

Assessment of various traditional Chinese medicine formulas on skin micro-circulatory perfusion

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Abstract. Laser Doppler imagery is a contemporary form biomedical science specializing in non-invasive micro-circulation investigation. This paper outlines the process and results of applying twelve different test samples to mouse skin to collect data variation of micro-circulation, then judge the effects of different formulas in skin health improvement. More specifically, it is said [15] that micro-circulation is associated with anti-aging (otherwise known as micro-circulation acceleration) by the way of improvement of nutrition supply and hazard substance elimination, has some effects of skin anti-aging. In this paper, compared with Group 0 (the blank group), Group 1, and Group 2 formulas have statistically significant differences in skin micro-circulation perfusion, which implies that those two groups are provided with better anti-aging formulas. So, Group 1's formula and Group 2's formula have the most significant positive effect on the skin health.

Keywords: Laser doppler imager, skin micro-circulatory perfusion, skin health, Chinese medicine formulas

1. Introduction

“Flavonoid” is a term that represents a group of plant polyphenol secondary metabolites, which exist in fruits, medicinal plants, and teas [1], and have a wide range of critical biological functions, including anti-allergenic antioxidant, anti-inflammatory, anti-carcinogenic, and antiviral effects, both when in vitro or in vivo [2-4]. However, there are less than 5% flavonoids with low bioavailability, which reduces some of metabolic transporters activity and enzymatic activity [5-7]. Thus, Flavonoids configuration and its ratio could contribute to the final bioactivity. Skin vessels are arranged into deep and superficial horizontal plexuses and they are provided with thermo-regulation, nutritional and oxygen support. The skin has been proven to be the largest organ in the body, and be utilized for a range of functions. Indeed, the skin can be used as a marker of estimation physiological and pathological status, such as aging, irritation and acne etc [8].

A Laser Doppler imager (LDI) is a piece of widely used technology; an optical and non-contacting equipment, focused at non-invasively monitoring micro vascular blood perfusion [9]. Supported with laser Doppler technology, the Laser Doppler Imager (LDI) is a blood perfusion imager, could transfer imaged tissue blood perfusion to signal digitization, in which way creates a direct measurement

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method. LDI scans the tissue with digital laser beam, to create color-coded images and reflect micro-circulatory perfusion activity.

In this paper, gel covered with 10 selected traditional Chinese medicine formulas is applied to BALB/C mice with back skin. To confirm their ability to accelerate skin micro-circulation perfusion, the Laser Doppler Imager will be used to detect BALB/C mice back skin micro-circulation change, and observation will be conducted 30 minutes after wipe.

2. Materials and methods

2.1. Materials

Eight-week-old female BALB/c mice, weighing 21–25g, were purchased from Jin Muyang animal vivarium (Beijing, China).

2.2. Method

Thirty six mice were housed in a room with constant humidity ($55\pm 5\%$) conditions and temperature ($20\pm 2^\circ\text{C}$). Meanwhile, the mice were reared in an isolator caging system (Niki Shoji, Tokyo), which is a 12-h light/dark cycle. Water and food were accessible *ad libitum*. All the experimental procedures used in the present study were approved by the Committee of Animal Experiments of Science School, Beijing Technology and Business University.

2.3. Traditional Chinese medicine formulas

Ten samples of traditional Chinese medicinal formulas, each featuring its own custom flavonoid content as a biological activator, are mixed with the same base material (gel) in ratio of 1:100, whereas water is deemed as blank control, and the base material (Gel) is negative control. COLLGUARD™ (BASF, China), with the claimed functions of antioxidant and acceleration metabolism is used as the positive control. The base material (Gel) is provided by China Cosmetic Research Center, China. The twelve groups' information is depicted in Table 1.

2.4. Grouping, dosing and testing subjects

Table 1
No. 0-11 Group information

Group No.	Sample	Flavonoid content(mg/mL)
0	Water(blank control)	0
1	Traditional Chinese medicine formula 1	0.3360
2	Traditional Chinese medicine formula2	0.4440
3	Traditional Chinese medicine formula3	0.318
4	Traditional Chinese medicine formula4	0.391
5	COLLGUARD™(positive control)	0.0889
6	Traditional Chinese medicine formula5	0.540
7	Traditional Chinese medicine formula6	0.461
8	Traditional Chinese medicine formula7	0.220
9	Traditional Chinese medicine formula8	0.441
10	Traditional Chinese medicine formula9	0.240
11	Gel (negative control)	0

Thirty-six experimental mice, are placed in 25°C constant temperature and humidity laboratory, and all of them are divided into 12 groups evenly (corresponding to Group 0-11), namely 3 mice for one will be placed on the mouse pad and scanned by the laser Doppler imager to explore skin micro-circulatory perfusion rate.

2.5. Statistic analysis

Next, we identified two independent variables; micro-circulatory perfusion number respectively gained at pre-dose and post-dosed. Get the mean of three mice within a group to inspect whether samples, in this way to reduce the test system impact. In this experiment, one-way analysis of variance method is used.

