

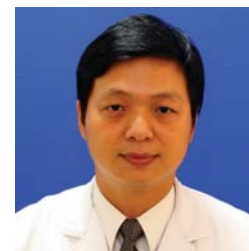
The Effect in Topical Use of Lycogen™ via Sonophoresis for Anti-aging on Facial Skin

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Abstract: *Background:* Anti-aging skin care is a growing popular topic in cosmetic and aesthetic fields, and skin care rather than makeup tips draw more attention nowadays. The phenomenon of skin aging includes thinning of skin losses of elasticity and moisture, pigmented spot formation, and wrinkle development. Along with growth in age, the decreased rates of epithelium renewal and cellular recovery as well as the reduced contents of elastin, collagen, and glycosaminoglycans all contribute to creases or folds of skin. Available strategies for wrinkle treatments include topical use of skin care products with anti-aging contents, dermabrasion, laser, Botox injection, fillers injection, and facelift. Though all of these above options can provide different degrees of improvement in facial wrinkles, the cost-effect, pain of intervention therapy, and necessity of repetitive treatment may impact on choices made. Topical use of anti-aging skin products is the most convenient and cheap way to achieve skin anti-aging effect. Lycogen™ is an antioxidant, which can prevent the down-regulation of pro-collagen I, intracellular accumulation of malondialdehyde (MDA) and achieve the aim of skin rejuvenation. *Methods:* Twenty-six female patients were included in our study with ages between 30 and 45. They were randomly assigned to two groups: the vehicle control group and the experimental group. Patients in the control group applied a skin care product without Lycogen™ to the face via sonophoresis after facial cleanser use in the morning and at night. The experimental group applied a Lycogen™-containing skin care product via sonophoresis in the same time schedule. We evaluated results, including pigmented spots, wrinkles, texture, pores, and red area by VISIA on weeks 0, 1, 2, 4, 6, 8, and 10 respectively. *Results:* In the aspect of pigmented spots, the experimental group showed significant difference in comparison with the vehicle control group on weeks 2, 6, 8, and 10. For wrinkles, the experimental group had better results on weeks 1, 2, 4, 8, and 10. Measured by texture, the experimental group had better results on weeks 1, 2, 4, 6, 8, and 10. Determined by pores, the experimental group had better results on weeks 2, 4, 6, 8, and 10. Concerning red areas, the experimental group had better results on weeks 6, 8, and 10. ($p < 0.05$). *Conclusion:* In our study, we applied a Lycogen™-containing product by sonophoresis as the experimental group in comparison with a skin care product without Lycogen™. VISIA (Canfield Imaging Systems, Fairfield, NJ) was used to evaluate facial skin in aspects of pigmented spots, wrinkles, texture, pores, and red area. Overall, Lycogen™ had proven effectiveness on anti-oxidation as patients who used the Lycogen™-containing product had better outcomes.

Keywords: Anti-aging, cosmetics, Lycogen™, lycopene, sonophoresis.

INTRODUCTION

Aging of skin causes many changes, including thinning, dryness, laxity, fragility, enlarged pores, fine lines and

wrinkles [1]. In addition, pigmentation is a common age-associated change because of exposure to ultraviolet irradiation from sun light [2, 3]. Along with the aging process, fewer moisture barriers such as ceramides, lipids, and fatty acids are produced, which leads to increased trans-epidermal water loss and hence dryness of skin. The aging process leads to less collagen formation and increases in collagenase, which breaks down collagen, resulting in overall decrease of collagen content in the dermis. Exposure to free radicals and ultraviolet rays can both damage collagen fibers and stimulate matrix metalloproteinases (MMPs) activity, which

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results in the collapse of collagen [4]. The number of epidermal cells decreases by 10% per decade, which makes the skin thinner and more vulnerable. Moreover, the rete-ridges of the dermal-epidermal junction flatten out, making less nutrients to penetrate from dermis to epidermis. The aging changes make the skin difficult to repair. Inevitable biological aging lets our cell renewal rate decrease and makes aging skin more susceptible to environmental damage [5]. A wrinkle, a rhytide, is a crease, ridge or fold on the surface of skin. Age wrinkling on the skin begins to form due to the reasons mentioned above such as aging, smoking, habitual facial expressions, sun damage or poor hydration.

