# THE EFFICACY OF 2% COPPER PEPTIDE SERUM FOR EYEBROW HYPOTRICHOSIS: A RANDOMIZED, DOUBLE-BLIND, VEHICLE-CONTROLLED, SPLIT-FACE COMPARATIVE STUDY

**SITT LIN BO**

**MASTER OF SCIENCE**
**IN**
**DERMATOLOGY**

**SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE**
**MAE FAH LUANG UNIVERSITY**
**2025**

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**THESIS APPROVAL**

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**Thesis Title:** The Efficacy of 2% Copper Peptide Serum for Eyebrow Hypotrichosis: A Randomized, Double-blind, Vehicle-controlled, Split-face Comparative Study

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Sitt Lin Bo

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| **Advisor** | Professor Thamthiwat Nararatwanchai, Ph. D. |

## ABSTRACT

Background: Eyebrows are essential for protecting the eyes and play a vital role in facial aesthetics, expression, and personal identity. Eyebrow hypotrichosis, characterized by sparse or thinning eyebrow hair, may result from aging, genetic predisposition, over-plucking, or medical conditions, and can negatively affect self-confidence and social interactions. Current options, including cosmetic procedures such as microblading and topical medications, have limitations, highlighting the need for more effective and non-invasive approaches. Copper peptides, particularly glycyl-L-histidyl-L-lysine copper complex (GHK-Cu), have demonstrated wound-healing, anti-inflammatory, and hair growth–promoting properties. However, the efficacy of topical copper peptide (GHK-Cu) formulations for eyebrow hypotrichosis has not been well established.

Objective: This study aims to evaluate the efficacy of 2% copper peptide (GHK-Cu) serum in increasing eyebrow hair density in individuals with eyebrow hypotrichosis.

Materials and Methods: A randomized, double-blind, placebo-controlled, split-face clinical trial was conducted with 18 participants aged 18–40 years. Each participant applied 2% copper peptide (GHK-Cu) serum to one eyebrow and a placebo serum to the other twice daily for 12 weeks. The application side was randomized, and neither the participants nor the investigators were aware of the allocation. Primary outcomes included eyebrow hair count and hair diameter measured using the Visioscan® VC 20plus. Secondary outcomes included eyebrow density assessed by global photographic evaluation by three independent medical doctors using standardized photographs

captured with the VISIA® Skin Analysis System, participant satisfaction, and adverse effects monitored through questionnaires.

Results: The GHK-Cu serum–treated side demonstrated a statistically significant increase in eyebrow hair count and diameter compared with the placebo side, particularly at week 12. Hair count significantly increased on the treated side at week 12 compared with baseline ($p = 0.005$), week 4 ($p = 0.003$), and week 8 ($p = 0.030$), while no significant changes were observed on the placebo side. Hair diameter also showed a significant increase at week 12 compared with baseline ($p = 0.040$). Global photographic assessment scores were significantly higher on the GHK-Cu–treated side at weeks 4, 8, and 12 ($p < 0.05$). Patient satisfaction scores were consistently higher on the treated side, with a significant difference observed at week 12. No serious adverse effects were reported.

Conclusion: Topical 2% copper peptide (GHK-Cu) serum demonstrated significant efficacy in improving eyebrow hair count, diameter, and overall density compared with placebo. The treatment was well tolerated and associated with high participant satisfaction, suggesting that 2% GHK-Cu serum may serve as a safe and effective non-invasive option for enhancing eyebrow hair growth in individuals with eyebrow hypotrichosis.

**Keywords:** Eyebrow Hypotrichosis, Copper Peptide (GHK-Cu), Eyebrow Hair Growth, Split-face Study, Randomized Controlled Trial, Topical Serum

# TABLE OF CONTENTS

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Eyebrows possess the ability to protect our eyes from perspiration and dust, serving a protective function. They also play a significant role in facial expression, nonverbal communication, and personal identity. Together with other coordinated facial movements, eyebrows are capable of expressing a variety of emotions, including surprise, anger, and happiness. Additionally, they assist in facial attractiveness and contribute to sexual dysmorphism through their distinct color, shape, intensity, and distribution [(1)]. For those reasons, eyebrows are an important part of the facial characteristics, and people often undergo aesthetic procedures to recreate them.

Eyebrow hypotrichosis is an undesirable condition that could impact a person's self-confidence and also have an effect on psychosocial interactions [(2)]. Although eyebrow hypotrichosis can have a negative influence on physical characteristics, it does not get as much attention as scalp hair loss. In clinical practice, it could become a challenging issue as a primary complaint or a positive finding during a routine physical examination [(3)].

Causes of eyebrow hypotrichosis may vary from idiopathic or secondary to underlying conditions, such as primary dermatoses, endocrine disorders, autoimmune diseases, infections, trauma, neoplasms, nutritional disorders, etc. [(3)]. Currently, there are only a few options available for eyebrow hypotrichosis treatment because of the limitations of the recommended medications. Cosmetic camouflage techniques such as microblading, tattooing, eyebrow transplantation, and topical medications are among the potential therapeutic approaches; however, their effectiveness can vary greatly [(4)].

The human copper-binding peptide GHK-Cu (glycyl-L-histidyl-L-lysine-$Cu^{2+}$) is a small, naturally occurring tripeptide found in human plasma, saliva, and urine. It can be released during proteolytic degradation of proteins in the extracellular matrix following tissue injury [(5)]. Numerous studies have demonstrated its efficacy in wound

healing and its ability to stimulate the synthesis of collagen, elastin, proteoglycans, and glycosaminoglycans. It also possesses anti-inflammatory, antioxidant, and hair growth-promoting properties [6]. Due to its exceptional benefits, synthetically derived GHK-Cu complexes are widely utilized in various skin care products, including those for rejuvenation, anti-aging, anti-wrinkle, after-sun care, skin moisturization, and hair growth [6-7].

The positive impact of GHK-Cu on hair growth has been shown through various mechanisms. One study demonstrates its potency as a stimulator of follicle growth and preservation in animal models [8]. Moreover, numerous studies suggest that GHK-Cu stimulates the proliferation of dermal papilla cells, which play a crucial role in regulating hair follicle growth cycles. Additionally, it can increase the expression of growth factors, such as vascular endothelial growth factor (VEGF), which promotes blood vessel formation and thus supports the nourishment and growth of hair follicles [9].

Overall, GHK-Cu holds promise as a potential therapeutic agent for promoting hair growth and addressing hair loss concerns. However, its efficacy specifically for eyebrow hair stimulation remains unknown. Conducting a study would provide valuable information regarding the potential therapeutic benefits of GHK-Cu for eyebrow hypotrichosis and possibly providing an effective solution for people who want to improve the appearance of their eyebrows.

## 1.2 Research Questions

Main Question: Can a 2% copper peptide (GHK-Cu) serum increase the overall eyebrow density?

## 1.3 Research Objectives

1.3.1 General Objective

To study the efficacy of a 2% copper peptide (GHK-Cu) serum for eyebrow hypotrichosis.

1.3.2 Specific Objectives

1.3.2.1 Primary Objective

To compare the efficacy of the 2% copper peptide (GHK-Cu) serum and that of the placebo-based serum on eyebrow hair growth by evaluating the number and diameter of eyebrow hairs.

1.3.2.2 Secondary Objectives

1. To compare the efficacy of the 2% copper peptide (GHK-Cu) serum and the placebo-based serum for increasing eyebrow density by using the global photographic assessment score and the participants' satisfaction score.

2. To identify the side effects of the 2% copper peptide (GHK-Cu) serum and placebo-based serum by using a research questionnaire.

## 1.4 Research Hypothesis

1.4.1 Primary Hypothesis:

The 2% copper peptide (GHK-Cu) serum has a higher efficacy than the placebo serum in increasing the number and diameter of eyebrow hairs.

1.4.2 Secondary Hypotheses:

1. The 2% copper peptide (GHK-Cu) serum significantly improves the global photographic assessment score and participant satisfaction score compared to the placebo.

2. The 2% copper peptide (GHK-Cu) serum is safe for use, with a minimal occurrence of side effects similar to the placebo group.

## 1.5 Results Used for the Evaluation

1.5.1 Primary outcomes

1. Number of eyebrow hairs
2. Diameter of eyebrow hairs

1.5.2 Secondary outcomes

1. Global photographic assessment score

2. Participants' satisfaction score

1.5.3 Safety evaluation for side effects

## 1.6 Conceptual Framework

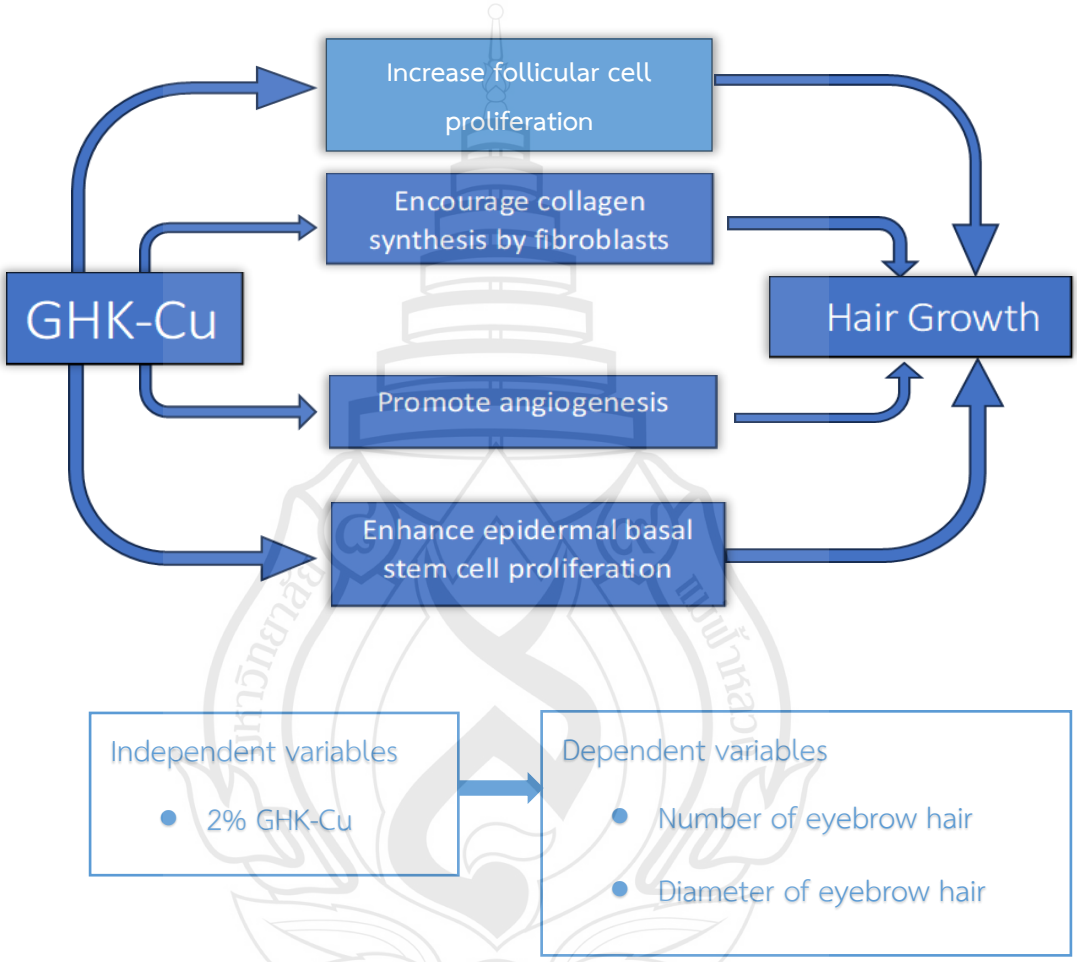



**Figure 1.1** Conceptual framework

## 1.7 Definition of Specific Terms

1.7.1 Efficacy

Efficacy is operationally defined in this study as the serum containing 2% copper peptide (GHK-Cu) performing better than the placebo serum in treating eyebrow hypotrichosis. This is demonstrated by its ability to increase the number and diameter of eyebrow hairs, improve the global photographic assessment score, receive higher

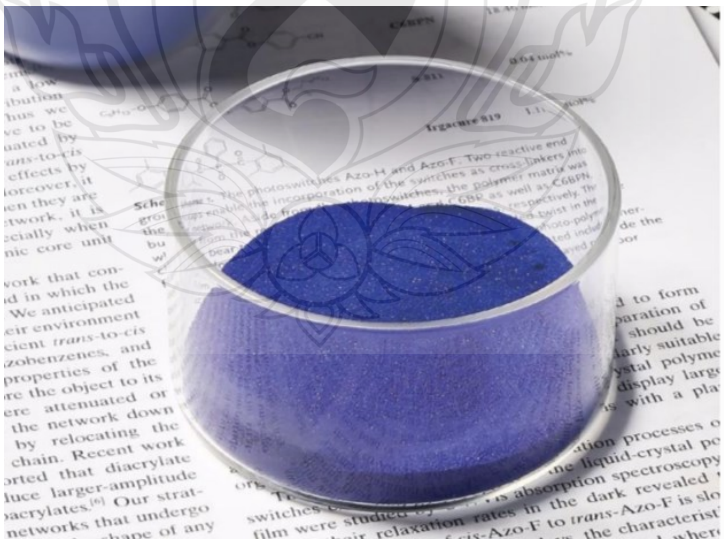
satisfaction scores from participants, and exhibit only a minimal occurrence of side effects.

1.7.2 Eyebrow Hypotrichosis

The term "eyebrow hypotrichosis" refers to a condition in which eyebrow hair diameter and density are less than normal, occurring in healthy individuals without any significant underlying diseases or disorders. It is defined as either Grade 1 or 2 on the Allergan Global Eyebrow Assessment (GEBA) scale, which employs a photonumeric guide. The GEBA tool is a validated 4-point grading system for eyebrow fullness (1 = very sparse, 2 = sparse, 3 = full, and 4 = very full) [10].

1.7.3 Serum Containing 2% Copper Peptide

This serum contains copper peptide complexes (GHK-Cu), consisting of glycyl-L-histidyl-L-lysine tripeptides (GHK) and divalent copper. GHK-Cu is a naturally occurring peptide originally found in human serum and has been the topic of significant study for almost four decades due to its capacity to repair tissue and promote wound healing. A lot of research has also revealed its potential to stimulate hair growth by increasing the size of hair follicles. GHK peptide can be synthesized chemically using well-established methods, particularly solid-phase synthesis techniques [11]. The serum will be formulated using a solvent, complexing agent, humectant, thickener, copper peptide, and preservative.



**Source** [12]

**Figure 1.2** Copper peptide powder (GHK-Cu)

1.7.4 Placebo Serum

The placebo serum will be formulated using the same ingredients, appearance, odor, color, and packaging as the 2% copper-peptide serum. The only difference will be the absence of the active compound—copper peptide.

1.7.5 Global Photographic Assessment Score

This score indicates whether the overall density of eyebrow hair increased or decreased following the administration of the serum. This assessment is based on a photograph taken by the Visia® Complexion Analysis System. Three doctors will serve as the evaluators, and the scores range from −3 to +3.

1.7.6 Number of Eyebrow Hairs

The number of eyebrow hairs will be counted from a photo of a region approximately 1 $cm^2$ in size, captured by a Visioscan® VC 20plus. To capture an image, the researcher will establish an anatomical point at the area of the eyebrow that is directly on the mid-pupillary line where the eyebrow meets. And, this point will be used at every visit to analyze the number of eyebrow hairs.

1.7.7 Diameter of Eyebrow Hairs

The diameter of the eyebrow hairs can be calculated from a photo taken with Visioscan® VC 20plus by measuring the width of the eyebrow hairs at the base of each follicle. The same anatomical point, determined by drawing a perpendicular line from the center of the mid-pupil to the point where the eyebrow meets, will be used to measure the mean diameter of the eyebrow hairs.

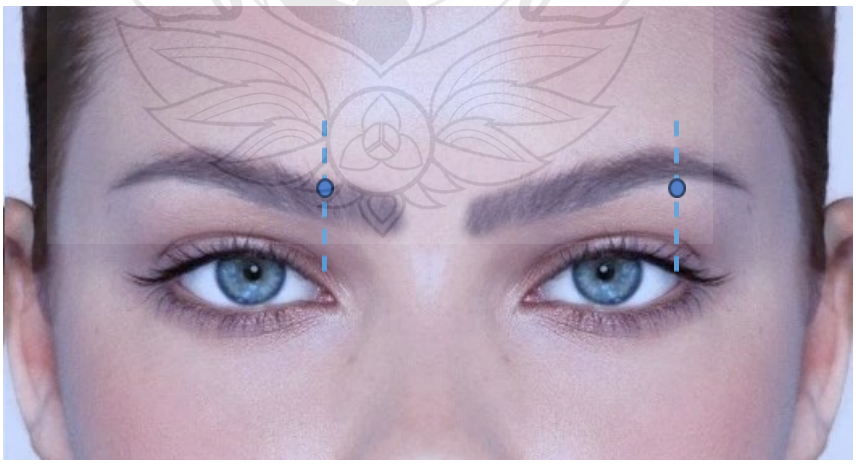


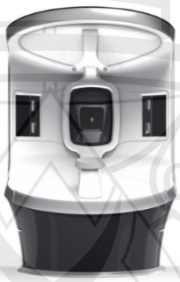
**Figure 1.3** Anatomical reference points used for measuring eyebrow hair count and diameter

1.7.8 Participants' Satisfaction Score

It evaluates participant satisfaction with treatment outcomes by means of questionnaires. Participants will be rated on their satisfaction on each side of the eyebrow by evaluating the satisfaction score, and the score ranges from −3 to +3.

