at 1 cm below and lateral to the alar of the nose landmark in each visit

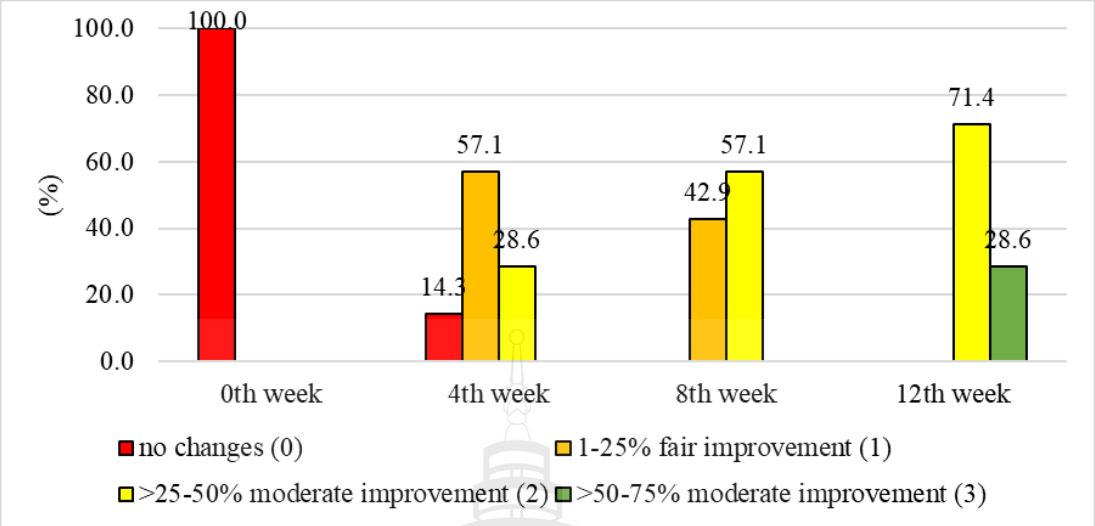


Figure F4 Bar graph showing percentage of facial assessment wrinkle score by VISIA

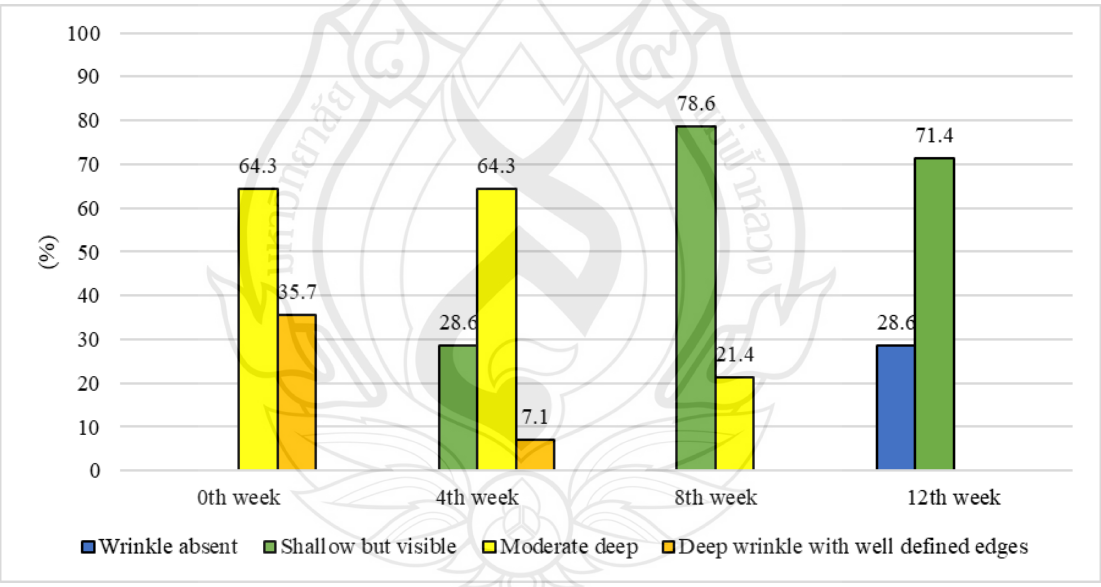


Figure F5 Bar graph showing percentage of Rao-Goldman 5-points visual score by dermatologist

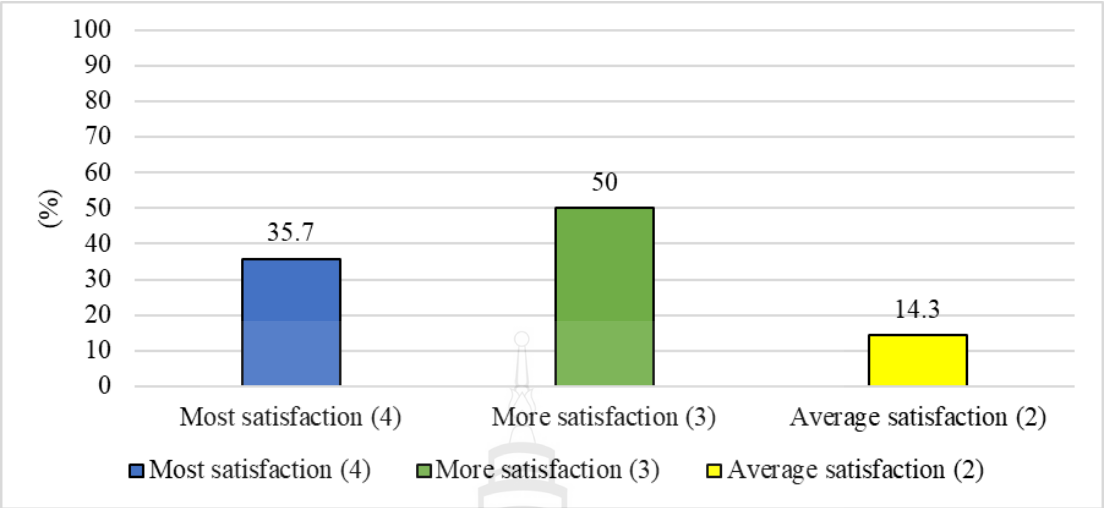


Figure F6 Bar graph showing percentage of patients' satisfactory score