Topical application of antioxidants is a non-invasive method for anti-aging. Lycopene is classified as a carotene, which is rich in many fruits and vegetables, such as pink grapefruit, papaya, wolfberry, and tomatoes. It is a powerful antioxidant, which can help protect skin from damage of free radicals [6]. However, lycopene exists naturally in vegetables and fruits and the cost is high if commercial product of lycopene is yielded from pure natural plant extracts. There are studies that report photosynthetic bacteria can be microbial sources of industrial production of lycopene [7]. However, small-scale bioreactors were yielded due to different metabolic pathways. Lycogen™, derived from the extracts of *Rhodobacter sphaeroides*, exerts several biological effects similar to that of lycopene, which can prevent the down-regulation of pro-collagen I, intracellular accumulation of malondialdehyde (MDA), and achieve anti-aging effect [8]. Furthermore, Lycogen™ had potential effects on pigmentation improvement as well [9]. In order to demonstrate an easy way to achieve anti-aging effect by applying skin care product efficiently, this study was designed to apply Lycogen™ to the skin via sonophoresis and to evaluate the outcomes by VISIA complexion analysis objectively.

MATERIAL AND METHODS

All participants were informed about the whole study and gave signed permission for the IRB (Institutional Review Board, KMUHIRB-2013-06-02(I)). Afterwards, skin stimulation test of sampling was performed for all to ensure there was no allergic reaction. Participants were randomized into two groups: the vehicle control group (N=12) and the experimental group (N=14). The vehicle control group members used a skin care product mainly consisting of moisture without Lycogen™. There were twelve women in the control group with ages between thirty and forty-five. There were fourteen women with ages between thirty and forty-five within the experimental group. Members in the experimental group applied a Lycogen™ skin care product. All participants administered the skin care product of 0.5 ml via sonophoresis everyday after the face was washed in the morning and at night, separately. The study continued for 10 weeks. All members enrolled in the study had skin analysis by VISIA (Canfield Imaging Systems, Fairfield, NJ, US) complexion analysis on weeks 0, 1, 2, 4, 6, 8, and 10 respectively. VISIA complexion analysis uses three point standard white light, ultraviolet UV light and cross polarized light as three light imagings to evaluate skin condition in the aspects of pigmented spots, wrinkles, texture, pores, and red area [10]. All the results were collected and analyzed to evaluate the outcome.

RESULTS

After signed permissions for the IRB, all participants were randomly assigned to two groups for testing. Skin condition was evaluated by VISIA complexion analysis with fixed facial position of left 37°, center 0°, right 37° respectively on regular time in the morning and at night on weeks 0, 1, 2, 4, 6, 8, and 10. Comprehensive results of skin condition were recorded in aspects of pigmented spots, wrinkles, texture, pores, and red area. In every subgroup of skin condition evaluation, the results were collected in different facial positions respectively, and an overall result was processed by sum of all three facial positions. The results were presented as figures after statistical analysis by means of paired t-test. The X axis was the time of the experiment, and the Y axis was the percentile calculated by VISIA complexion analysis result in comparison to baseline (week 0).

Pigmented spots are visible focal melanocyte accumulation. Results are presented in (Fig. 1). In this subgroup, the control group had decreased results with left, front, and right facial positions at week 1, which may be interpreted as the skin care product without Lycogen™ not being able to suppress the formation of pigmented spots. In the experimental group, the results with left, front, and right facial positions at week 1 showed improvement, but the results with right and front facial positions had decreased by week 8. After statistical analysis, the experimental group had better results than did the control group ($p < 0.05$) with right facial position on week 8, with front facial position on weeks 8 and 10, and with left facial position on week 10 respectively. In overall results, the experimental group had better results than did the control group on weeks 2, 6, 8, and 10.

Wrinkles are furrows, folds or creases in the skin that increase over time as a result of aging and sun exposure. Wrinkles are associated with decreased collagen and elastin amount in dermis. Results are presented in (Fig. 2). In the control group, results with right and left facial positions had decreased at week 1, but improved by week 6. With front facial position, the control group had better results compared to week 0. The experimental group had better results with left, front, and right facial positions on weeks 1, 2, 4, 6, 8, and 10. After statistical analysis, the experimental group had better results than did the control group with right facial position on week 1, with front facial position on week 4, and with left facial position on week 8. In overall results, the experimental group had better results than did the control group on weeks 1, 2, 4, 8, and 10.