1.7.9 Visia® Complexion Analysis System

This is a modern high-resolution digital camera system for facial analysis and capture known as the Visia® from Canfield Scientific, Inc., based in New Jersey, USA (https://www.canfieldsci.com). Its live image overlay and multi-point positioning system make it simple to capture precisely registered photos to monitor development over time. It can be beneficial for independent comparisons and for providing objective follow-up for various interventions [13].



**Source** [14]

**Figure 1.4** Visia® complexion analysis system

1.7.10 Visioscan® VC 20plus

Visioscan® VC 20plus is a high-resolution UVA-light video camera specifically designed for studying the skin's surface. It is capable of precise measurement and analysis of various parameters, including skin texture, hydration levels, pigmentation, and hair growth. This device is commonly utilized in dermatology and cosmetic research settings to evaluate the effectiveness of skincare products, hair treatments, and cosmetic procedures. With its multi-functional software (SELS® parameters), Visioscan® VC 20plus is an extremely versatile device that provides easy, accurate, and cost-effective characterization of skin surface conditions.

Dimensions of the camera: 5.0 × 5.8 × 12.6 cm; weight: approx. 230 g; cable length: approx. 1.5 m

Sensor resolution: 1/2″ B/W CMOS sensor, 1.3 MP (1280 × 1024 pixels)

Image size: approx. 10 mm × 8 mm

Objective: 20 mm

Aperture: 2.8 mm

Depth of focus: (calculated) approx. ±0.05 mm

Measurement uncertainty for length measurements: < 50 µm (vertically: 0.44%, horizontally: 0.50%, but minimum 2 pixels)


**Source** [15]

**Figure 1.5** Visioscan® VC 20plus

1.7.12 Side Effect Evaluation

To evaluate the side effects, symptoms such as burning, redness, itching, flaking, and abnormal hair growth in an undesirable region after applying the serum will be monitored, and the severity of the condition will be rated from mild to moderate if it is present.

## 1.8 Scope of the Research

Men and women from Thailand, aged 18 to 40, who would like to thicken their eyebrow hair and are experiencing thin eyebrows due to idiopathic hypotrichosis or other unknown causes will be recruited for a voluntary research project. A total of 18 participants will be enrolled for treatment, evaluation, and monitoring at Mae Fah Luang University Hospital in Bangkok for a period of 12 weeks. Each participant will receive two serums: a 2% copper-peptide serum and a placebo serum to apply to the eyebrow area. The "block randomization" method will be used to determine which side of the eyebrow receives which serum. The serums need to be applied twice daily for 12

weeks throughout the project. There will be 4 visits in total: the baseline visit before serum application and monthly visits (weeks 4, 8, and 12) after serum application.

The primary outcome will be an objective evaluation of the number and diameter of eyebrows. Secondary outcomes will include the global photographic assessment score and participants' satisfaction score. The side effects will also be recorded. Changes in the overall eyebrow density will be evaluated by three medical doctors using photos recorded with the Visia® camera. Images captured by Visioscan® VC 20plus will be used to estimate the diameter and number of eyebrows. Questionnaires will be used to assess participant satisfaction, and throughout the study, a history will be taken, and the eyebrow region will be examined for side effects or complications.

# CHAPTER 2

# LITERATURE REVIEW

In this chapter, the researcher has studied and reviewed related literature according to the following topics:

2.1 The Functions and Importance of Eyebrows

2.2 Eyebrow Anatomy and Growth

2.3 Causes of Hypotrichosis

2.4 Treatment of Thin Eyebrows

2.5 Copper Peptide (GHK-Cu)

## 2.1 The Functions and Importance of Eyebrows

Besides being crucial for appearance, eyebrow hair serves a variety of vital biological functions, including protection from the weather, sensory transmission, and shielding the eyes from sweat and rain [16]. Salt, a component of sweat, may contribute to irritation and harm to our eyes. Raising our eyebrows can help direct sweat streams down the sides of our faces and away from our eyes. This protection is crucial for maintaining clear vision and eye health.

Eyebrows play a crucial role in facial expression and contribute to defining the eyes, working in cooperation with the nose, lashes, cheekbones, and hairline to create a distinctive facial feature [16]. The impact of emotions revealed on the face can be enhanced by the eyebrows. Raised eyebrows, for instance, can indicate surprise or worry, while furrowed eyebrows can indicate concentration or anger. These delicate gestures support the emotional message being expressed [1].

As the eyes often stand out as the main feature of the face, well-groomed eyebrows can accentuate the color, shape, and emotion of the eyes by framing them. Nowadays, many cosmetic treatments target the appearance of the eyebrows in an attempt to change the perception that they are essential to beauty. To shape and highlight the arch, common methods include depilating or tweezing, as well as applying

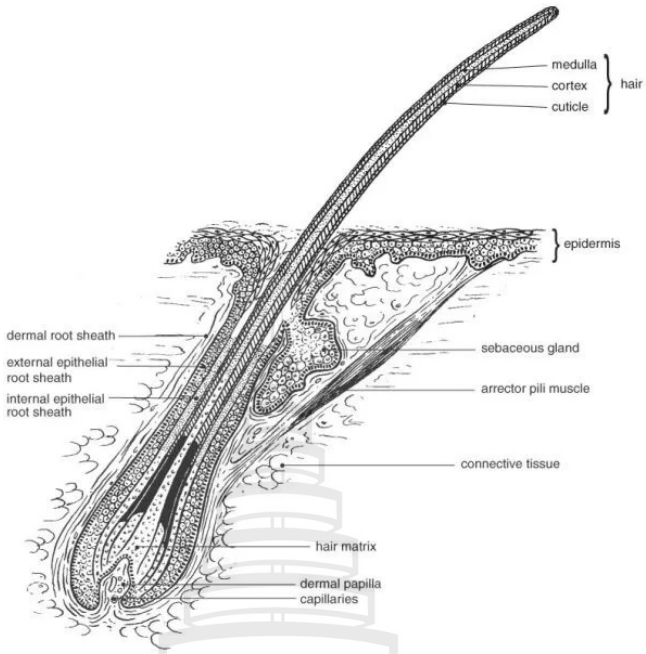
cosmetics to change the length and color. Cosmetic surgery techniques such as Botox injections, permanent tattoos, surgical tucks, and lifts expressly target eyebrow looks, demonstrating the eyebrows' ongoing significance in facial aesthetics [1].

Eyebrows are also important for facial identification. A study has demonstrated that they provide recognition characteristics that aid in facial recognition and the identification of specific individuals. Volunteers in the study were tasked with identifying celebrity photos by the researchers. Using Photoshop, the eyebrows of these celebrities were eliminated. Only a small percentage of the well-known individuals were correctly identified. This highlights the significance of the hair above the eyes in the recognition of each individual [1]. As evidenced by their diverse functions, eyebrows emerge not only as an integral aspect of facial characteristics but also as essential features in daily human interactions and overall well-being.

## 2.2 Eyebrow Anatomy and Growth

### 2.2.1 Eyebrow Anatomy

The body's hair follicles differ in size and form, but they all have the same fundamental structure [16]. Since the anatomical features of eyebrows and body hair are similar, this topic will discuss the anatomy of hair. A hair follicle comprises three segments: lower, middle, and upper [17]. The lower segment, encompassing the bulb and suprabulbar areas, extends from the follicular base to the point where the arrector pili muscle inserts. The short middle segment, known as the isthmus, extends from the meatus of the sebaceous gland to the point of insertion of the arrector pili muscle. Lastly, the infundibulum, or upper segment of the hair follicle, extends from the meatus of the sebaceous gland duct to the follicular opening.




**Source** [18]

**Figure 2.1** Schematic of a longitudinal section of a hair follicle

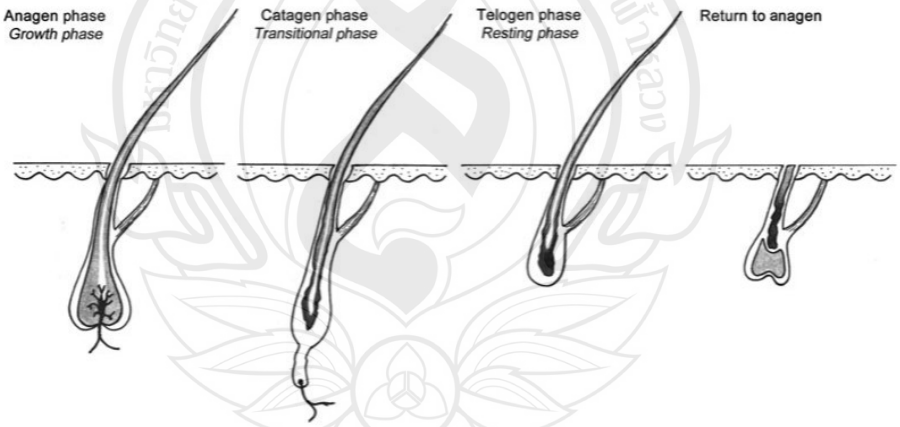
There are three major circular sections that make up each individual hair shaft during the growing period: the cuticle, cortex, and medulla [17]. The innermost layer of hair is known as the medulla, where medullary granules containing citrulline and vacuoles rich in glycogen are observed in the cells. The cortex, the middle layer of the hair shaft, primarily comprises keratin and contains pigment cells. It also stores moisture and determines hair color based on the distribution and type of melanin granules present within. The outer layer, known as the cuticle, provides protection and shine to the hair. It consists of overlapping flat cells that interlock with the inner root sheath, resembling roof shingles when viewed under electron microscopy.

2.2.2 Physiology of Eyebrow Growth

Multiple keratinocyte layers inside a hair follicle interact to form a hair. The process of hair development is dynamic and cyclical, regulated by various hormones and cytokines that control the length of growth cycles. Factors such as age, developmental stage, dietary habits, and environmental changes also influence the duration of growth cycles [19]. Human hair can be categorized into two types. The first is terminal hair, found on hormone-dependent body regions (such as the scalp, beard, chest, axilla, and pubic region) as well as on androgen-independent body regions (such

as eyebrows and lashes). These hairs are made up of hair shafts that are long (> 2 cm), thick (> 60 μm in diameter), pigmented, and medullated. The second type is vellus hair (androgen-independent hair), which covers the remaining part of the body in adults. These hairs are typically unpigmented, short (< 2 cm), and thin (< 30 μm in diameter) [20].

The eyebrow hair cycle has three phases: anagen, catagen, and telogen, similar to any other hairs. The anagen phase is the growing stage of hair, during which stem cells from the bulge play a crucial role in initiating hair growth and forming new hair shafts. The regression stage of follicles is known as catagen. During this phase, follicular keratinocytes undergo apoptosis, leading to the degeneration of the lowest part of the hair follicles. The dermal papilla migrates upward and settles near the hair bulge towards the end of the catagen phase [16]. The resting stage of hair follicles is known as telogen. The percentage of follicles in the telogen stage varies significantly depending on the body area. Following telogen, the lower part of the follicle regenerates. The old hair sheds, and the cycle repeats when the new hair shaft emerges [4].




**Source** [21]

**Figure 2.2** Phases of human hair

The anagen phase of scalp hairs typically lasts an average of 2 to 7 years, the catagen phase approximately 2 to 4 weeks, and the telogen phase approximately 3 months. However, eyebrow hair follicles have a shorter anagen phase lasting only 2 to 4 weeks, with catagen lasting 2 to 3 weeks and telogen typically lasting 2 to 3 months

[14]. The length of hair directly correlates with the duration of anagen. Human brow hair follicles have a limited duration of 2 to 4 weeks in anagen, whereas the scalp follicles can remain in anagen for several years, allowing for longer growth potential. Approximately 10–15% of eyebrow follicles are in anagen phase, while the majority of follicles (85–90%) are in telogen phase [17].

## 2.3 Eyebrow Hypotrichosis

A condition known as "eyebrow hypotrichosis" is characterized by an undesirable eyebrow hair pattern, primarily marked by a reduction in the amount of hair and an absence of hair growth [4]. In clinical practice, eyebrow hypotrichosis poses an important and challenging issue. The loss of eyebrows can be highly distressing for patients since they play significant roles in both social and cosmetic appearance. The prevalence of eyebrow hypotrichosis is not well documented. Studies on alopecia areata report varying rates, with 19.8% affected in a Japanese study and 62.8% in a Danish cohort. Among the Danish patients, severity ranged from minimal thinning to complete loss in over a third of cases [22]. Eyebrow hypotrichosis can be categorized as idiopathic or the result of various kinds of underlying conditions, including primary dermatoses, endocrinopathies, autoimmune conditions, infections, neoplasms, trauma, exogenous agents, and genetic diseases [3].

The underlying disorders associated with eyebrow hypotrichosis can be summarized as follows [4]:

2.3.1 Primary dermatoses: Atopic dermatitis, seborrheic dermatitis

2.3.2 Endocrine disorders: Hypothyroidism, hyperthyroidism

2.3.3 Autoimmune diseases: Alopecia areata, discoid lupus erythematosus, frontal fibrosing alopecia, Graham Little syndrome

2.3.4 Infections: Leprosy, secondary syphilis, herpes zoster

2.3.5 Trauma: Trichotillomania, chemical/electrical/thermal burns, post-surgery, alopecia artefacta, eyebrow tattoo removal

2.3.6 Neoplasms: Folliculotropic mycosis fungoides, Sézary syndrome, squamous cell carcinoma, basal cell carcinoma, malignant melanoma, systemic mastocytosis

2.3.7 Exogenous agents: Acitretin, barbiturates, busulfan, carboplatin, cetuximab, cocaine, cyclophosphamide, docetaxel, heparin, isotretinoin, paclitaxel, propranolol, and thallium poisoning

2.3.8 Nutritional disorders: Chronic marasmus, chronic zinc deficiency, biotin deficiency, iron deficiency, hypoproteinemia

2.3.9 Genodermatoses: Familial eyebrow diffuse alopecia, keratosis follicularis spinulosa decalvans, ulerythema ophryogenes, ectodermal dysplasia, Fraser syndrome, Meige syndrome, Omenn syndrome, Netherton syndrome, and Rothmund-Thomson syndrome

## 2.4 Treatment of Eyebrow Hypotrichosis

There is currently no conventional, evidence-based treatment for eyebrow hypotrichosis apart from surgical transplantation, microblading, cosmetic concealment, eyebrow tattooing, and topical medicines [(2)]. An eyebrow restoration can significantly improve a patient's quality of life because it addresses an important cosmetic concern. However, it is essential for doctors not only to address the patient's cosmetic issues but also investigate possible underlying medical conditions when treating individuals experiencing eyebrow hair loss [(3)].

The term "cosmetic camouflage" for eyebrows refers to the application of cosmetics to conceal or enhance the appearance of sparse or absent eyebrow hair. This typically involves using brow pencils, powders, gels, or pomades to fill in gaps, define the shape, and create a natural-looking brow. For individuals seeking longer-lasting results, cosmetic procedures such as microblading or tattooing can provide more permanent alternatives. However, cosmetic camouflage has several drawbacks, including its temporary nature, time-consuming application process, need for regular maintenance, and potential to cause skin irritation or trigger allergies.

Microblading is a form of superficial micropigmentation where a manual tool with a blade made of stacked needles is used to deposit pigment in the papillary dermis. This technique creates hair-like incisions that are subtle and defined, resembling genuine eyebrow hair. However, the results are semi-permanent, typically lasting between 12 and 18 months. Microblading serves as an intermediate option between surgical transplantation and tattooing. Setting up microblading is simple, but the process requires significant practice and skilled hands [23].

Cosmetic tattooing involves injecting tattoo ink into the dermis to simulate micropigmentation [24]. This process can trigger an inflammatory response due to the deposition of foreign substances, leading to histopathological findings such as lymphocytic infiltration in the dermis. After studying the safety of permanent tattoos, the U.S. FDA discovered several side effects, including granulomas and keloids, allergic responses, and infections [25].

For patients who desire a more natural, thicker, and fuller eyebrow, hair transplantation is a permanent treatment option. Hair transplantation involves transplanting individual hair follicles by harvesting donor hair follicles. This can be accomplished through two methods: either extracting each follicle individually using the follicular unit extraction method (FUE) or amputating a strip of hair-bearing skin, known as the follicular unit transplantation method (FUT). Hair transplantation serves as an alternative treatment method for individuals with severe hair loss or abnormalities of the eyebrows, eyelashes, or both. However, it is a complex procedure, and the approach depends largely on the surgeon's experience and the specific case presentation [26].

Regarding topical therapy, 0.03% bimatoprost, primarily used for treating glaucoma and ocular hypertension, has FDA approval for treating eyelash hypotrichosis [27]. Numerous studies have shown that bimatoprost can also improve eyebrow hypotrichosis, demonstrating improvements in eyebrow growth and patient satisfaction with minimal side effects [4]. Another topical treatment option is minoxidil, which has been found to promote hair development by limiting and regressing the telogen phase of hair follicles while extending the anagen phase, resulting in increased hair follicle size [28]. According to a study, a 2% minoxidil lotion significantly outperformed a

placebo in enhancing eyebrows, proving to be a successful and well-tolerated treatment for eyebrow hypotrichosis [29].