Texture measures the skins smoothness by identifying the hills and valleys of the skin. Raised areas are pimples, milia, blackheads, dead skin cells, shown in yellow. Indentions are fine lines, open pores, open hair follicles shown in blue. The results are presented in (Fig. 3). In the control group, results with front facial position improved on weeks 1 and 2, but became worse than baseline on weeks 4, 6, 8, and 10. The experimental group had better results with left, front, and right facial positions on weeks 1, 2, 4, 6, 8, and 10. After statistical analysis, the experimental group had better results than did the control group with right facial position on weeks 1 and 2, with front facial position on weeks 4, 8, and 10, and with left facial position on weeks 2, 8, and 10. In overall results, the experimental group had better results than did the control group on weeks 1, 2, 4, 6, 8, and 10.

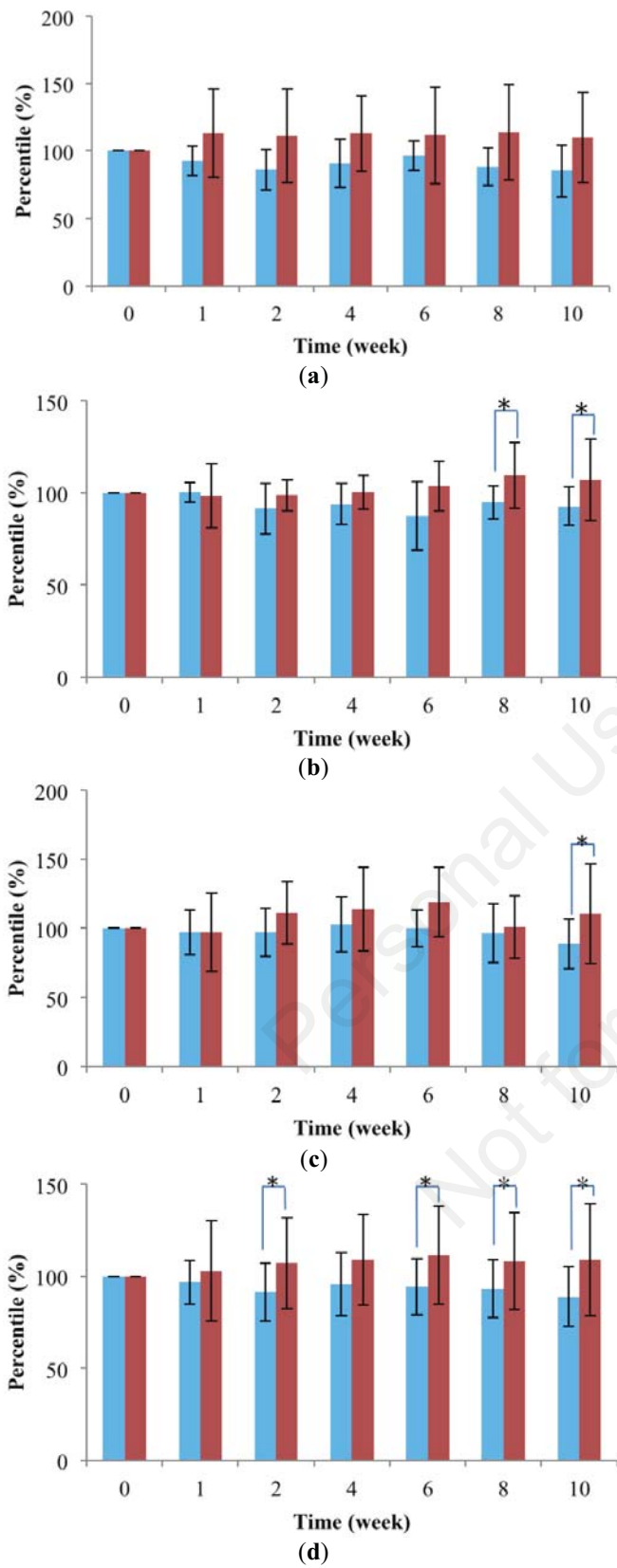


Fig. (1). Pigmented spots on face evaluations through right side (a), front (b), left side (c) and overall facial position (d). Blue color, the vehicle control group; and red color, the experimental group ($p < 0.05$).