Loss of eyebrows may be an isolated occurrence with no medical implications (idiopathic), but it can also be an indicator of a more serious medical condition. Therefore, it is important to make a proper diagnosis and implement appropriate treatment that focuses on the underlying etiologies [4]. In the case of atopic dermatitis, treatment with emollients and topical corticosteroids has been reported to lead to partial eyebrow regrowth in one case report [30]. Treating seborrheic dermatitis in the eyebrows is safe and effective using topical antifungals, low-potency corticosteroid creams, or topical calcineurin inhibitors [22]. According to findings from a small-scale study, it has been observed that implementing appropriate treatment for hypothyroidism can potentially facilitate the restoration of normal telogen-anagen hair proportions [31]. The treatment of infectious conditions has several variations on alopecia of the eyebrows and eyelashes, while topical and intralesional steroids have long been utilized for autoimmune conditions causing eyebrow alopecia [22].

## 2.5 Copper Peptide (GHK-Cu)

2.5.1 Introduction

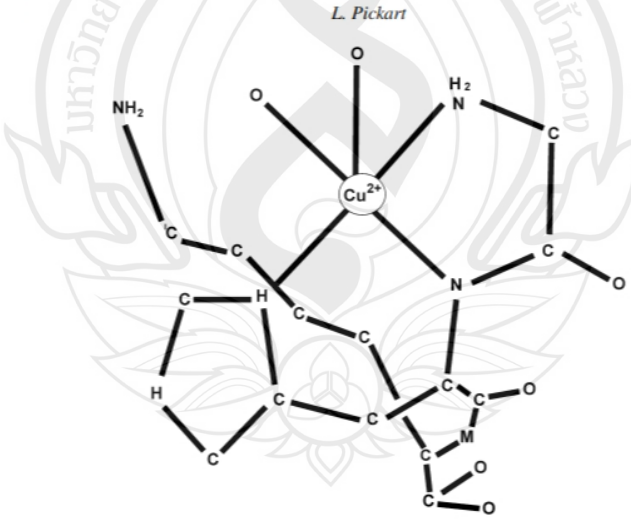
GHK (glycyl-L-histidyl-L-lysine), discovered in 1973 by Pickart and Thaler, is a naturally occurring peptide initially identified in human serum, possessing cellular protective and growth-promoting properties [32]. The molecule possesses a very high affinity for copper (II) and binds with Cu to form GHK-Cu. Both GHK and GHK-Cu have been demonstrated to play a crucial role in anti-inflammatory and tissue remodeling processes. Because of its potent protective and regenerative properties, GHK-Cu is widely used in numerous skincare and hair care products [33].

The copper peptide GHK-Cu has demonstrated numerous benefits for both skin and hair health. These include tightening loose skin, reversing aging-related thinning, repairing skin barrier proteins, improving firmness, elasticity, and clarity, reducing fine lines and wrinkles, smoothing rough skin, diminishing photodamage and hyperpigmentation, stimulating wound healing, protecting against UV radiation,

reducing inflammation and free radical damage, and promoting hair growth, thickness, and follicle size [34].

2.5.2 Biochemical Properties of GHK-Cu

GHK is a natural component found in human plasma, saliva, and urine. It is produced during the proteolytic degradation of extracellular matrix proteins following tissue injury and probably during normal tissue turnover. The plasma level of GHK is approximately 200 ng/mL at the age of 20, but decreases to around 80 ng/mL by the age of 60. GHK stands out in biochemistry due to its small size, facilitating its interaction with membrane receptors, and its unique ability to bind copper, enabling the transfer of copper in and out of cells. It exhibits a high affinity for $Cu^{2+}$ and forms the chelate GHK-Cu. The affinity of GHK for copper is comparable to that of albumin, and even in the presence of albumin, a significant amount of copper can bind to it. Although albumin and GHK can exchange copper with tissues, albumin, being more abundant in plasma, primarily governs copper transport. GHK's effects, such as stimulating collagen synthesis, are likely mediated by specialized receptors, as albumin with copper cannot mimic the actions of GHK-Cu on cellular functions [5].




**Source** [35]

**Figure 2.4** The solution structure of GHK-Cu at pH 7.0

2.5.3 Tissue Remodeling and Wound Healing Ability of GHK-Cu

GHK-Cu has been extensively studied for its wound-healing properties, demonstrating its potential to promote wound healing through various pathways. One study suggested that GHK-Cu has the capacity to regulate the expression and activation of various matrix metalloproteinases through different stages of wound healing, exerting a significant influence on the healing process [36]. In another experiment, animals received either saline or GHK-Cu injections into wound chambers. Analysis revealed that GHK-Cu injections led to a concentration-dependent increase in dry weight, DNA, total protein, collagen, and glycosaminoglycan contents, along with a notable stimulation of collagen synthesis. These findings indicate that GHK-Cu has the ability to enhance extracellular matrix accumulation in wounds in vivo [37].

An open study used a 2% GHK gel to treat diabetic neuropathic ulcers, revealing a significant improvement in ulcer closure rate, particularly in larger ulcers. The closure rate was observed to be three times faster compared to standard care, with a notable reduction in ulcer infection incidence when the gel was applied immediately after debridement [38].

2.5.4 Angiogenesis Ability of GHK-Cu

Angiogenesis plays a crucial role in skin regeneration and healing. GHK-Cu, along with other copper carrier proteins, has been shown to stimulate angiogenesis in rabbit eye models [39]. Additionally, it can enhance the expression of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) in irradiated human dermal fibroblasts, thereby promoting blood vessel formation and facilitating blood flow into injured tissues [40]. SPARC is an extracellular matrix-binding protein that is transiently expressed and modulates cellular morphology and endothelial cell proliferation. According to a report, GHK and other peptides containing the GHK sequence, such as KGHK, released from SPARC during the degradation of the extracellular matrix, contribute to tissue remodeling by upregulating angiogenic levels [41].

2.5.5 Anti-inflammatory and Antioxidant Ability of GHK-Cu

The antioxidant and anti-inflammatory properties of GHK and its copper (II) complex, GHK-Cu, have been extensively studied. In an animal study, GHK demonstrated a reduction in the inflammatory response, thereby preventing the

progression of bleomycin-induced fibrosis in mice. Treatment with GHK significantly improved collagen deposition and restored MMP-9/TIMP-1 balances in lung tissue. Moreover, it decreased the expression of TNF-α and IL-6 in bronchoalveolar lavage fluid (BALF) and MPO in lung extracts [42].

GHK has demonstrated its ability to reduce the levels of reactive oxygen species (ROS), such as hydroxyl (·OH) and peroxyl (ROO·) radicals, in Caco-2 cells. This reduction was measured using flow cytometry and the electron spin resonance (ESR) spin-trapping technique. The capacity of GHK to lower ROS levels, particularly ·OH and ROO·, indicates its potential as an endogenous antioxidant in biological systems. Additionally, GHK exhibited a more potent effect on reducing ·OH compared to other known antioxidative peptides, including reduced glutathione and carnosine [43].

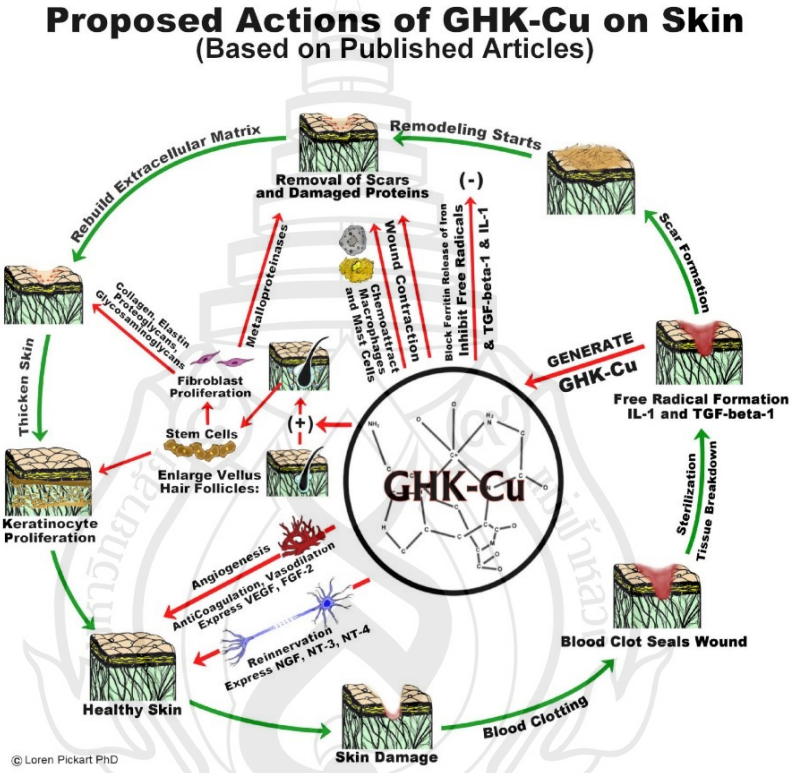
GHK aids in natural cell protection alongside glutathione (GSH), the skin's inherent antioxidant, to mitigate damage caused by reactive carbonyl species (RCS) and UVB radiation. This peptide functions as a scavenger of particular RCS, such as HNE and acrolein, thereby inhibiting the glycation process of proteins like collagen and elastin. By preventing the formation of advanced glycation end-products (AGEs), GHK helps maintain the integrity and functionality of skin proteins [44].

2.5.6 Skin Repairing Ability of GHK-Cu

GHK-Cu-containing cosmetics are widely used for skin rejuvenation. Following a 12-week application to the facial skin of 71 women presenting mild to advanced evidence of photoaging, a GHK-Cu facial cream reduced visible signs of aging, improved skin flexibility, clarity, and appearance, diminished fine lines and wrinkles, and increased skin thickness and density [45]. Additionally, a study with twenty women assessed collagen production after daily application of creams containing Cu-GHK, vitamin C, or retinoic acid to the thighs for a month. Using immunohistology methods and skin biopsy samples, new collagen production was measured. Results indicated that after a month, 70% of those treated with Cu-GHK showed increased collagen levels, compared to 50% for vitamin C and 40% for retinoic acid [46].

Over the course of twelve weeks, 41 women were assessed with a GHK-Cu eye cream compared to a placebo control and an eye cream containing vitamin K. The GHK-Cu cream outperformed both controls in terms of reducing wrinkles, enhancing

overall attractiveness, and improving skin density and thickness [47]. Additionally, GHK-Cu was assessed in an 8-week randomized, double-blind clinical study involving female participants' facial skin. The findings indicated that GHK-Cu enhanced the synthesis of collagen and elastin while upregulating the expression of the MMP1 and MMP2 genes as well as TIMP1. Application of GHK-Cu in lipid-based nanocarriers showed a significant reduction in wrinkle volume compared to Matrixyl® 3000 and control serum, demonstrating its effectiveness as a wrinkle reducer [48].




**Source** [49]

**Figure 2.5** Effects of GHK-Cu on skin

2.5.7 Hair Growth Ability of GHK-Cu

Copper peptides positively influence the growth and regulation of hair follicles. An effective dosage of peptide-copper complexes can be administered through injection or topical application to promote hair growth. A study has demonstrated that intradermal injection of copper peptide complexes stimulates hair follicles in C3H mice [8]. Additionally, research has shown that AHK-Cu, one of the tripeptide-copper complexes, exhibits stimulating effects on human hair follicle growth in vivo and

dermal papilla cell proliferation in vitro. This effect may be attributed to the modulation of the Bcl-2/Bax ratio and caspase-3/PARP levels, ultimately reducing apoptosis and promoting proliferation [50].

In a 1993 study by Uno and Kurata, a similar copper-binding peptide (PC1031) was applied to the back skin of fuzzy rats, leading to the promotion of follicular growth. The study employed three methods to examine hair stimulation: (1) phototrichogram analysis; (2) folliculogram (micro-morphometric analysis); and (3) assessment of DNA synthesis rate in follicular cells. The findings demonstrated stimulated proliferation of follicular cells, resulting in either the maintenance of piebald terminal follicles or the transition of vellus follicles to the terminal type through enlargement during the anagen phase. The researchers concluded that PC1031's effectiveness resembled that of topical minoxidil, an FDA-approved medication for hair growth [51]. Additionally, findings from a study in Korea suggest that copper-GHK treatment may enhance the proliferative potential of basal keratinocytes by influencing the expression of integrins, p63, and PCNA. Moreover, elevated expression of p63, a potential skin stem cell marker, suggests that copper-GHK supports basal stem cell survival in the skin [52].

In a randomized, double-blind, 6-month prospective study, the efficacy and safety of a complex of 5-Aminolevulinic Acid (5-ALA) and Glycyl-Histidyl-Lysine (GHK) Peptide for male pattern hair loss were evaluated. Significant increases in hair count were observed in the treated groups compared to the placebo groups after 6 months of application. The researcher concluded that a complex of 5-ALA and GHK peptides could be considered complementary agents for the treatment of male pattern hair loss [11]. This study will be conducted over a 3-month period to evaluate the depth of efficacy. Although previous studies reported results at 6 months, they also showed a noticeable increase in hair count within 3 months, suggesting that early effects can be observed within this timeframe.

A commercial product known as Graftcyte®, which contains an analog of GHK-Cu, has been clinically investigated and proven to improve the healing process and outcomes of hair transplant procedures. Studies have indicated that it accelerated the growth of new hair and decreased the skin crusting and shedding of hair transplants following hair transplantation [5].

In conclusion, GHK-Cu exhibits promising potential for stimulating hair growth through multiple mechanisms, including the stimulation of dermal fibroblasts and increased expression of vascular endothelial growth factor, which promote follicular enlargement. Additionally, GHK-Cu encourages collagen synthesis within the hair, strengthens hair follicles lacking thickness, and stimulates hair follicle development. Furthermore, GHK-Cu initiates various biological processes associated with wound remodeling, such as chemoattraction of wound healing cells, anti-inflammatory properties, increased protein synthesis, and cellular proliferation [5].

2.5.8 GHK-Cu: Concentration, Formulation and Safety

There are various types of GHK-Cu products available, including topical creams and serums, microneedling solutions, and injectable formulations. Among these, topical application is the most widely used and well-tolerated, particularly in cosmetic formulations targeting anti-aging and hair growth. While different vendors recommend a broad dosage range of 1–10% for topical use, concentrations of 1–2% are generally considered optimal for anti-aging and cosmetic purposes due to their proven efficacy and lower risk of skin irritation [53]. When applied consistently over a period of 12 to 24 weeks, GHK-Cu has demonstrated significant efficacy in promoting hair growth. These effects are supported by multiple in vitro and in vivo studies, as well as safety data from various commercial sources, all of which report minimal irritation or adverse effects at recommended topical concentrations [8, 11].

GHK-Cu is commonly formulated in serums, creams, gels, or microneedling solutions for topical cosmetic use. As it is sensitive to both highly acidic and alkaline environments, maintaining a stable formulation pH is essential. The peptide is often combined with humectants such as hyaluronic acid or glycerin to enhance skin hydration and promote better dermal penetration. To ensure microbial stability without compromising peptide integrity, preservatives compatible with peptides—such as phenoxyethanol or ethylhexylglycerin—are typically used. The final product should be stored in opaque, airtight packaging to protect it from light and oxidation, which can degrade the active compound.

# CHAPTER 3

# RESEARCH METHODOLOGY

## 3.1 Study Design

This study is a randomized, double-blinded, placebo-controlled, split-face clinical trial. A placebo-controlled study helps isolate the true effects of GHK-Cu serum by providing direct comparisons between treated and untreated areas within the same subject, thereby strengthening the study's reliability and ensuring accurate, scientifically validated measurements of the serum's effects.

## 3.2 Study Population

Men and women residing in Thailand, a total of 18 aged between 18, and 40, who are experiencing idiopathic eyebrow hypotrichosis and desire to enhance the appearance of their eyebrows.

## 3.3 Study Location

Mae Fah Luang University Hospital, Bangkok, Thailand

## 3.4 Sample Size Calculation

The reference data were obtained from a double-blind study comparing a lotion containing 2% minoxidil to a placebo lotion to enhance eyebrow appearance [29].

According to the study, in the minoxidil-treated group, the mean number of eyebrow hairs at baseline is 41.08 ± 9.31, and the mean change in the number of eyebrow hairs from baseline after 16 weeks is 5.05 ± 5.24. In the placebo group, the

mean number of eyebrow hairs at baseline is 41.23 ± 9.33, and the mean change in the number of eyebrow hairs from baseline after 16 weeks is 0.97 ± 4.67.

Calculate the sample size using the formula for dependent means,

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \sigma_d^2}{(\mu_d)^2}$$

$\alpha = 0.05$ (two-tailed) $Z_{0.025} = 1.96$

$\beta = 0.10$ $Z_{0.100} = 1.28$

$n_1 = 40$, $n_2 = 40$

$\mu_1 = 41.08 - 5.05 = 36.03$ $S_1 = 5.24$

$\mu_2 = 41.23 - 0.97 = 40.26$ $S_2 = 4.67$

$$\sigma_d^2 = S_p^2 = \frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2}$$

$$S_p^2 = \frac{(40-1)(5.24)^2 + (40-1)(4.67)^2}{40+40-2}$$

$S_p^2 = 24.6332$

$$n = \frac{(1.96+1.28)^2 \times 24.6332}{(36.03-40.26)^2}$$

$n = 14.45 \approx 15$

The number of research participants in this study should be at least 15.