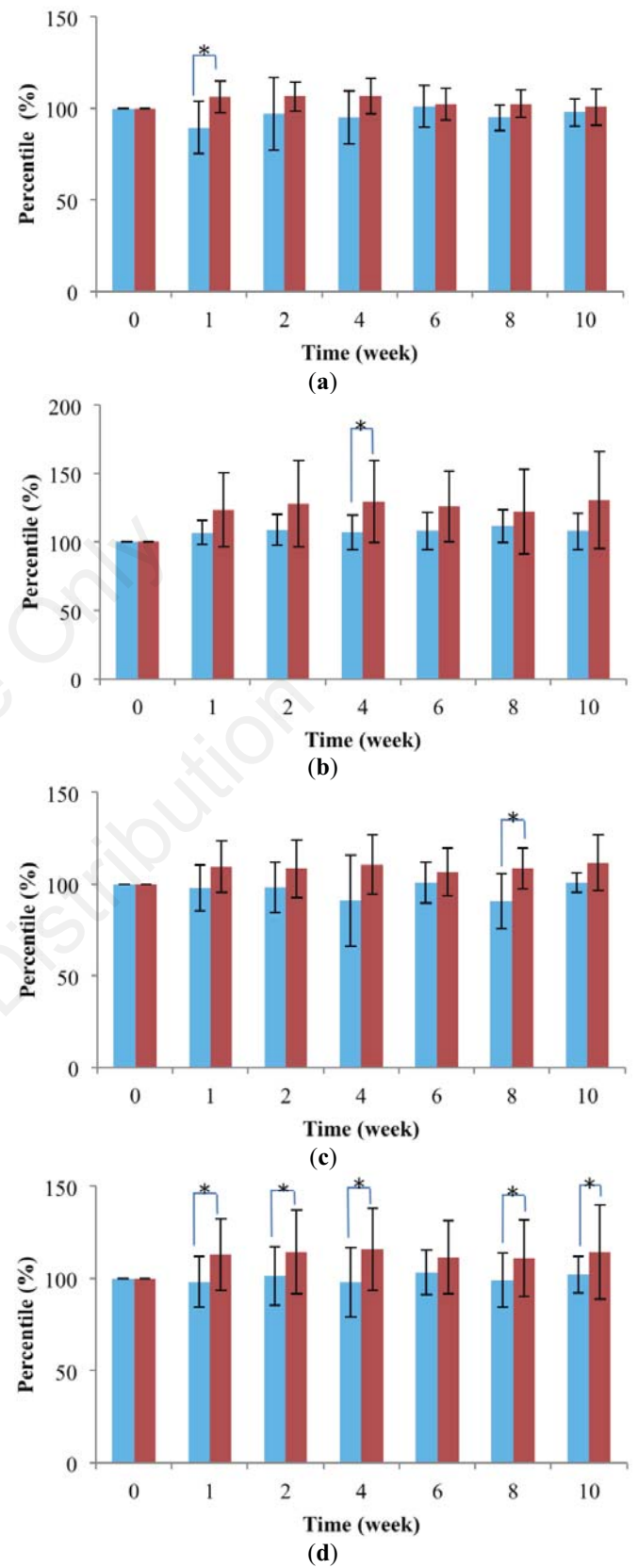


Fig. (2). Wrinkles on face evaluations through right side (a), front (b), left side (c) and overall facial position (d). Blue color, the vehicle control group; and red color, the experimental group ($p < 0.05$).