The expected dropout rate is 20%; n = 15+3 = 18.

So, 18 participants will be recruited.

## 3.5 Inclusion Criteria

3.5.1 People in the age range of 18 to 40.

3.5.2 Healthy male and female volunteers from Bangkok, Thailand.

3.5.3 Volunteers who want to increase their eyebrow hair growth and density.

3.5.4 People who are assessed by a research doctor as having eyebrow hypotrichosis with Grade 1 or 2 on the GEBA (Global Eyebrow Assessment) scale a without significant medical cause. The GEBA scale, calibrated with 4 points, is utilized to assess the fullness of the eyebrow (1=very sparse, 2=sparse, 3=full, and 4=very full).

3.5.5 People who can follow up on the study for 3 consecutive months and voluntarily consent to participate.

## 3.6 Exclusion Criteria

3.6.1 People who have been diagnosed with any of the following diseases in the past or who currently have any of the following medical illnesses:

1. Primary dermatoses, including atopic dermatitis and seborrheic dermatitis, especially those affecting the area around the eyebrows.

2. Disorders resulting from thyroid hormone imbalances that exhibit clinical signs and symptoms.

3. Autoimmune disorders that may lead to hair loss, such as alopecia areata and discoid lupus erythematosus (DLE), frontal fibrosing alopecia, and Graham Little syndrome.

4. Infectious diseases, including leprosy and secondary syphilis.

5. Disorders associated with mental health conditions such as trichotillomania, characterized by a strong urge to pull out or break one's own hair, and trichotemnomania, involving repetitive shaving or cutting of hair.

6. Genetic diseases that are associated with thinning eyebrows, such as ectodermal dysplasia.

3.6.2 Individuals with a history of taking the following medications within the past 6 months before participating in the study:

1. Medications that promote hair growth, whether in oral or topical form, include minoxidil, finasteride, dutasteride, spironolactone, etc.

2. Medications that have side effects causing excessive hair growth (hypertrichosis), such as phenytoin, cyclosporin, and steroids, whether in oral or topical form.

3.6.3 People who have a history of undergoing chemotherapy or radiotherapy.

3.6.4 People with a history of underlying medical illnesses such as heart diseases, renal diseases, liver disease, and malignancy.

3.6.5 People who have experienced an accident or burn resulting in a scar around the eyebrow.

3.6.6 People who have undergone eyebrow cosmetic procedures, such as eyebrow tattooing or microblading.

3.6.7 People who have previously undergone eyebrow transplant surgery.

3.6.8 Pregnant women, those suspected of being pregnant, or breastfeeding women.

3.6.9 People who have a history of copper peptide allergy.

## 3.7 Withdrawal Criteria

3.7.1 People who wish to discontinue participation in the study.

3.7.2 Participants who experiences an allergic reaction to the medication.

3.7.3 People who become pregnant during the research.

3.7.4 If a participant is found to have a severe allergic reaction, such as Stevens–Johnson syndrome, anaphylaxis, and severe contact dermatitis to the medication.

## 3.8 Discontinuation Criteria

3.8.1 If ≥30% of participants experience serious adverse reactions (e.g., severe allergic reactions such as Steven-Johnson Syndrome, anaphylaxis and severe contact dermatitis) directly attributed to the study product, the trial will be discontinued to prevent further harm.

3.8.2 If no significant improvement in eyebrow growth, as measured by any of the study parameters, is observed by Week 8, the study will be terminated early. This will prevent unnecessary exposure to the treatment if it does not demonstrate meaningful benefits.

## 3.9 Recruitment of Research participants

3.9.1 Potential participants will be invited by researchers through designated recruitment channels, including digital posters on social media platforms such as Facebook, Instagram, and Line, as well as direct communication via email or messages. Additionally, posters will be placed on university bulletin boards and in the university hospital for those interested.

3.9.2 Invitations will be sent out at the beginning of the recruitment phase and will continue until the target sample size is reached. To ensure informed decision-making, interested participants will have at least (7) days to review the study details before enrolling.

## 3.10 Materials and Equipment Used in Research

3.10.1 Documents detailing the personal histories and general information of research participants.

3.10.2 Documents describing the methods and data of the research.

3.10.3 Informed consent form.

3.10.4 Side effects recording form.

3.10.5 Questionnaire to evaluate patient satisfaction with the outcome of the treatment.

3.10.6 Serum containing 2% copper peptide.

3.10.7 Placebo serum.

3.10.8 VISIA® Skin Analysis System.

3.10.9 Visioscan® VC 20plus.

## 3.11 Product Overview of Copper Peptide (GHK-Cu) Serum and Placebo Serum

3.11.1 Formulation and Percentage Composition of Ingredients

1. Copper Peptide (GHK-Cu) Serum

The serum is scientifically formulated to promote hair growth. Its key ingredient, Copper Tripeptide-1 (GHK-Cu), is well researched in dermatological and trichological studies for its anti-aging properties. This serum is specifically designed to provide targeted benefits for eyebrow hair growth. The formulation is non-toxic, non-irritating, and suitable for a wide range of skin types, supporting safe daily use.

**Table 3.1** Ingredients and percentage by weight (% w/w) of the copper peptide (GHK-Cu) serum

| No. | Trade Name / INCI Name | % w/w |
|---|---|---|
| 1 | Deionized Water | 94.25 |
| 2 | Glycerine | 2.00 |
| 3 | Butylene Glycol | 1.00 |
| 4 | Xanthan Gum | 0.15 |
| 5 | Copper Peptide (GHK-Cu) (1000 ppm Solution) | 2.00 |
| 6 | Euxyl PE 9010 (Phenoxyethanol and Ethylhexylglycerin) | 0.60 |
| | Total | 100 |

2. Placebo Serum

The placebo serum will be formulated using the same ingredients, appearance, odor, color, and packaging as the 2% copper-peptide serum. The only difference will be the absence of the active compound—copper peptide. The use of a placebo group is essential to establish a control that helps determine the true efficacy of the 2% copper peptide serum. The placebo, which lacks the active ingredient but mimics the serum's appearance and application, allows researchers to compare the results on the same individual by analyzing the side of the face treated with the placebo against the side treated with the actual serum. This comparison helps ensure that any

observed effects are due to the copper peptide itself, rather than psychological factors or other variables.

**Table 3.2** Ingredients and percentage by weight (% w/w) of the placebo serum

| No. | Trade Name / INCI Name | % w/w |
|---|---|---|
| 1 | Deionized Water | 96.25 |
| 2 | Glycerine | 2.00 |
| 3 | Butylene Glycol | 1.00 |
| 4 | Xanthan gum | 0.15 |
| 5 | Euxyl PE 9010 (Phenoxyethanol (and) Ethylhexylglycerin) | 0.60 |
| | Total | 100 |

3.11.2 Safety Information of Ingredients (per FDA and COSING Standards)

The serum is formulated with ingredients that comply with FDA and COSING safety standards, selected for their established efficacy and safety profiles. Additionally, the product contains no GMO-derived ingredients, aligning with global safety and quality standards, and includes no animal by-products that conflict with halal or cruelty-free principles. Below is the detailed breakdown of each key ingredient:

1. Deionized water (CAS 7732-18-5) [54] is an FDA GRAS-approved cosmetic solvent that acts as a pure, contaminant-free base for dissolving and delivering active ingredients, and it is non-toxic, non-irritating, and safe for all skin types, including sensitive skin.

2. Glycerin (CAS 56-81-5) [55] is an FDA-approved cosmetic humectant that draws moisture into the skin, supports the skin barrier, and improves hydration, and is widely considered non-toxic, non-irritating, and safe even for sensitive skin.

3. Butylene glycol (CAS 107-88-0) [56] is an FDA-approved cosmetic humectant and solvent that enhances skin hydration and improves the absorption of active ingredients; it is generally safe, well tolerated, and associated with a low risk of irritation at typical use levels.

4. Copper Tripeptide (GHK-Cu) (CAS 89030-95-5) [57] is a cosmetic peptide used for anti-aging, skin repair, wound healing, and hair growth support; it stimulates collagen production, improves skin elasticity, enhances hair follicle activity, and

reduces inflammation. It is permitted for use in cosmetics under FDA regulations and is considered non-toxic, non-irritating, and safe at recommended concentrations.

5. Xanthan gum (CAS 11138-66-2) [58] is a safe, non-irritating thickener and stabilizer used in cosmetics to improve texture and keep formulations consistent, suitable even for sensitive skin.

6. Euxyl® PE 9010 is a cosmetic preservative blend of phenoxyethanol (CAS 122-99-6) [59] and ethylhexylglycerin (CAS 70445-33-9) [60] that prevents microbial growth; it's permitted for cosmetic use (phenoxyethanol ≤1%) and is generally safe, with a low risk of mild irritation in very sensitive skin.

3.11.3 Preparation Methods

The preparation of both the Copper Peptide (GHK-Cu) Serum and the placebo serum involve meticulously controlled steps to ensure product efficacy, stability, and safety. The formulation process will be developed and tested by CourseMetic Lab Co. Ltd., ensuring high-quality and precise manufacturing standards for the products.

1. Preparation method for Copper Peptide (GHK-Cu) Serum

The formulation begins with the preparation of Phase A, where each raw material is weighed individually and added to the main vessel, ensuring thorough dissolution after each addition. Phase A consists of deionized water (solvent), glycerin (humectant), butylene glycol (humectant), and xanthan gum (thickener). Once all the ingredients in Phase A are fully combined, Phase B, which includes phenoxyethanol (preservative) and ethylhexylglycerin (preservative), is gradually added to the Phase A mixture with continuous mixing until a homogeneous mixture is achieved. Finally, Phase C, which contains copper peptide GHK-Cu (active ingredient), is introduced into the Phase AB mixture. The combined phases are stirred until fully blended and homogeneous, ensuring consistency and effectiveness in the final serum formulation.

**Table 3.3** Description of the Copper Peptide (GHK-Cu) Serum

| Test Description | Specification | Result |
|---|---|---|
| Appearance | Low viscous liquid | Low viscous liquid |
| Color | Transparent | Transparent |
| Odor | Characteristic | Characteristic |
| pH @ 25°C | 6.80–7.50 | 7.38 |
| Viscosity @ 25°C (rpm) | Spindle 1, rpm 60 | 132, %T= 66.07 |

2. Preparation method for Placebo Serum

The placebo serum formulation is prepared by first balancing the raw materials in Phase A, which consists of deionized water (solvent), glycerin (humectant), butylene glycol (humectant), and xanthan gum (thickener). Each ingredient is added to the main vessel one at a time, ensuring that each one is fully dissolved before adding the next, to achieve proper mixing and stability. Once all Phase A ingredients are fully combined, Phase B, which includes phenoxyethanol (preservative) and ethylhexylglycerin (preservative), is gradually incorporated into the mixture with continuous mixing. This process continues until a homogeneous and uniform serum is formed, ensuring consistency in texture and composition.

**Table 3.4** Description of the placebo serum

| Test Description | Specification | Result |
|---|---|---|
| Appearance | Low viscous liquid | Low viscous liquid |
| Color | Transparent | Transparent |
| Odor | Characteristic | Characteristic |
| pH @ 25°C | 6.80–7.50 | 6.92 |
| Viscosity @ 25°C (rpm) | Spindle 1, rpm 60 | 132, %T= 66.07 |

3.11.4 Quality Control and Standards

1. Raw Material Testing: All raw materials used in the formulation, including distilled water, humectants, and active ingredients, undergo rigorous quality control testing before use. Each ingredient is tested for purity, microbial contamination, and consistency in accordance with FDA and COSING standards. Copper Peptide (GHK-Cu) is specifically tested for its peptide content and biological activity to ensure optimal efficacy.

2. In-Process Testing: During preparation, the formulation is continuously monitored for pH, viscosity, and homogeneity to ensure the desired texture and stability. Regular checks are performed during mixing and cooling stages to prevent inconsistencies in the serum's composition.

3. Microbial Testing: Once formulated and packaged, the serum undergoes microbial testing to ensure compliance with industry hygiene and safety standards. It is

tested for bacteria, yeast, and mold using established protocols, and only products that pass these tests are approved for release.

4. Stability Testing: Stability testing is conducted to assess the serum's performance under various conditions, such as temperature, humidity, and light exposure. This testing ensures the serum maintains its integrity, potency, and safety over time. Physical stability (color, texture, odor) and chemical stability (active ingredient retention) are regularly tested to confirm long-term efficacy.

3.11.5 Packaging

The products are packaged in high-quality, airtight containers designed to protect them from light, air, and contamination, ensuring the active ingredients remain stable and effective throughout their shelf life. The packaging process occurs in a sterile environment to prevent contamination, with all containers and caps sterilized before use to maintain product integrity. Each container is labeled with essential information, including the product name, ingredients, batch number, manufacturing date, and expiration date, ensuring traceability and compliance with regulatory standards. Once packaged, the products are stored in a temperature-controlled environment, away from direct sunlight and excessive humidity, preserving their stability until they reach consumers.


**Figure 3.1** Packaging design for the copper peptide (GHK-Cu) serum and the placebo serum

3.11.6 THFDA Registration Data

The product is officially registered under the Thai Food and Drug Administration (THFDA) as Harmonia BrowGlow Serum, with registration number 12-1-6700019196, approved on June 14, 2024. This registration confirms that the product complies with THFDA safety, formulation, and labeling standards for cosmetic use in

Thailand. Manufactured by Cosmetic Lab Co., Ltd., located at 69/9 Moo 1, Ban Mai, Pak Kret, Nonthaburi 11120, Thailand, the serum is classified as a cosmetic product specifically formulated for eyebrow application. It is designed for twice-daily use (morning and evening) and meets THFDA microbiological and safety regulations, ensuring high-quality standards and consumer safety.

## 3.12 Research Procedure

The researcher will identify and select volunteers based on the inclusion and exclusion criteria. Potential participants will be recruited through various channels, including social media, university bulletin boards, and clinical settings. Interested individuals will be provided with detailed study information, and given sufficient time to decide whether to participate. Recruitment will continue until the target sample size is reached.

Potential participants will receive a comprehensive explanation of the study. The consent process will ensure that participants fully understand the study before agreeing to take part. Eligible participants will be invited to an information session, where a researcher will explain the study's purpose, procedures, duration, potential risks, and benefits. They will receive an informed consent form and an information sheet about the study, allowing them time to review the details and ask questions. The researcher will emphasize their right to withdraw at any time without penalty. Once they decide to participate, they will sign the consent form and receive a copy for their records. Only then will they proceed with the study, following ethical guidelines to ensure voluntary participation.

After obtaining informed consent, participants will undergo facial marking to identify the application area—specifically, a 1 $cm^2$ region on each eyebrow, aligned with the mid-pupil line. This ensures consistent and accurate product application throughout the study. Participants will then receive labeled serum tubes indicating the left or right eyebrow, based on their assigned randomization. Detailed instructions will be provided, including application method, dosage, possible side effects, and precautions to ensure proper and safe use of the product.

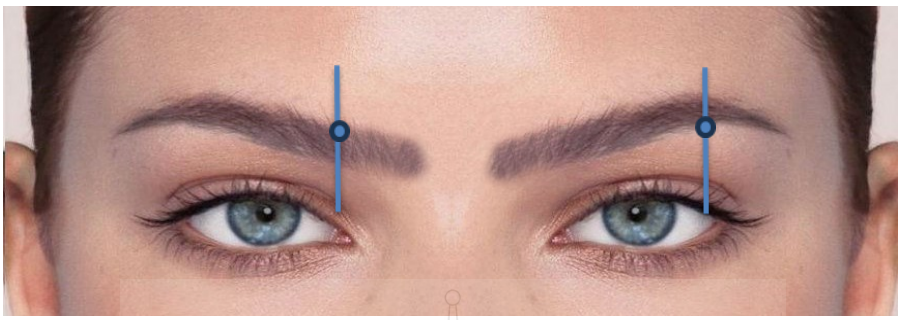


**Figure 3.2** Method for determining anatomical reference points for measuring the number and diameter of eyebrow hairs

As a baseline measurement, a straight-angle photo of each participant's eyebrow will be taken using the VISIA® Skin Analysis System to assess the global photographic score. Additionally, a 1 $cm^2$ region of the eyebrow on both sides, located at the mid-pupil line, will be photographed using the Visioscan® VC 20plus. Each hair in the image will be counted, and the width of the eyebrow at the base of each follicle will be measured to determine eyebrow diameter. The same measurement process will be repeated at the 4th, 8th, and 12th week after serum administration to track changes in eyebrow density, hair count, and diameter over time.

For randomization, two identical tubes will be prepared: one containing a serum with 2% copper peptide (GHK-Cu) (Serum A) and the other a placebo serum (Serum B) with a similar texture, color, and odor. To ensure a balanced and unbiased assignment of Serum A and Serum B to each side of the participants' faces, a fixed-block randomization method with a block size of 2 will be used. Each block will consist of two participants (four treatment areas: two left and two right sides) to maintain an equal distribution of treatment conditions throughout the study. With a total of 18 participants, the study will involve 36 treatment areas (left and right sides of the face for each participant).