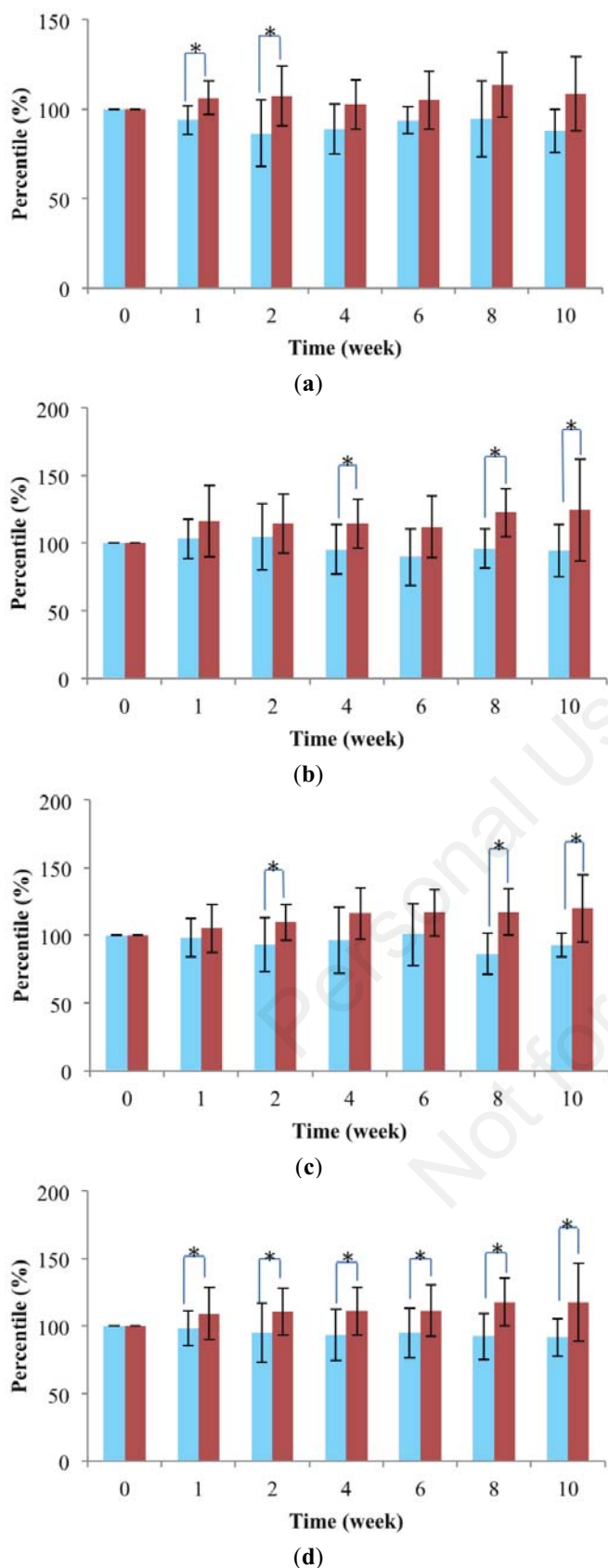


Fig. (3). Textures on face evaluations through right side (a), front (b), left side (c) and overall facial position (d). Blue color, the vehicle control group; and red color, the experimental group ($p < 0.05$).

Pores are the circular surface openings of sweat and oil gland ducts, and the sebum from pores can help thermal regulation and prevent skin being dried out. The size of pores may be bigger in oily-skinned people, the elderly, and males. Because dust and dirt can easily clog the pores, an inadequate facial cleaning process may enlarge the pores over days. The VISIA system distinguishes pores from spots based on size. Pores are much smaller than spots. The results are presented in (Fig. 4). After statistical analysis, the experimental group had better results than did the control group with right facial position on weeks 1, 2 and 8, with front facial position on weeks 2, 6, and 10, and with left facial position on weeks 4, 6, 8, and 10. In overall results, the experimental group had better results than did the control group on weeks 2, 4, 6, 8, and 10.

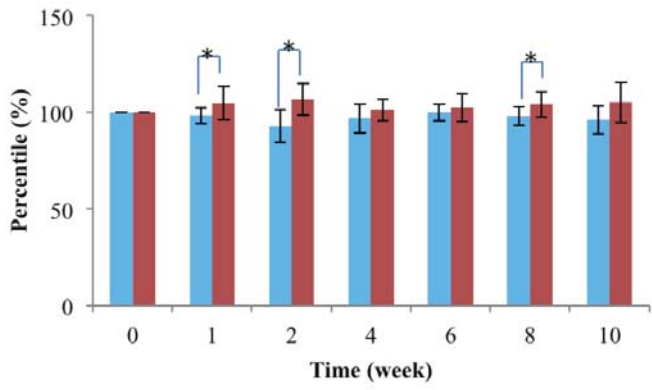
Red areas are weakened and broken capillaries. This profile shows sub-surface vascular conditions such as inflammation, spider veins, rosacea or acne. The results are presented in (Fig. 5). After statistical analysis, the experimental group only had better results than did the control group with right facial position on week 2. In overall results, the experimental group had better results than did the control group on weeks 6, 8, and 10 ($p < 0.05$).

DISCUSSION

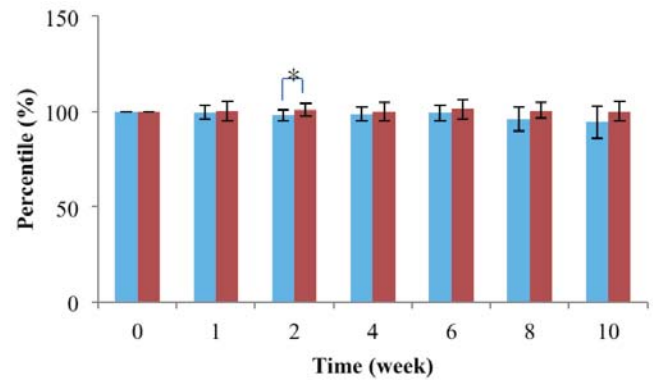
Skin aging is a complex process and can be influenced by both intrinsic and extrinsic factors. Intrinsic factors are mostly determined by genetics or hormones and cannot be stopped. Extrinsic causes include environmental and mechanical factors, excessive sunlight exposure, pollutants, nutrient deficiency, and smoking [11].

Ultraviolet light penetrates into the skin and affects different cells based upon its wavelength and can be subdivided into UVA (320-400 nm) and UVB (290-320 nm). UVB, predominantly absorbed by the epidermis, may trigger sunburns. UVA penetrates deeper to the dermis where collagen is located and may result in decreased collagen production [4]. UVA has been demonstrated to reduce type I collagen [12, 13], the most abundant collagen [4], and inhibits the biosynthesis of pro-collagen synthesis in human dermal fibroblasts [12, 13]. UVA triggers photo-aging via two major pathways, i.e., induction of MMPs expressions and mutations in mitochondrial collagen-related DNA [14]. The resultant generation of reactive oxygen species (ROS) has been shown to mediate effects including transcription factor activation [2], lipid peroxidation [15], and DNA double-strand breakages [16].

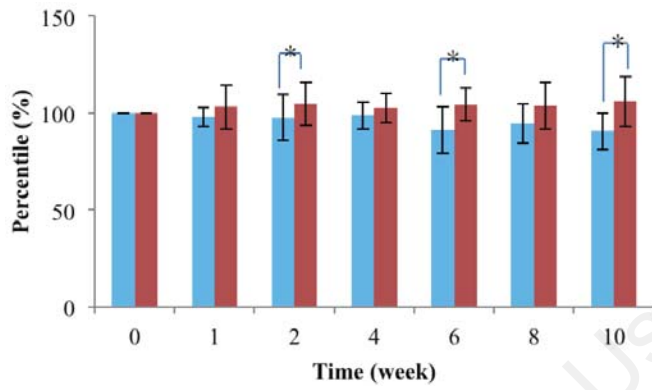
Many phytonutrients have been described as promising photoprotectants in cell culture, animal and clinical studies [17]. The possible mechanisms of these natural products may involve scavenging free radicals, protecting the skin matrix via the inhibition of enzymatic degradation, or promoting the synthesis of collagen in the skin [18]. Lycopene, a member of the carotenoid family, is the red-colored pigment predominantly found in fruits and vegetables such as tomato, papaya, watermelon, etc. It has been shown that cutaneous concentration of lycopene correlates with the roughness of the skin [19]. Actually, some evidence has further demonstrated that topical application of lycopene is a convenient way to restore the UV-depleted antioxidants from the skin



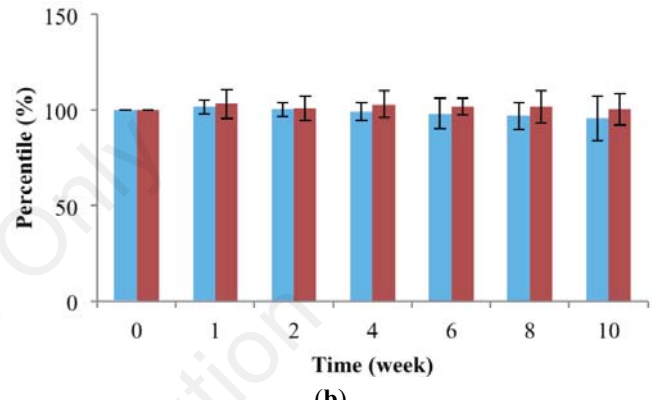
(a)



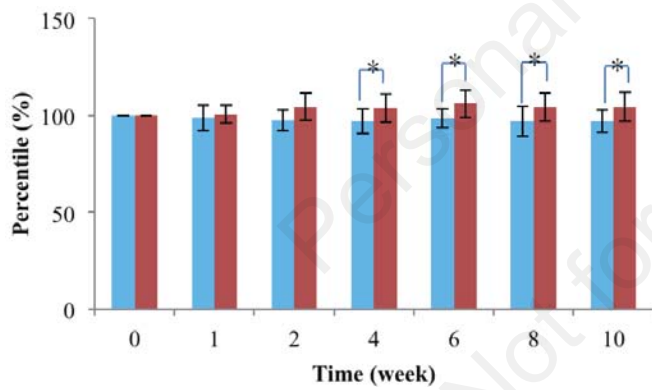
(a)