For each block, the assignment sequences will be randomly generated, ensuring that:

1. One participant receives Serum A on the right and Serum B on the left
2. The other participant receives Serum A on the left and Serum B on the right

These sequences will be randomly arranged in blocks of two participants at a time to maintain balance. A third-party individual, uninvolved in participant assessments, will generate and assign these sequences. To preserve the double-blind nature of the trial, all assignments will be sealed in opaque envelopes and remain undisclosed until the study is completed.

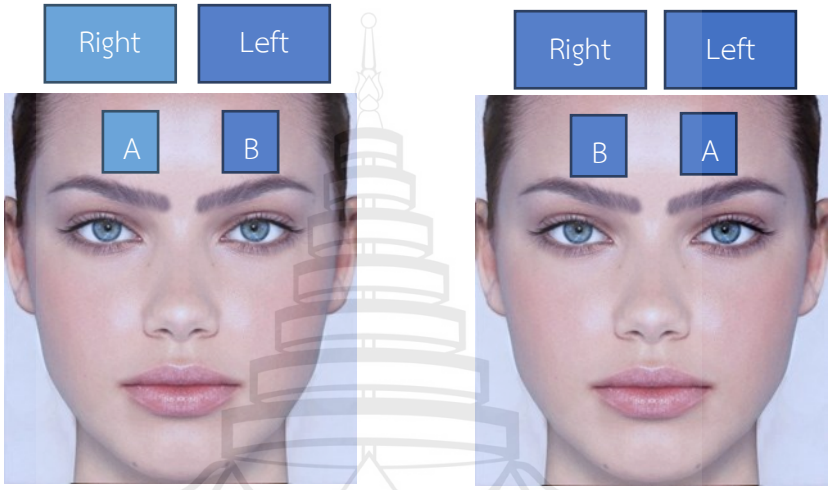



**Figure 3.3** Possible ways to apply Serum A and Serum B

After completing the consent process, the research participants will receive tubes labeled to indicate whether they should be applied to the left or right eyebrow. The labeling will be done by the randomizer according to each participants assigned randomization outcome. The labeled tubes will then be passed to the research doctor, who will oversee the next steps of the study. The researcher will provide each participant with their assigned tubes, along with detailed instructions on how to apply the serum, potential side effects, and necessary precautions.

The serum should be applied to the eyebrow using a wand and then spread throughout the area. Both sides of the eyebrow need to be treated as directed by the label. Washing the face or wiping the area where the serum will be applied with alcohol should be avoided for at least 4 hours after application. The serum needs to be applied twice daily, in the morning and at night, for 12 weeks. Participants can apply makeup to their eyebrows once the serum has completely dried. Ensuring clear labeling of all serum tubes will be essential to facilitate proper identification and prevent any confusion. Separate wands and hands should be used for each side of the eyebrow during application to prevent cross-contamination. For example, apply Serum A to the

right eyebrow using the right hand, and apply Serum B to the left eyebrow using the left hand.

**Figure 3.4** Photograph showing the eyebrow area for serum application

A patch test will be performed before starting the serum to help prevent allergic reactions. A small amount of the product will be applied to a discreet area, such as the inner forearm or behind the ear, and left on for 24 hours while avoiding contact with water. After this period, the area will be evaluated for any signs of irritation, redness, itching, burning sensation, swelling, rash, or other unusual reactions. The Cosmetic, Toiletry, and Fragrance Association (CTFA) 5-point scale will be used to evaluate skin irritation based on visible signs of reaction. The scale is as follows: 0 = no reaction, 1 = barely perceptible reaction, 2 = mild reaction, 3 = moderate reaction, and 4 = severe reaction. Participants with a CTFA score of 2 or higher (i.e., mild, moderate, or severe reaction) will be excluded from further participation in the study to ensure safety. The patch test will be conducted during the first visit, and the research doctor will assess the results the following day. If no adverse reactions are observed within the 24-hour period, the product is considered safe for use. However, if any of the mentioned symptoms occur, the area should be rinsed immediately with lukewarm water, the use of the serum should be discontinued, and the participant will be excluded from the study. All participants will receive compensation for travel expenses related to the evaluation of patch test results, and those who experience adverse reactions will receive medical care until the tested area has fully recovered.

All participants, including those in the placebo group, will be closely monitored throughout the study to promptly identify and manage any adverse reactions or

unexpected outcomes. The study protocol will undergo thorough review and approval by an ethics committee to ensure that the potential benefits of the research justify any risks to participants. If the 2% copper peptide serum is proven effective, participants who received the placebo will be offered access to the active treatment after the study concludes, ensuring they also benefit from the research findings.

To ensure the confidentiality of data when photography is used, several protective measures will be implemented. Identifiable features, such as the eyes, will be masked by a black strip to maintain anonymity. Additionally, each participant will be assigned a unique picture code in place of their name, ensuring that no personal information is linked to the images. All photographic data will be securely stored, with access restricted to authorized personnel only, further protecting participants' confidentiality.

After the completion of the research, participants will be provided with information on the outcomes of the study and any relevant findings. If applicable, participants may also be given access to the treatment that demonstrated efficacy during the study or provided with affordable options for continued use of the copper-peptide serum. Additionally, each research volunteer will receive a minimum of 300 baht per visit to cover travel expenses.

If research participants experience side effects from applying the serum, the research doctor will first assess the severity of the adverse effects. In cases where the symptoms are mild, such as slight burning or itching, the research can continue as long as the individuals voluntarily choose to participate. However, if symptoms worsen and become more severe, such as excessive redness, swelling, or dermatitis, the researcher will reassess and ask the participants to discontinue applying the serum and withdraw from the study. The researcher will then provide treatment for the participants for any side effects at no cost. Subsequently, the researcher will gather information, evaluate the results, and discuss and summarize the findings.

## 3.13 Follow-up Evaluation

Participants will be provided with an appointment card and instructed to visit the research location at weeks 4, 8, and 12 following serum application. During each visit, a researcher will use the VISIA® Skin Analysis System to capture a straight-angle photo of the participant's face in order to assess the global photographic score. To determine the eyebrow hair count, a measurement point will be marked on both sides of the eyebrow along the mid-pupillary line. At this marked point, a photo of a region approximately 1 $cm^2$ in size will be taken using the Visioscan® VC 20plus. Each hair in the photo will be counted, and hair density will be recorded within a 1 $cm^2$ area of the measurement point along the mid-pupil line. Additionally, eyebrow diameter will be determined by measuring at the base of the follicle.

The researcher will conduct a medical history assessment with each participant to evaluate any post-medication side effects, such as burning, redness, itching, and abnormal hair growth in unwanted areas. Additionally, the physical appearance of both eyebrows will be examined to identify signs of erythema, scaling, edema, and blistering. Should any of these signs or symptoms develop, both the research participants and the research doctor will classify them as mild, moderate, or severe, and record any adverse effects accordingly.

To assess compliance, the research doctor will ask participants to bring back the serum tubes at every follow-up visit, and the amount of serum will be checked. After applying the serum for 12 weeks, the research participants will be asked to rate their satisfaction with both medications. At the end of the research, the research doctor will provide each participant with a serum tube containing 2% copper peptide. This is aimed at restoring the density of eyebrow hair on the placebo side and achieving even density on both sides.

## 3.14 Data Evaluation

The outcomes of this study that need to be assessed include:

1. Number and Diameter of Eyebrow Hair at Measurement Point

2. Global Photographic Assessment Score

3. Research Participants' Satisfaction Score

4. Safety Evaluation for Side Effects

3.14.1 Number and Diameter of Eyebrow Hair at Measurement Point

Ensure that the research participant sits upright in the chair and instruct them to focus their eyes on a point while maintaining a neutral facial expression. A line will be drawn perpendicular from the center of the mid-pupil to the point where the eyebrows meet, and photos of a region approximately 1 $cm^2$ in size will be captured using Visioscan® VC 20plus. The number of eyebrow hairs will be determined by counting the hairs visible in the photo. The diameter will be measured by measuring the width at the base of each follicle, and these measurements will be averaged. This process will be repeated for both eyebrows of each participant at every follow-up appointment. Representative values of the number and diameter of eyebrow hair will be recorded for subsequent statistical analysis.

3.14.2 Global Photographic Assessment Score

Three doctors who are not participating in the study will be assigned as evaluators. The research doctor will prepare photos of the eyebrows for assessment by capturing straight-angle photos of the participants' faces using the VISIA® Skin Analysis System during the four visits. To compare the eyebrows on the same side, the Microsoft PowerPoint 2010 program will be used to align the photos. The left photo will show the eyebrow prior to serum application, while the right photo will show the eyebrow after serum application. The evaluating doctors will receive comparative photos of the eyebrows taken during the four visits. The evaluation will involve comparing each brow individually and considering the overall eyebrow density from the photos. The results of eyebrow changes will be provided as a score.

**Table 3.5** Global photographic assessment score

| Score | Description |
|---|---|
| +3 | Significant increase in the overall composition of the eyebrows compared to the photograph before serum application. |
| +2 | Moderate increase in the overall composition of the eyebrows compared to the photograph before serum application. |

**Table 3.5** (continued)

| Score | Description |
|---|---|
| +1 | Slight increase in the overall composition of the eyebrows compared to the photograph before serum application. |
| 0 | No change in the overall composition of the eyebrows compared to the photograph before serum application. |
| -1 | Slight decrease in the overall composition of the eyebrows compared to the photograph before serum application. |
| -2 | Moderate decrease in the overall composition of the eyebrows compared to the photograph before serum application. |
| -3 | Significant decrease in the overall composition of the eyebrows compared to the photograph before serum application. |

3.14.3 Research Participants' Satisfaction Score

The research doctor will give questionnaires to each participant during the 12-week follow-up to evaluate their satisfaction with the treatment. Participants will rate their satisfaction with each side of the eyebrow by providing a satisfaction score. The scoring criteria are as follows:

**Table 3.6** Research participants' satisfaction score

| Score | Description |
|---|---|
| +3 | Very satisfied with the treatment result |
| +2 | Moderately satisfied with the treatment result |
| +1 | Slightly satisfied with the treatment result |
| 0 | No change with the treatment result |
| -1 | Slightly unsatisfied with the treatment result |
| -2 | Moderately unsatisfied with the treatment result |
| -3 | Very unsatisfied with the treatment result |

3.14.4 Safety Evaluation for Side Effects

The research doctor will take a history of any signs and symptoms such as burning, redness, itching, flaking, dryness, and abnormal hair growth in an undesired area following serum application. Any symptoms suspected to be related to serum's side effects will be recorded, and study participants will be asked to rate the severity of these symptoms, categorizing them into three levels: mild, moderate, and severe.

The research doctor will conduct a physical inspection of each research participant's eyebrow to identify any lesions indicative of serum side effects. These lesions will then be categorized as follows: vesicles, edema, erythema, and scaling. If any such lesions are found, they will be classified as mild, moderate, or severe based on an assessment of their severity. All research participants will undergo assessments at 4, 8, and 12 weeks following serum administration.

**Table 3.7** Safety evaluation for side effects

| Score | Description |
|---|---|
| 0 | No symptoms |
| 1 | Mild symptoms |
| 2 | Moderate symptoms |
| 3 | Severe symptoms |

## 3.15 Data Analysis

The results of the research participants will be presented using descriptive analysis, including numbers, percentages, means, and standard deviations. Statistical evaluation will be conducted using the SPSS® program, Version 20.

3.15.1 Global Photographic Assessment Score

The changes in global photographic assessment scores from baseline to weeks 4, 8, and 12 following serum application will be compared in both the copper peptide-treated group and the placebo group. A repeated measures ANOVA will be utilized when the data are normally distributed, or the Wilcoxon matched-pairs signed-rank test

will be used when the data are not normally distributed, with a significance level of 0.05.

3.15.2 Number and Diameter of Eyebrow Hair

The changes in the number of eyebrow hairs from baseline to weeks 4, 8, and 12 following serum application will be compared in both the copper peptide-treated group and the placebo group. The repeated measures ANOVA will be considered when the data are normally distributed, or the Wilcoxon matched-pairs signed-rank test will be used when the data are not normally distributed, using a significance level of 0.05.

The changes in the averaged diameter of eyebrow hairs from baseline to weeks 4, 8, and 12 following serum application will be compared in both the copper peptide-treated group and the placebo group. The repeated measures ANOVA will be considered when the data are normally distributed, or the Wilcoxon matched-pairs signed ranks test will be used when the data are not normally distributed, using a significance level of 0.05.

3.15.3 Research Participants' Satisfaction

Comparing the research participants' satisfaction data scores between the copper peptide-treated group and the placebo group after 12 weeks will be done using the paired t-test.

3.15.4 Safety Evaluation

The comparison of the side effect data between the copper peptide and placebo groups will be conducted using the McNemar test to assess the significance of the change.

## 3.16 Ethical Consideration

This work strictly complies with the Good Clinical Practice (GCP) criteria established by the International Conference on Harmonisation (ICH). These criteria represent a globally accepted ethical and scientific standard for conducting, designing, recording, and reporting trials involving human beings.

For a better understanding, the following considerations will be taken into account.

3.16.1 The consent process will be conducted by a researcher at a designated study location before any study procedures begin. The researcher will explain the study's purpose, procedures, duration, potential risks, and benefits. Participants will receive an informed consent form and an information sheet, allowing them time to review the details and ask questions. Consent will be voluntary, and participants have the right to withdraw at any time without penalty. Those who choose to participate will sign the consent form and receive a copy for their records before proceeding with the study.

3.16.2 The researcher will emphasize the voluntary nature of participation and make it clear that participants' decisions will not influence their medical care or their relationship with the research doctor, ensuring that no deferral vulnerability arises. To further protect against potential coercion or conflict of interest, the researcher will not obtain informed consent from their own patients.

3.16.3 Take measures to reduce any risk of participant harm, either physical or psychological.

3.16.3.1 Physical risks include:

1. Skin irritations such as redness, itching, a burning sensation, swelling, and rash

2. Severe allergic reactions, including Stevens-Johnson Syndrome, anaphylaxis, and severe contact dermatitis from the medication

3. Uneven eyebrow distribution due to the split-face comparative study design

3.16.3.2 Psychological risks include:

1. Emotional distress related to self-confidence, particularly if eyebrow thickness is uneven during the study

2. Privacy concerns, as participants may feel anxious about the confidentiality of their personal information and data

3. Stress from the frequent serum application twice daily, monthly visits, and participation in assessments.

3.16.4 The researcher will not allow doctors or nurses to recruit participants to prevent deferential vulnerability.

3.16.5 Ensure the study will be professionally carried out with an unbiased and impartial approach.

3.16.6 Prior to starting the study, approval will be obtained from the relevant ethics review board.

3.16.7 Ensure that all data will be securely stored, and that only individuals with permission may access it.

3.16.8 Avoid apparent biased reporting and data manipulation to support any notions.

3.16.9 This study will be provided without charge. The researcher and the participants will have no conflicts of interest.

3.16.10 Confidentiality will be maintained through photo de-identification, participant ID usage, and secure, restricted-access data storage. Data will be retained for 3 years after publication per ethical guidelines and securely deleted thereafter.

3.16.11 At the end of the research, each participant will receive a serum tube containing 2% copper peptide, intended to restore eyebrow hair density on the placebo side and achieve even density on both sides.

3.16.12 Before conducting the study, the researcher will ensure that 2% copper peptide (GHK-Cu) is approved by the Thai FDA and will conduct a patch test to avoid side effects such as allergies and hypersensitivity reactions.

# CHAPTER 4

# RESULT

## 4.1 General Characteristics of the Participants

**Table 4.1** Demographic data of the study participants

| Demographic data | n=18 |
|---|---|
| Sex | |
| Male | 6 |
| Female | 12 |
| Age range (years) | |
| 20–29 | 9 |
| 30–40 | 9 |
| Mean ± SD | 28.94±3.24 |
| Occupation | |
| Government staff | 3 |
| Office staff | 3 |
| Own business | 1 |
| School teacher | 3 |
| Student | 8 |
| Underlying disease | |
| Yes | 0 |
| No | 18 |
| Global Eyebrow Assessment scale | |
| 1 | 8 |
| 2 | 10 |

According to the baseline characteristics of the 18 participants, the majority were female (12), while 6 were male. The mean age was 28.94 ± 3.24 years. Nine participants were aged 20–29 years, and the remaining nine were in the 30–40 year age

group. In terms of occupation, there were 3 government staff members, 3 office staff, 1 business owner, 3 school teachers, and 8 students. None of the participants had any underlying medical conditions. Based on the Global Eyebrow Assessment (GEBA) scale, 10 participants had a score of 2, while 8 participants had a score of 1.

## 4.2 Clinical Evaluation

4.2.1 Number of eyebrow hairs

**Table 4.2** Statistical comparison of the number of eyebrow hairs between the active serum–treated side and the placebo-treated side at baseline and at weeks 4, 8, and 12 (n = 18)

| **Number of eyebrow hair** | **(GHK-Cu) active serum side** | **Placebo side** | **Paired differences ± SE** | **p-value[a]** |
|---|---|---|---|---|
| | **Mean ± SD** | **Mean ± SD** | | |
| Baseline | 28.89 ± 5.910 | 28.78 ± 4.545 | 0.111 ± 0.804 | 0.892 |
| Week 4 | 28.78 ± 5.976 | 28.67 ± 4.728 | 0.111 ± 0.867 | 0.899 |
| Week 8 | 29.28 ± 5.899 | 28.11 ± 4.549 | 1.167 ± 0.715 | 0.121 |
| Week 12 | 30.50 ± 5.773 | 28.67 ±4.102 | 1.833 ± 0.864 | 0.049 |
| p-value[b] | p = 0.001 | p = 0.075 | | |

**Note** Statistical analysis was performed using a paired t-test and repeated measures ANOVA.

[a]Paired Samples Test, [b]Repeated measurement ANOVA

Based on the statistical analysis shown in the table, the mean number of eyebrow hairs on the active serum–treated side at baseline and at weeks 4, 8, and 12 were 28.89 ± 5.910, 28.78 ± 5.976, 29.28 ± 5.899, and 30.50 ± 5.773, respectively. Analysis revealed a significant increase in eyebrow hair count on the active serum side over time at the 0.05 level of significance ($F = 11.01$, $p = 0.001$). In comparison, the placebo-treated side showed mean eyebrow hair counts of 28.78 ± 4.545, 28.67 ± 4.728, 28.11 ± 4.549, and 28.67 ± 4.102 at baseline and at weeks 4, 8, and 12, respectively.

Statistical testing indicated that changes in eyebrow hair count on the placebo side were not significant over the study period ($F = 2.43$, $p = 0.075$).

When the two sides were compared at each assessment point, no statistically significant differences were detected at baseline ($p = 0.892$), week 4 ($p = 0.899$), or week 8 ($p = 0.121$). However, by week 12, the mean eyebrow hair count on the active serum side was significantly greater than that of the placebo side, with a paired mean difference of $1.833 \pm 0.864$ ($p = 0.049$). These findings indicate that the active serum progressively increased eyebrow hair count during the 12-week treatment period, with a significant advantage over placebo observed at the final follow-up visit.

**Table 4.3** Post-hoc multiple comparison analysis of the number of eyebrow hairs

| **Number of eyebrow hair** | **Compare to** | **(GHK-Cu) active serum side** |
|---|---|---|
| | | **p - value** |
| Baseline | Week 4 | 1 |
| Baseline | Week 8 | 1 |
| Baseline | Week 12 | 0.005 |
| Week 4 | Week 8 | 0.645 |
| Week 4 | Week 12 | 0.003 |
| Week 8 | Week 12 | 0.030 |

**Note** Post-hoc comparisons were conducted using the Wilcoxon signed-rank test with Bonferroni correction.

According to the post-hoc multiple comparison analysis of eyebrow hair count, changes across different visits were evaluated separately for the active serum side and the placebo side. For the active serum side, no statistically significant differences were observed between baseline and week 4 (mean difference = $-0.111 \pm 0.227$, $p = 1$), baseline and week 8 (mean difference = $-0.389 \pm 0.282$, $p = 1$), or week 4 and week 8 (mean difference = $-0.500 \pm 0.294$, $p = 0.645$). However, significant increases in eyebrow hair count were detected at later follow-up visits. Compared with baseline, the number of eyebrow hairs at week 12 was significantly higher (mean difference = $-1.611 \pm 0.397$, $p = 0.005$). In addition, week 12 values were significantly greater than those at week 4 (mean difference = $-1.722 \pm 0.394$, $p = 0.003$) and week 8 (mean difference

= −1.222 ± 0.384, p = 0.030). These findings suggest that the beneficial effect of the active serum became more apparent after longer treatment duration, particularly by week 12.

For the placebo side, no statistically significant differences were observed between any of the time points after adjustment for multiple comparisons. The results indicate that the number of eyebrow hairs on the placebo side remained relatively stable throughout the study period.

4.2.2 Diameter of eyebrow hair

**Table 4.4** Statistical comparison of the diameter of eyebrow hairs between the active serum–treated side and the placebo-treated side at baseline and at weeks 4, 8, and 12 (n = 18)

| **Diameter of eyebrow hair** | **(GHK-Cu) active serum side** | **Placebo side** | **Paired differences ± SE** | **p-value[a]** |
|---|---|---|---|---|
| | **Mean ± SD** | **Mean ± SD** | | |
| Baseline | 1.83 ± 0.28 | 1.77 ± 0.21 | 0.054 ± 0.04 | 0.213 |
| Week 4 | 1.83 ± 0.29 | 1.77 ± 0.21 | 0.062 ± 0.043 | 0.175 |
| Week 8 | 1.85 ± 0.31 | 1.76 ± 0.21 | 0.083 ± 0.041 | 0.055 |
| Week 12 | 1.88 ± 0.30 | 1.78 ± 0.22 | 0.10 ± 0.044 | 0.036 |
| p-value[b] | p = 0.005 | p = 0.236 | | |

**Note** Statistical analysis was performed using a paired t-test and repeated measures ANOVA.

[a]Paired Samples Test, [b]Repeated measurement ANOVA

Based on the statistical analysis presented in the table, the mean diameter of eyebrow hairs on the active serum–treated side measured 1.83 ± 0.28 at baseline, 1.83 ± 0.29 at week 4, 1.85 ± 0.31 at week 8, and 1.88 ± 0.30 at week 12. A statistically significant increase in eyebrow hair diameter over time was observed on the active serum side at the 0.05 significance level (F = 4.79, p = 0.005). In contrast, the placebo-treated side showed mean eyebrow hair diameters of 1.77 ± 0.21 at baseline, 1.77 ± 0.21 at week 4, 1.76 ± 0.21 at week 8, and 1.78 ± 0.22 at week 12. The changes in hair

diameter across the study period on the placebo side were not statistically significant ($F = 1.46$, $p = 0.236$).

When the two sides were compared at each study visit, no significant differences were identified at baseline ($p = 0.213$), week 4 ($p = 0.175$), or week 8 ($p = 0.055$), although the week-8 comparison suggested a possible trend toward significance. However, at week 12, the active serum–treated side demonstrated a significantly greater mean eyebrow hair diameter than the placebo-treated side, with a paired mean difference of $0.10 \pm 0.044$ ($p = 0.036$). Overall, these findings indicate that application of the active serum gradually increased eyebrow hair thickness over the 12-week treatment period, and a statistically significant superiority compared with the placebo was observed at the final follow-up visit.

**Table 4.5** Post-hoc multiple comparison analysis of the diameter of eyebrow hairs

| **Diameter of eyebrow hair** | **Compare to** | **(GHK-Cu) active serum side** |
|---|---|---|
| | | **p-value** |
| Baseline | Week 4 | 1 |
| Baseline | Week 8 | 1 |
| Baseline | Week 12 | 0.04 |
| Week 4 | Week 8 | 1 |
| Week 4 | Week 12 | 0.077 |
| Week 8 | Week 12 | 0.089 |

**Note** Post-hoc comparisons were conducted using the Wilcoxon signed-rank test with Bonferroni correction.

Based on the post-hoc multiple comparison analysis of eyebrow hair diameter, variations across the study visits were examined for both the active serum side and the placebo side. For the active serum side, no statistically significant differences were identified between baseline and week 4 ($p = 1$), baseline and week 8 ($p = 1$), or week 4 and week 8 ($p = 1$). However, a significant increase in eyebrow hair diameter was observed when baseline was compared with week 12 (mean difference = $–0.056 \pm 0.018$, $p = 0.040$), indicating statistical significance at the 0.05 level. Comparisons between week 4 and week 12 ($p = 0.077$) and week 8 and week 12 ($p = 0.089$) showed

a tendency toward increased diameter, although these differences did not remain statistically significant after correction for multiple comparisons.

For the placebo side, no statistically significant differences in eyebrow hair diameter were found among any of the evaluated time points. Overall, these results indicate that a significant increase in eyebrow hair diameter was observed only after 12 weeks on the active serum side, whereas no meaningful changes occurred on the placebo side throughout the study period.

4.2.3 Global Photographic Assessment Score (GPAS score)

**Table 4.6** Statistical comparison of Global Photographic Assessment Scores (GPAS) between the active serum–treated side and the placebo-treated side at baseline and at weeks 4, 8, and 12 (n = 18)

| **GPAS score** | **(GHK-Cu) active serum side** | **Placebo side** | **Paired mean difference** | **p-value[(a)]** |
|---|---|---|---|---|
| | **Mean ± SD** | **Mean ± SD** | **± SE** | |
| Week 4 | 0.83 ± 0.514 | 0.33 ± 0.485 | 0.5 ± 0.146 | 0.003 |
| Week 8 | 1.11 ± 0.417 | 0.56 ± 0.511 | 0.556 ± 0.166 | 0.004 |
| Week 12 | 1.44 ± 0.705 | 0.78 ± 0.548 | 0.667 ± 0.229 | 0.010 |
| p-value[(b)] | P < 0.001 | P = 0.028 | | |

**Note** Statistical analysis was performed using a paired t-test and repeated measures ANOVA.

[(a)]Paired Samples Test, [(b)]Repeated measurement ANOVA

Based on the statistical analysis shown in the table, the mean GPAS scores on the active serum side at week 4, week 8, and week 12 were 0.83 ± 0.514, 1.11 ± 0.417, and 1.44 ± 0.705, respectively. A statistically significant change in GPAS scores was observed over time on the active serum side ($p < 0.001$), indicating a gradual and continuous clinical improvement during the follow-up period. On the placebo side, the mean GPAS scores at week 4, week 8, and week 12 were 0.33 ± 0.485, 0.56 ± 0.511, and 0.78 ± 0.548, respectively. The GPAS scores on this side also showed a statistically significant change over time ($p = 0.028$), suggesting a mild level of clinical improvement throughout the study.

When the two sides were compared at each follow-up visit, the active serum side demonstrated significantly higher GPAS scores than the placebo side at week 4 (paired mean difference = 0.50 ± 0.146, p = 0.003), week 8 (paired mean difference = 0.556 ± 0.166, p = 0.004), and week 12 (paired mean difference = 0.667 ± 0.229, p = 0.01).

**Table 4.7** Post-hoc multiple comparison analysis of global photographic assessment scores (GPAS)

| GPAS score | Compare to | (GHK-Cu) active serum side | Placebo serum side |
|---|---|---|---|
| | | p-value | p-value |
| Week 4 | Week 8 | 0.061 | 0.311 |
| Week 4 | Week 12 | 0.005 | 0.048 |
| Week 8 | Week 12 | 0.088 | 0.645 |

**Note** Post-hoc comparisons were conducted using the Wilcoxon signed-rank test with Bonferroni correction.

Based on the post-hoc multiple comparison analysis of GPAS scores, changes between follow-up visits were evaluated for both the GHK-Cu active serum–treated side and the placebo-treated side. On the active serum side, no statistically significant differences were observed between week 4 and week 8 (p = 0.061) or between week 8 and week 12 (p = 0.088). However, the GPAS score at week 12 was significantly higher than that at week 4 (p = 0.005), indicating a significant overall improvement over the longer treatment interval ($p < 0.05$).

On the placebo side, no statistically significant differences were found between week 4 and week 8 (p = 0.311) or between week 8 and week 12 (p = 0.645). However, a statistically significant increase in GPAS score was observed between week 4 and week 12 (p = 0.048), suggesting a modest overall improvement. Overall, these findings suggest that the active serum produced a more consistent and sustained improvement in GPAS scores over the 12-week treatment period, whereas the placebo side demonstrated only limited improvement over time.

4.2.4 Research Participants' Satisfaction Score

**Table 4.8** Frequency distribution of patient satisfaction scores at the 12th-week follow-up comparing (GHK-Cu) active serum and placebo sides

| Satisfaction Scores | (GHK-Cu) active serum side | Placebo side |
|---|---|---|
| Very Satisfied (+3) | 5 | 0 |
| Moderately satisfied (+2) | 5 | 0 |
| Slightly satisfied (+1) | 8 | 12 |
| No Changes | 0 | 6 |
| Slightly unsatisfied (-1) | 0 | 0 |
| Moderately Unsatisfied (-2) | 0 | 0 |
| Very unsatisfied (-3) | 0 | 0 |

**Table 4.9** Statistical analysis of patient satisfaction scores at the 12th-week follow-up comparing (GHK-Cu) active serum and placebo sides

| Evaluation on follow-up 12th week | (GHK-Cu) active serum side | Placebo side | Mean Difference ± SE | p-value |
|---|---|---|---|---|
| | Mean(SD) | Mean(SD) | | |
| | 1.83(0.86) | 0.67 (0.49) | 1.167 + 0.218 | p = 0.001 |

**Note** Statistical analysis was performed using the paired samples t-test.

At the 12-week follow-up assessment, the mean patient satisfaction score for the GHK-Cu active serum–treated side was 1.83 ± 0.86, whereas the placebo-treated side showed a lower mean score of 0.67 ± 0.49. Statistical analysis revealed that patient satisfaction was significantly higher on the active serum side compared with the placebo side, with a mean paired difference of 1.167 ± 0.218 (p = 0.001). These findings suggest that participants were considerably more satisfied with the effects of the active serum than with the placebo treatment at the conclusion of the 12-week study period.

4.2.5 Safety Evaluation

Safety assessment was conducted at each follow-up visit by evaluating the presence of local cutaneous reactions and participant-reported symptoms on both the active serum side and the placebo side. The parameters assessed included burning

sensation, erythema, itching, dryness, scaling, vesicle formation, edema, flaking, abnormal hair growth, and any other treatment-related adverse effects. Throughout the 12-week study period, no adverse events or local skin reactions were observed in any participant on either the copper peptide (GHK-Cu) serum side or the placebo side. Clinical examination revealed no evidence of irritation, inflammation, or hypersensitivity reactions at any evaluation time point. Participants also did not report discomfort, intolerance, or complications associated with the application of the study products.

# CHAPTER 5

# DISCUSSION, CONCLUSION, SUGGESTION

## 5.1 Discussion

This research study evaluated the efficacy of 2% copper peptide (GHK-Cu) serum compared with placebo for eyebrow enhancement. According to the study, most of the participants were female (66.7%), while males accounted for 33.3%. The mean age of the participants was 28.94±3.24 years, with an age range of 22–35 years. Regarding occupation, the majority were students (44.4%), followed by government staff (16.7%), office staff (16.7%), and school teachers (16.7%), while a small proportion operated their own business (5.6%). None of the participants had underlying medical diseases. Based on the Global Eyebrow Assessment Scale at baseline, 44.4% of participants were graded as score 1, and 55.6% were graded as score 2. These demographic and baseline characteristics indicate that the study population consisted mainly of young, healthy adults.

Standardized photographic and clinical evaluation methods were used for assessing eyebrow hair count, hair diameter, global photographic assessment scores, and patient satisfaction, comparing the 2% GHK-Cu serum–treated side with the placebo-treated side. Photographic assessments were performed using the VISIA® Skin Analysis System, while hair count and diameter were measured using the Visioscan® VC 20plus. The results demonstrated a statistically significant increase in both eyebrow hair count and diameter on the GHK-Cu serum–treated side compared with the placebo side, particularly at the 12th week of follow-up. Furthermore, clinical assessment scores and patient satisfaction outcomes indicated superior efficacy of the GHK-Cu serum compared with placebo. Overall, these findings suggest that 2% copper peptide (GHK-Cu) serum is effective in enhancing eyebrow hair growth and thickness over the treatment period.

The findings of this study can be summarized as follows:

No allergic reactions were observed during patch testing of the 2% copper peptide (GHK-Cu) serum, and no adverse events were reported throughout the study period. Therefore, under the conditions of this study, the 2% GHK-Cu serum was demonstrated to be safe for topical application to the eyebrow area.

Regarding the number of eyebrow hairs, a statistically significant increase was observed on the GHK-Cu serum–treated side at the 12th week compared with baseline ($p = 0.005$), the 4th week ($p = 0.003$), and the 8th week ($p = 0.030$), indicating that meaningful improvement became evident by week 12. No statistically significant differences were found between baseline and the 4th week ($p = 1$), baseline and the 8th week ($p = 1$), or between the 4th and 8th week ($p = 0.645$). In contrast, the placebo-treated side showed no statistically significant differences in eyebrow hair count at any time point after adjustment for multiple comparisons (all $p > 0.05$), demonstrating stability throughout the study period.

For eyebrow hair diameter, a statistically significant increase was observed on the GHK-Cu serum–treated side at the 12th week compared with baseline ($p = 0.040$), suggesting that improvement in hair thickness required prolonged application. No statistically significant differences were detected between baseline and the 4th week ($p = 1$), baseline and the 8th week ($p = 1$), or between the 4th and 8th week ($p = 1$). Although comparisons between the 4th and 12th week ($p = 0.077$) and the 8th and 12th week ($p = 0.089$) showed an increasing trend, they did not reach statistical significance after adjustment. The placebo-treated side demonstrated no statistically significant changes in hair diameter at any time point (all $p > 0.05$).

In terms of GPAS scores, the GHK-Cu serum–treated side showed a statistically significant change over time ($p = 0.001$), and the placebo side also demonstrated a statistically significant change ($p = 0.028$). However, direct comparison between the two sides revealed significantly higher GPAS scores on the GHK-Cu serum side at the 4th week ($p = 0.003$), 8th week ($p = 0.004$), and 12th week ($p = 0.01$), indicating superior clinical improvement with the active serum throughout the follow-up period.

Furthermore, patient satisfaction was assessed at the 4th, 8th, and 12th weeks of follow-up. The mean satisfaction scores on the GHK-Cu serum–treated side were higher than those on the placebo-treated side at each follow-up visit, with a statistically significant difference observed at the 12th week.