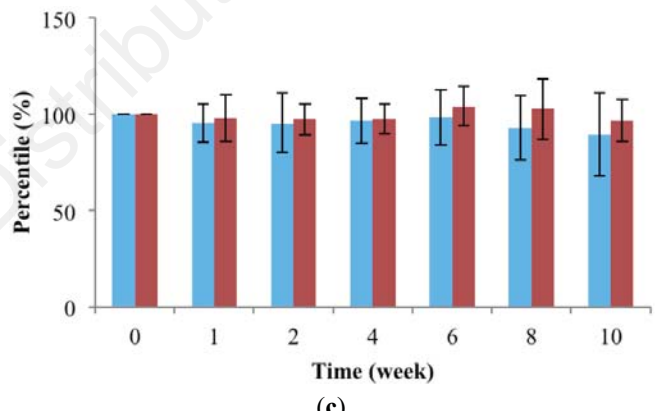
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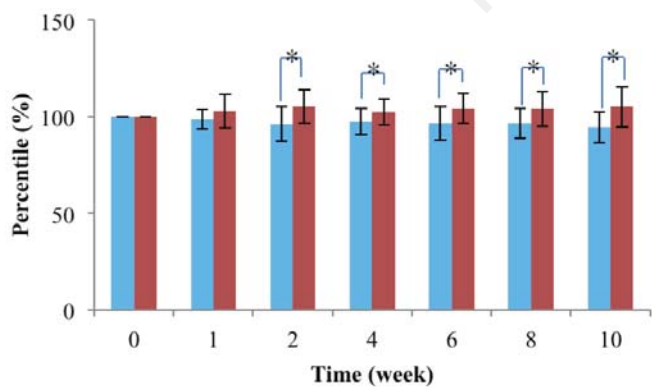
(b)



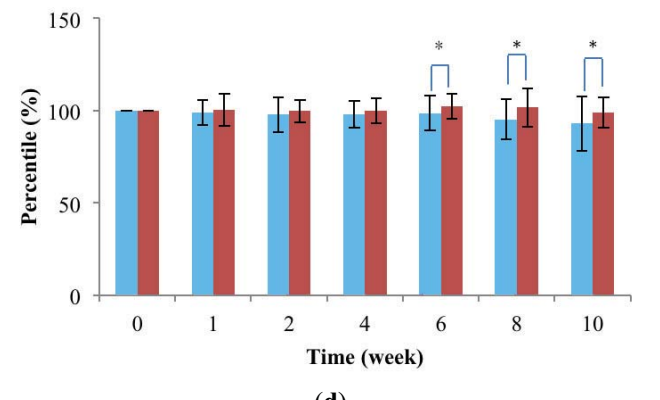
(c)



(c)



(d)



(d)

Fig. (4). Pores on face evaluations through right side (a), front (b), left side (c) and overall facial position (d). Blue color, the vehicle control group; and red color, the experimental group ($p < 0.05$).

Fig. (5). Red areas are weakened and broken capillaries. Results of red areas on face evaluations through right side (a), front (b), left side (c) and overall facial position (d). Blue color, the vehicle control group; and red color, the experimental group ($p < 0.05$).

and can protect skin against premature aging and Cancer [20]. Also, lycopene exhibits antioxidant and anti-inflammatory actions in macrophages [21], inhibits angiogenesis *in vitro* and *in vivo* [22] and decreases cancer risk [23], and may provide protection against cardiovascular diseases [24]. Although several lines of evidence have demonstrated the therapeutic potential of lycopene, several limitations have hindered its development and clinical utility. For example, lycopene is hydrophobic but highly soluble in organic solvents. Extraction of lycopene using organic solvents is usually toxic, expensive, and hazardous. Alternatively, using supercritical CO₂ as a solvent to extract lycopene from waste tomato skin carries less toxicity and other hazards. Nevertheless, the dissolution of lycopene in supercritical CO₂ is still compelling, although its measurement is difficult due to its instability [25]. Therefore, studies have aimed at a reliable method to extract lycopene or identify novel bioactive compounds that exhibit functions similar to that of lycopene.

Microorganisms such as fungi and bacteria may produce metabolites that have been used in cosmetic and pharmaceutical applications. Some of these bacteria-derived metabolites/compounds have been shown to possess prominent therapeutic potentials that may have clinical utility. *Rhodobacter sphaeroides* is a group of bacteria that can generate energy through photosynthesis. An extract of *Rhodobacter sphaeroides* named Lycogen™ is a dark-red compound. The patent strain (WL-APD911) of Lycogen™ was developed by Asia-Pacific Biotech Developing Inc. (Kaohsiung, Taiwan). Lycogen™ has attracted significant attention due to its dramatic biotechnological availability [9, 26]. For example, Lycogen™ can serve as an anti-inflammatory agent that ameliorates dextran sodium sulfate-induced colitis in mice [26]. In addition, it also inhibits melanogenesis through the MEK/ERK signaling pathway [9]. UVA leads to various unfavorable outcomes of skin aging. Although Lycogen™ possesses several biological actions similar to that exerted by lycopene, the known phyto-protectant in skin health, it still remains an open question whether Lycogen™ also protects skin from noxious insult caused by UVA.