According to the study findings, a significant increase in eyebrow hair was observed on the GHK-Cu serum–treated side. This effect may be attributed to the biological mechanisms of GHK-Cu, including stimulation of dermal fibroblast activity and enhancement of collagen synthesis, which help maintain the structural support of the dermal environment surrounding hair follicles. In addition, GHK-Cu promotes dermal papilla cell proliferation and upregulates growth factors such as vascular endothelial growth factor (VEGF), thereby enhancing angiogenesis and improving blood supply to the hair follicles. These combined actions contribute to follicular enlargement and strengthening of thinner hair shafts, ultimately resulting in increased eyebrow hair count and thickness following GHK-Cu application.

In a 1993 experimental study, Uno and Kurata investigated a copper-binding peptide (PC1031) applied to the dorsal skin of fuzzy rats and demonstrated promotion of hair follicle growth. The peptide stimulated follicular cell proliferation, maintained terminal follicles, and induced enlargement of vellus follicles into terminal-type follicles during the anagen phase. The authors reported that its hair growth–promoting effect was comparable to topical minoxidil [51]. In another randomized double-blind clinical trial, a combination of 5-aminolevulinic acid (5-ALA) and glycyl-histidyl-lysine (GHK) peptide was evaluated for male pattern hair loss, and after six months the treated groups showed a statistically significant increase in hair count compared with placebo, suggesting the complex could serve as a complementary option for hair growth improvement [11].

Previous studies have shown that GHK-Cu possesses multiple biological activities, including stimulation of dermal fibroblasts, enhancement of collagen synthesis within hair structures, and promotion of dermal papilla cell proliferation and follicular enlargement. In addition, GHK-Cu increases vascular endothelial growth factor (VEGF) expression, promotes angiogenesis, and improves blood supply to hair follicles. Collectively, these mechanisms contribute to increased hair thickness. The findings of the present study are consistent with previous research demonstrating the hair growth–promoting effects of GHK-Cu. The significant increases in eyebrow hair count and diameter observed correspond with the underlying biological mechanisms reported in earlier studies, further supporting the role of GHK-Cu in enhancing hair growth and density.

Various topical agents, including minoxidil and bimatoprost, have also been extensively investigated for eyebrow hair enhancement. A randomized controlled trial comparing minoxidil 2% with bimatoprost 0.01% and 0.03% showed that all treatments improved eyebrow density, with bimatoprost—particularly at the higher concentration—demonstrating superior efficacy [2]. However, these agents may be associated with adverse effects such as local irritation and hyperpigmentation, which can limit long-term compliance. In contrast, the present study evaluated the efficacy and tolerability of a GHK-Cu peptide-based serum using a placebo-controlled design. Owing to its distinct mechanism in promoting hair follicle activity and tissue remodeling, GHK-Cu may offer a more favorable safety profile. Overall, the results of this study are in agreement with prior in vitro and in vivo evidence, supporting the potential of GHK-Cu as an effective alternative therapeutic option for eyebrow hypotrichosis.

Under the conditions of this study, the 2% GHK-Cu serum was safe and well tolerated, with no treatment-related adverse effects such as burning, redness, itching, flaking, or vesicle formation reported throughout the study period. Overall, the serum significantly increased eyebrow hair count and diameter, likely due to its combined biological actions, including stimulation of dermal fibroblasts, promotion of dermal papilla cell proliferation, enhancement of collagen synthesis, and upregulation of growth factors that support follicular development and hair thickening.

## 5.2 Suggestion

5.2.1 The findings from this study can serve as baseline data for further research and may particularly support the development of GHK-Cu in cosmetic formulations, such as scalp hair serums, eyelash enhancers, and combination products for improving hair density. Additionally, these results may provide valuable reference data for future studies on hair growth disorders, including eyebrow thinning and alopecia-related conditions.

5.2.2 The duration of this study was 12 weeks (3 months). A longer follow-up period beyond 12 weeks may provide additional information regarding the sustained

efficacy and long-term safety of GHK-Cu serum. Extended treatment duration may demonstrate more pronounced improvements in eyebrow hair count and thickness.

5.2.3 This study suggests that GHK-Cu serum may serve as an alternative or complementary topical option for eyebrow hair enhancement. The data obtained may also be used for comparison with other hair growth–promoting agents in future clinical research.

## 5.3 Conclusion

From the findings of the clinical study, it can be concluded that the 2% GHK-Cu serum significantly increases eyebrow hair count and diameter without causing harmful side effects among the participants. The formulation was well tolerated throughout the study period. GHK-Cu serum may be considered a safe and effective topical option for enhancing eyebrow hair growth and density.

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# APPENDIX A

## INFORMED CONSENT FORM

I, _____, have decided to participate in [The Efficacy of 2% Copper Peptide (GHK-Cu) Serum for the Eyebrow Hypotrichosis: A Randomized, Double-Blind, Vehicle-Controlled, Split-Face Comparative Study]. I have received information and explanations about this research, and I have had the opportunity to ask questions and receive satisfactory answers. I have had sufficient time to read and understand the information provided in the documents thoroughly and have decided to participate in this research.

I understand that I have the freedom to choose not to participate in this research, and I can withdraw from this research at any time without any impact on my care or rights that I am entitled to.

By signing this document, I do not waive any rights that I am entitled to under the law, and after signing, I will receive a copy of the information sheet and the informed consent.

Participant's Signature _____ Date _____

(_____)

..... (For illiterate participants who can understand though listening) .....

I cannot read, but the researcher has read this information sheet and informed consent form to me, and I understand it well. Therefore, I voluntarily sign or place my fingerprint.

Participant's signature/fingerprint _____ Date _____

(_____)

Signature of the person requesting consent _____ Date_____

(_____)

**Testimony of a witness who is not a stakeholder in the research** (If the participant cannot read but can understand through listening)

I have been present during the consent request process and confirm that the person requesting consent has read / explained the information sheet to _____. The aforementioned person had the opportunity to ask questions and decided to participate voluntarily after understanding the information in this document.

Witness's signature _____ Date _____

(_____)

If the participant is a minor, under the age of 18, or with mental or intellectual disabilities, special protections must be provided.

I, as …….[relationship to the participant]…… have read the information above and fully understand the content. I agree to let Mr. / Mrs. / Miss _____ participate in the research voluntarily. Therefore, I sign this informed consent form.

Legal representative / guardian's signature_____ Date _____

(_____)

# APPENDIX B

## RESEARCH PARTICIPANT PROFILE (CONFIDENTIAL)

THE EFFICACY OF 2% COPPER PEPTIDE (GHK-Cu) SERUM FOR THE EYEBROW HYPOTRICHOSIS: A RANDOMIZED, DOUBLE-BLIND, VEHICLE-CONTROLLED, SPILT-FACE COMPARATIVE STUDY

Volunteer Number: ……..

**General information**

Date: ……..……..…

1. Sex: ●Male ●Female

(Pregnancy/Lactation……..)

2. Age: ……..years

3. Occupation:

   1. Government official
   2. Employees
   3. Private sector businesses
   4. Housewife
   5. Students
   6. Others individuals: …….

4. Pre-existing medical conditions:

   - YES
   - NO

   If YES, please specify:

   1. ……
   2. ……
   3. ……

5. Regularly administered medications and supplements (including both oral and topical medications)

1. ……

2. ……

3. ……

6. History of allergic reactions to medications, food, or chemicals: • YES • NO

……

……

7. History of accidents or injuries in the eyebrow area: • YES • NO

……

……

8. History of treatment received for eyebrow hypotrichosis:

8.1 History of Eyebrow transplant surgery:

- YES
- NO

If YES, please specify: ……

……

8.2 History of Eyebrow Tattooing or Microblading:

- YES
- NO

If YES, please specify: ……

……

8.3 History of Medications, Including both oral and topical forms

- Medications known to promote hair growth (e.g., minoxidil, finasteride, dutasteride, spironolactone)
- Medications known to cause excessive hair growth as a side effect (e.g., phenytoin, cyclosporine, steroids)

- YES
- NO

If YES, please specify:

(1) Name of medicine: ……. Duration used: ……

□ Currently using □ Stopped using it for a period of time: ……

(2) Name of medicine: ……. Duration used: ……

□ Currently using □ Stopped using it for a period of time: ……

(3) Name of medicine: …… Duration used: ……..

□ Currently using □ Stopped using it for a period of time: ……

9. Currently using facial products: …….

……..……

…….

## Records of the Experimental Outcomes

### 1. Global Photographic Assessment Score

Scoring criteria: -3 to +3

| Score | Description |
|---|---|
| +3 | Significant increase in the overall composition of the eyebrows compared to the photograph before serum application. |
| +2 | Moderate increase in the overall composition of the eyebrows compared to the photograph before serum application. |
| +1 | Slight increase in the overall composition of the eyebrows compared to the photograph before serum application. |
| 0 | No change in the overall composition of the eyebrows compared to the photograph before serum application. |
| -1 | Slight decrease in the overall composition of the eyebrows compared to the photograph before serum application. |
| -2 | Moderate decrease in the overall composition of the eyebrows compared to the photograph before serum application. |
| -3 | Significant decrease in the overall composition of the eyebrows compared to the photograph before serum application. |

**Table 1.1** Global Photographic Assessment Score evaluation by 1st doctor

| | Week 0 | | Week 4 | | Week 8 | | Week 12 | |
|---|---|---|---|---|---|---|---|---|
| | Left | Right | Left | Right | Left | Right | Left | Right |
| Global Photographic Assessment Score (-3 to +3) | | | | | | | | |

**Table 1.2** Global Photographic Assessment Score evaluation by 2nd doctor

| | Week 0 | | Week 4 | | Week 8 | | Week 12 | |
|---|---|---|---|---|---|---|---|---|
| | Left | Right | Left | Right | Left | Right | Left | Right |
| Global Photographic Assessment Score (-3 to +3) | | | | | | | | |

**Table 1.3** Global Photographic Assessment Score evaluation by 3rd doctor

| | Week 0 | | Week 4 | | Week 8 | | Week 12 | |
|---|---|---|---|---|---|---|---|---|
| | Left | Right | Left | Right | Left | Right | Left | Right |
| Global Photographic Assessment Score (-3 to +3) | | | | | | | | |

**2. Diameter of eyebrow hairs**

**Table 2.1** the diameters of all eyebrows hairs (micrometer)

| Number of Eyebrow hairs | Treatment Duration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Week 0 | | Week 4 | | Week 8 | | Week 12 | |
| | Left | Right | Left | Right | Left | Right | Left | Right |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | |

**Table 2.2** the average diameter of eyebrow hairs (micrometer)

| | Treatment Duration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Weel 0 | | Week 4 | | Week 8 | | Week 12 | |
| | Left | Right | Left | Right | Left | Right | Left | Right |
| Averaged value (μm) | | | | | | | | |

**3. Number of Eyebrow hairs**

**Table 3.1** the total number of eyebrow hairs within 1cm$^2$ area

| | Treatment Duration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Week 0 | | Week 4 | | Week 8 | | Week 12 | |
| | Left | Right | Left | Right | Left | Right | Left | Right |
| Total number of hairs | | | | | | | | |

**4. Side effects Evaluation**

The researcher will obtain a history of any symptoms and conduct a physical examination of each participant's eyebrow to identify any lesions following serum use, assigning scores ranging from 0 to 3 for each.

| Score | Description |
|---|---|
| 0 | No symptoms |
| 1 | Mild symptoms |
| 2 | Moderate symptoms |
| 3 | Severe symptoms |

**Table 4.1** The evaluation scores of drug side effects by the participants.

| Symptoms | Week 4 | | Week 8 | | Week 12 | |
|---|---|---|---|---|---|---|
| | Left | Right | Left | Right | Left | Right |
| Burning | | | | | | |
| Redness | | | | | | |
| Itching | | | | | | |
| Flaking | | | | | | |
| Dryness | | | | | | |
| Abnormal hair growth | | | | | | |

**Table 4.2** The evaluation scores of drug side effects by the investigator.

| Symptoms | Week 4 | | Week 8 | | Week 12 | |
|---|---|---|---|---|---|---|
| | Left | Right | Left | Right | Left | Right |
| Vesicles | | | | | | |
| Erythema | | | | | | |
| Oedema | | | | | | |
| Scaling | | | | | | |

**5. Research Participants' Satisfaction Score**

After 12 weeks, participants will rate their satisfaction with each side of the eyebrow following serum application by providing a satisfaction score. The scoring criteria are as follows:

| Score | Description |
|---|---|
| +3 | Very satisfied with the treatment result |
| +2 | Moderately satisfied with the treatment result |
| +1 | Slightly satisfied with the treatment result |
| 0 | No changes with the treatment result |
| -1 | Slightly unsatisfied with the treatment result |
| -2 | Moderately unsatisfied with the treatment result |
| -3 | Very unsatisfied with the treatment result |

**Table 5** Satisfactory evaluation by volunteers (Please draw the circle on the number)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Right | -3 | -2 | -1 | 0 | +1 | +2 | +3 |
| Left | -3 | -2 | -1 | 0 | +1 | +2 | +3 |

# APPENDIX C

# CLINICAL EVALUATION

**Table C1** Eyebrow hair count measured at baseline and during follow-up visits at Weeks 4, 8, and 12

| Number of hair | Week 0 (Rt) | Week 0 (Lt) | Week 4 (Rt) | Week 4 (Lt) | Week 8 (Rt) | Weel 8 (Lt) | Week 12 (Rt) | Week 12 (Lt) |
|---|---|---|---|---|---|---|---|---|
| P 01 | 31 | 35 | 32 | 35 | 33 | 32 | 32 | 31 |
| P 02 | 30 | 32 | 31 | 34 | 29 | 33 | 32 | 36 |
| P 03 | 32 | 34 | 30 | 34 | 32 | 33 | 31 | 34 |
| P 04 | 25 | 27 | 24 | 26 | 24 | 27 | 25 | 29 |
| P 05 | 24 | 23 | 24 | 24 | 23 | 25 | 24 | 26 |
| P 06 | 20 | 26 | 19 | 25 | 20 | 25 | 22 | 26 |
| P 07 | 34 | 29 | 34 | 29 | 35 | 28 | 35 | 28 |
| P 08 | 24 | 22 | 24 | 22 | 23 | 21 | 25 | 26 |
| P 09 | 24 | 23 | 24 | 23 | 23 | 23 | 25 | 24 |
| P 10 | 34 | 30 | 35 | 30 | 34 | 31 | 35 | 31 |
| P 11 | 29 | 34 | 29 | 34 | 30 | 35 | 30 | 36 |
| P 12 | 35 | 34 | 35 | 33 | 34 | 35 | 35 | 36 |
| P 13 | 25 | 20 | 25 | 20 | 24 | 23 | 25 | 23 |
| P 14 | 36 | 35 | 35 | 35 | 35 | 34 | 36 | 32 |
| P 15 | 24 | 21 | 24 | 21 | 23 | 20 | 25 | 19 |
| P 16 | 38 | 35 | 38 | 35 | 37 | 36 | 38 | 35 |
| P 17 | 24 | 28 | 23 | 28 | 24 | 29 | 23 | 30 |
| P 18 | 30 | 31 | 29 | 31 | 30 | 30 | 34 | 31 |

**Table C2** Average diameter of eyebrow hairs at baseline and at Weeks 4, 8, and 12

| Average diameter of eyebrow hair | Week 0 (Rt) | Week 0 (Lt) | Week 4 (Rt) | Week 4 (Lt) | Week 8 (Rt) | Weel 8 (Lt) | Week 12 (Rt) | Week 12 (Lt) |
|---|---|---|---|---|---|---|---|---|
| P 01 | 1.794 | 2.018 | 1.81 | 2.001 | 1.82 | 1.991 | 1.82 | 1.97 |
| P 02 | 1.688 | 1.945 | 1.68 | 2.101 | 1.688 | 2.01 | 1.788 | 2.112 |
| P 03 | 1.889 | 1.653 | 1.79 | 1.654 | 1.901 | 1.678 | 1.891 | 1.679 |
| P 04 | 1.432 | 1.553 | 1.459 | 1.503 | 1.456 | 1.543 | 1.401 | 1.6 |
| P 05 | 1.403 | 1.5 | 1.39 | 1.496 | 1.369 | 1.49 | 1.41 | 1.591 |
| P 06 | 1.303 | 1.512 | 1.39 | 1.501 | 1.369 | 1.5 | 1.398 | 1.51 |
| P 07 | 2.201 | 1.928 | 2.211 | 1.925 | 2.218 | 1.94 | 2.22 | 1.926 |
| P 08 | 1.789 | 1.56 | 1.789 | 1.453 | 1.66 | 1.422 | 1.823 | 1.62 |
| P 09 | 2.01 | 1.981 | 1.98 | 2.001 | 2.112 | 1.972 | 2.2 | 2.01 |
| P 10 | 2.19 | 1.891 | 2.11 | 1.9 | 2.161 | 1.88 | 2.2 | 1.89 |
| P 11 | 1.721 | 1.89 | 1.719 | 1.881 | 1.72 | 1.882 | 1.728 | 1.93 |
| P 12 | 2.019 | 2.009 | 2.011 | 2.18 | 2.013 | 2.199 | 2.02 | 2.201 |
| P 13 | 1.789 | 1.62 | 1.78 | 1.638 | 1.691 | 1.688 | 1.701 | 1.7 |
| P 14 | 2.204 | 2.001 | 2.201 | 2.011 | 2.198 | 2.01 | 2.203 | 2.008 |
| P 15 | 1.542 | 1.42 | 1.539 | 1.432 | 1.592 | 1.392 | 1.62 | 1.298 |
| P 16 | 2.228 | 2.109 | 2.23 | 2.101 | 2.218 | 2.1 | 2.221 | 2.191 |
| P 17 | 1.654 | 1.782 | 1.64 | 1.791 | 1.645 | 1.811 | 1.599 | 1.869 |
| P 18 | 1.891 | 1.875 | 1.895 | 1.872 | 1.851 | 1.87 | 1.921 | 1.876 |

**Table C3** Dermatologists' Evaluation Score by side at Weeks 4, 8, and 12

| GPAS | Week 4 (Rt) | Week 4 (Lt) | Week 8 (Rt) | Weel 8 (Lt) | Week 12 (Rt) | Week 12 (Lt) |
|---|---|---|---|---|---|---|
| P 01 | 0 | 0 | 1 | 1 | 1 | 1 |
| P 02 | 0 | 0 | 1 | 0 | 1 | 1 |
| P 03 | 1 | 0 | 1 | 0 | 1 | 1 |
| P 04 | 1 | 0 | 1 | 1 | 2 | 1 |
| P 05 | 1 | 0 | 2 | 0 | 2 | 1 |
| P 06 | 1 | 1 | 1 | 1 | 2 | 1 |
| P 07 | 1 | 1 | 1 | 1 | 2 | 0 |
| P 08 | 1 | 0 | 1 | 1 | 2 | 0 |
| P 09 | 1 | 1 | 1 | 0 | 2 | 1 |
| P 10 | 2 | 0 | 2 | 1 | 2 | 1 |
| P 11 | 1 | 0 | 1 | 0 | 1 | 1 |
| P 12 | 1 | 1 | 1 | 1 | 0 | 2 |
| P 13 | 1 | 1 | 1 | 1 | 1 | 1 |
| P 14 | 0 | 0 | 0 | 0 | 1 | 0 |
| P 15 | 1 | 1 | 1 | 1 | 1 | 0 |
| P 16 | 1 | 0 | 1 | 0 | 1 | 0 |
| P 17 | 1 | 0 | 2 | 0 | 3 | 1 |
| P 18 | 0 | 0 | 1 | 1 | 1 | 1 |

**Table C4** Volunteers' Evaluation Scores at Weeks 4, 8, and 12

| Evalution by volunteers | Week 12 (Rt) | Week 12 (Lt) |
|---|---|---|
| P 01 | 1 | 1 |
| P 02 | 0 | 2 |
| P 03 | 1 | 1 |
| P 04 | 0 | 1 |
| P 05 | 1 | 3 |
| P 06 | 3 | 1 |
| P 07 | 2 | 1 |
| P 08 | 0 | 2 |
| P 09 | 1 | 1 |
| P 10 | 2 | 1 |
| P 11 | 1 | 2 |
| P 12 | 1 | 3 |
| P 13 | 0 | 1 |
| P 14 | 1 | 1 |
| P 15 | 0 | 1 |
| P 16 | 1 | 1 |
| P 17 | 0 | 3 |
| P 18 | 3 | 1 |

**Table C5** Reported Side Effects During the Study Period

| Symptoms | Week 4 (Rt) | Week 4 (Lt) | Week 8 (Rt) | Weel 8 (Lt) | Week 12 (Rt) | Week 12 (Lt) |
|---|---|---|---|---|---|---|
| Burning | 0 | 0 | 0 | 0 | 0 | 0 |
| Redness | 0 | 0 | 0 | 0 | 0 | 0 |
| Itching | 0 | 0 | 0 | 0 | 0 | 0 |
| Flaking | 0 | 0 | 0 | 0 | 0 | 0 |
| Dryness | 0 | 0 | 0 | 0 | 0 | 0 |
| Abnormal Hr | 0 | 0 | 0 | 0 | 0 | 0 |
| Growth Signs | | | | | | |
| Vesicles | 0 | 0 | 0 | 0 | 0 | 0 |
| Erythma | 0 | 0 | 0 | 0 | 0 | 0 |
| Oedema | 0 | 0 | 0 | 0 | 0 | 0 |
| Scaling | 0 | 0 | 0 | 0 | 0 | 0 |

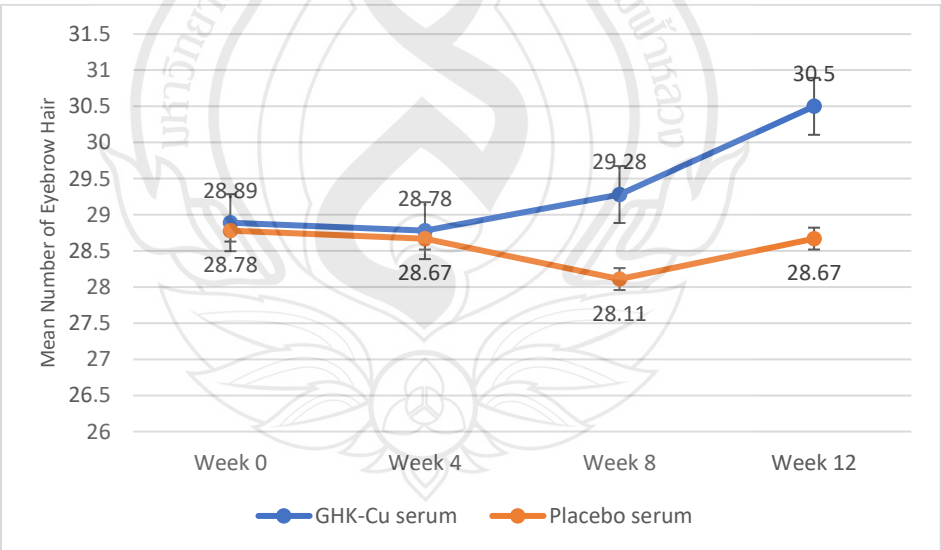



**Figure C1** Linear graph showing comparison of eyebrow hair number at each visit between the GHK-Cu serum–treated side and the placebo-treated side over the 12-week study period

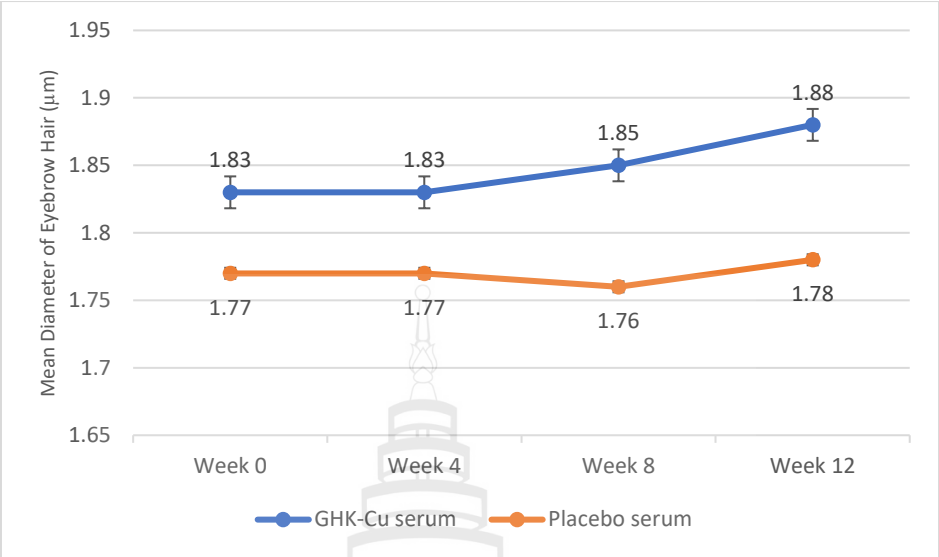



**Figure C2** Linear graph showing comparison of eyebrow hair diameter at each visit between the GHK-Cu serum–treated side and the placebo-treated side over the 12-week study period

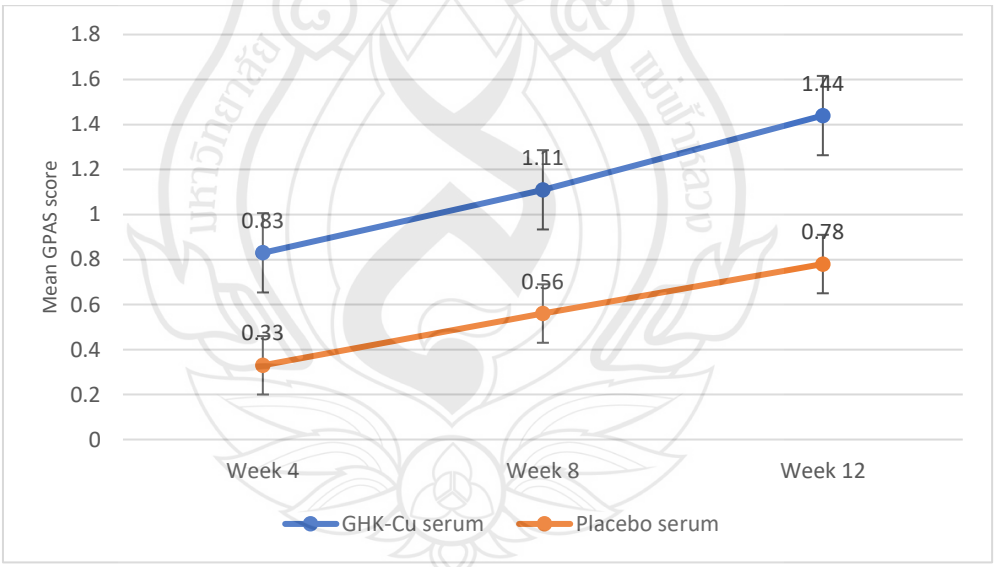



**Figure C3** Linear graph showing comparison of GPAS scores at each visit between the GHK-Cu serum–treated side and the placebo-treated side over the 12-week study period

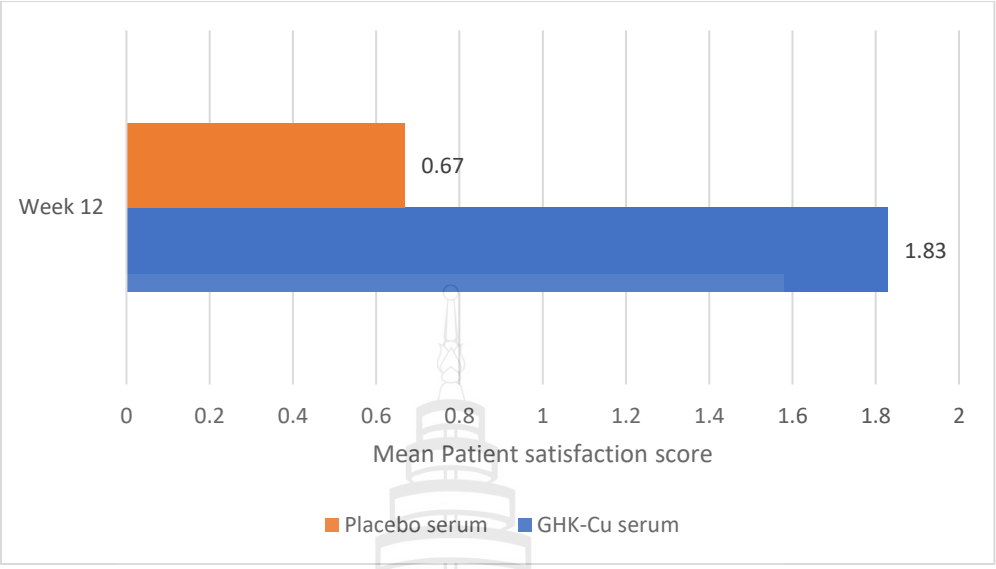



**Figure C4** Bar chart showing mean patient satisfaction scores at the 12-week follow-up for the GHK-Cu serum side and the placebo side.

# APPENDIX D

## STANDARDIZED PHOTOGRAPHS OF SUBJECTS

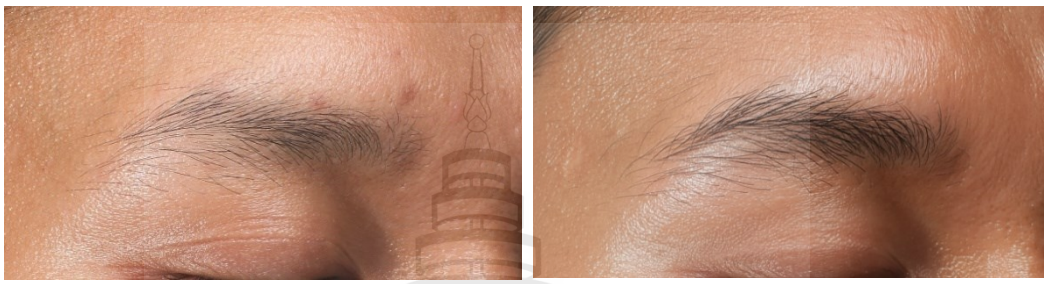



**Figure D1** Photos of subject showing before and after 12th week result on 2% copper peptide (GHK-Cu) serum treated side

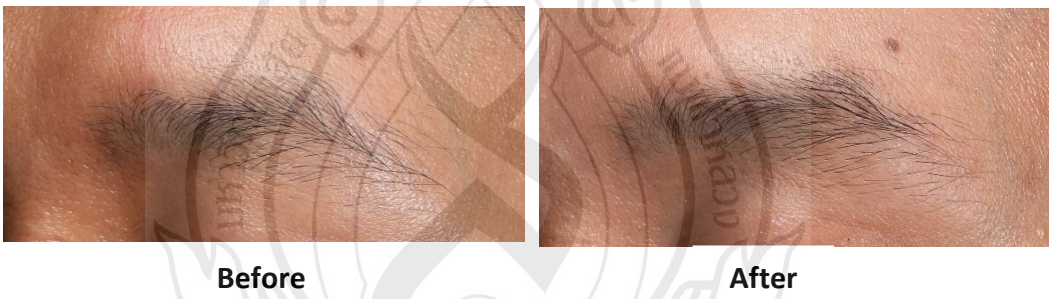
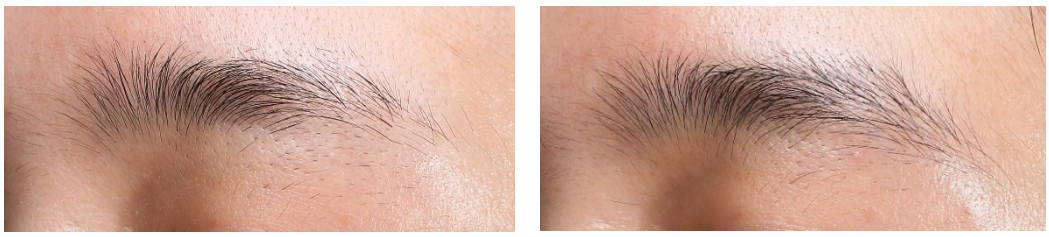
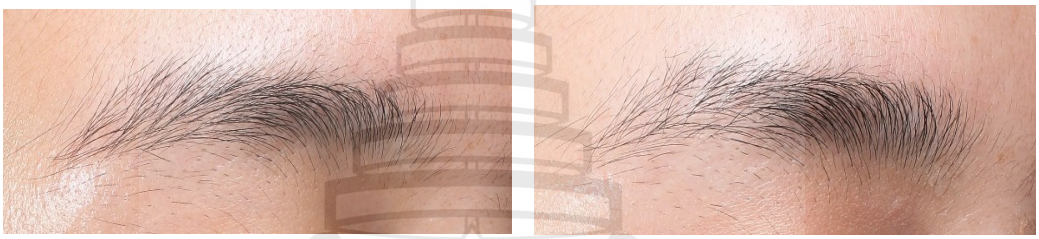



**Figure D2** Photos of subject showing before and after 12th week result on placebo treated side



Before After

**Figure D3** Photos of subject showing before and after 12th week result on 2% copper peptide (GHK-Cu) serum treated side

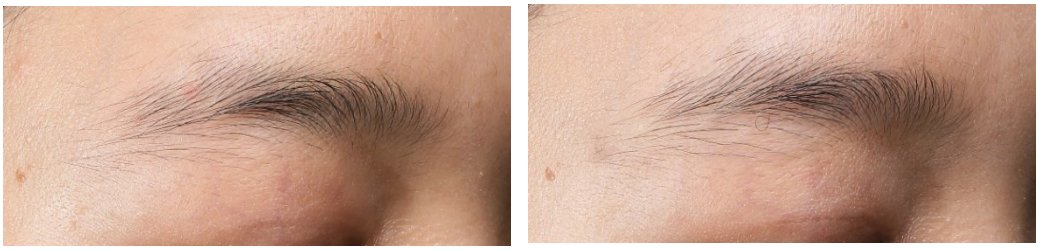


Before After

**Figure D4** Photos of subject showing before and after 12th week result on placebo treated side

Before After

**Figure D5** Photos of subject showing before and after 12th week result on 2% copper peptide (GHK-Cu) serum treated side



Before After

**Figure D6** Photos of subject showing before and after 12th week result on placebo treated side

# APPENDIX E

# MATERIAL


**Figure E1** Photo of 2% copper peptide (GHK-Cu) serum tube and placebo-based serum tube


**Figure E2** Photo of 2% copper peptide (GHK-Cu) serum tube and placebo-based serum tube

# CURRICULUM VITAE

| | |
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