In a previous study, administration of the extract of *Rhodobacter sphaeroide* Lycogen™ had shown ability to prevent the decreased cell viability, downregulation of pro-collagen I, the activation of MMP-associated pathways, and accumulation of the products of lipid peroxidation, which are largely induced or recruited by UVA exposure [8].

Despite previous studies showing Lycogen™ has great potential in anti-aging of the skin, including pigmentation, there are no studies evaluating the real effect of topical use of Lycogen™ on human skin. This study was designed to evaluate anti-aging effect of Lycogen™ by topical use.

Since 1930, medical equipment based on ultrasonic technology has been available. Various purposes can be achieved with different frequencies, including diagnostic sonography and therapeutic application. Absorption of topical skin care products via direct application on the skin can be inefficient due to stratum corneum (about 10–30 μm). Sonophoresis can facilitate transdermal delivery of compounds in skin care products based on ultrasonic cavitation and gas body activation.

In our study, skin care products with and without Lycogen™ were applied to the skin via sonophoresis to achieve efficient transdermal absorption. We chose VISIA as a tool to evaluate outcomes of skin condition. VISIA complexion analysis has high-tech RBX skin photo system where internal position and standard illumination ensure each image is consistent and repeatable. Also, it provides an informative comparison of women with the same age and ethnicity. Because VISIA complexion analysis is non-invasive, and has the characteristics of high resolution, specificity, quantitative, repeatability, a global large database, it is an ideal tool to evaluate our outcome objectively.

In our result of pigmented spots, the control group had fluctuating results during time of follow-up and some showed decreased value. On average, the control group had worsened results compared to baseline condition. In the experimental group, all the outcomes became better after week 4 compared to baseline, despite no absolute trend. In overall results, the experimental group had better results than did the control group on weeks 2, 6, 8, and 10 after statistic analysis. Pigmented spots may be affected by multiple factors, including photoaging, hormone status, and life style differences. In our patient group, most of them were nurses, so night shifts may affect these results. Lycogen™ can reduce melanin production *in vitro* and *in vivo* by targeting the ERK signaling pathway [9]. In our study, the result is compatible with this theory.

For wrinkles, the control group with front facial position had mildly improved results compared to baseline. Because the skin care product applied by sonophoresis had better transdermal efficiency, it may have resulted in improved results due to increased moisture even without Lycogen™. In overall results, the experimental group still had significantly better outcomes than did the control group on weeks 1, 2, 4, 8, and 10. This can be interpreted as Lycogen™ providing better efficacy in wrinkle-prevention because it can help increase overall collagen contents in the dermis.

For texture, the control group had varied results compared to baseline. In front facial position, the control group had improved on weeks 1 and 2, but decreased on weeks 4, 6, 8, and 10. This may be interpreted as a non-sustained effect of skin care product without Lycogen™. After statistical analysis of overall results (different facial positions), the experimental group had significantly better outcomes than did the control group on weeks 1, 2, 4, 6, 8, and 10.

Concerning pores, the control group had declined scores in comparison with baseline during follow-up in all different facial positions. In overall results, the experimental group had significantly better outcomes than did the control group on weeks 2, 4, 6, 8, and 10.

Many factors may influence scores of the red area, including inflammatory condition of skin or capillary injury. There was no obvious trend noted solely in control or experimental groups. However, the experimental group still had better scores than did the control group on weeks 6, 8, and 10 after analysis. Because the outcome of the red area may be influenced by lots of factors, the results could not be interpreted easily.

Among all these aspects of skin aging, topical use of Lycogen™-containing skin care product had great effect on wrinkles, texture, and pore prevention. Concerning pigmented spots and red areas, Lycogen™ still had better results, but not as overwhelming as in other aspects. However, the results on weeks 6, 8, and 10 all had significantly better outcomes, so the effects may be more prominent in longer follow-up. Overall, this study demonstrated the potential great ability of topical use of Lycogen™ in anti-aging of skin.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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