3. Result

After reviewing the conclusive results, within the same group of mice, there are no significant differences ($P > 0.05$), (see Table 2). However, when at the post-dose, among Group 1 and Group 2, subject's skin micro-circulatory perfusion rate is significantly increased when compared with blank control ($P < 0.05$). (see Table 2). Figures 1-12 illustrate micro-circulatory perfusion changing by wiping Group 0 and Group 1. For more specifics, we included a color-coded picture (left) representing micro-circulatory distribution information. The comparison between Figures 1 and 2 could refer to the factor that Group 1's formula is able to accelerate skin micro-circulatory perfusion rate. Lastly, the remaining figures record their corresponding groups and test samples. Flavonoid has the ability of reducing cholesterol level and decreasing resistance in plasma, leading to accelerated blood flow velocity [10]. For this test, flavonoid different configuration and its ratio instead of its content, do have an impact on micro-circulatory perfusion. As such, groups 1 and 2 could be recommended as specimens to be added to cosmetic bioactivities, which compared with the remaining traditional Chinese medicines.



Fig. 1. Group 0 Post-dose micro-circulatory perfusion.



Fig. 2. Group 1 post-dose micro-circulatory perfusion.



Fig. 3. Group 2 post-dose micro-circulatory perfusion.



Fig. 4. Group 3 post-dose micro-circulatory perfusion.



Fig. 5. Group 4 post-dose micro-circulatory perfusion.



Fig. 6. Group 5 post-dose micro-circulatory perfusion.



Fig. 7. Group 6 post-dose micro-circulatory perfusion.



Fig. 8. Group 7 post-dose micro-circulatory perfusion.



Fig. 9. Group 8 post-dose micro-circulatory perfusion.



Fig. 10. Group 9 post-dose micro-circulatory perfusion.



Fig. 11. Group 10 post-dose micro-circulatory perfusion.

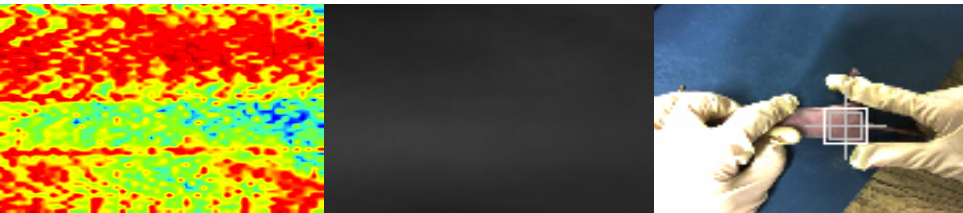


Fig. 12. Group 11 post-dose micro-circulatory perfusion.

Table 2

Group No.	Pre-dose & Post-dose Skin Micro-circulatory Perfusion, \pm s (n=3)	
	Pre-dose	Post-dose
0	374.23 \pm 70.73	293.71 \pm 49.66
1	498.52 \pm 118.21	505.15 \pm 28.42*
2	375.58 \pm 101.81	499.21 \pm 90.88*
3	335.15 \pm 56.84	399.11 \pm 62.39
4	342.45 \pm 96.69	315.74 \pm 36.01
5	311.12 \pm 29.26	354.00 \pm 39.65
6	429.10 \pm 161.41	357.57 \pm 58.70
7	353.87 \pm 115.57	352.22 \pm 59.67
8	311.71 \pm 22.89	375.99 \pm 88.99
9	314.83 \pm 144.78	340.47 \pm 112.57
10	363.55 \pm 115.59	423.66 \pm 87.79
11	302.36 \pm 18.76	433.71 \pm 91.83

Note: Comparing with group 0, * P <0.05

4. Discussion

Nowadays, various skin micro-circulation detection technologies are being used, such as Volume graphical method, Angioscope, Infrared Thermal Imaging, Xenon Washout Technology, etc. The Laser Doppler Imager, however, is the most widely used instrument for measuring skin-microcirculation. With stepwise moving laser beams over the tissue, color-coded images of the skin micro-circulation perfusion will be created [11].

The Laser Doppler Imager uses a 658 nm red laser, low-power blood perfusion measurements. By moving its mirror from one site to another, a laser beam is reflected down into the tissue, which is then followed by photo detector to collect blood perfusion information. Afterwards, when processing the data, a color camera is first set as the method of documentation. This camera can be set to extra high recording speeds in order to accurately analyze the test subject's skin micro-circulatory perfusion, which simultaneously ensuring the test instruments are stable during the measuring process. Additionally, each recording is made up of a serious of discrete measurement points, although laser beam scanning motion seems to be continuous [11].

As shown in Table 1, Group 1 and Group 2 do not have the highest amount of flavonoid, but they both significantly increase skin micro-circulatory perfusion. The reason for that is because various flavones configuration and the different ratio in traditional Chinese medicine formulas could trigger distinguished skin micro-circulation perfusion. For example, pentahydroxyflavone, rutin, and epigallocatechin gallate are all flavonoids which equip different abilities of free radical clearance and micro-circulatory acceleration [11].

As time passes, there are inevitable changes to ones physique and mental state that can be attributed to aging alone. Recent studies show that elastic tissue destruction was related to age in both males and females, and that skin micro-circulation is in variation in both the youth and the older. There are approximately sixty loops to every cm^2 in normal young people. With aging, there are less loops in skin. Tsuchida [12] recent study illustrates that in both sexes from twenty to seventy-two years, who are associated with high blood cholesterol. However, Ashimaoui [13] research shows that even when excluding the effects of high blood cholesterol, the older subjects had lower basal blood flow and impairment of maximal blood flow.

Micro-circulation has high potential for dominating the nutritional substance industry by promoting

energy metabolism, releasing bioactive substances within the skin, accelerating micro-circulation, increasing basal metabolic rates, and increasing nutritional substance transfers [14-18]. Thus, theoretically, skin health can be achieved by skin micro-circulation acceleration. According to the research of Yoshiya Ito [19], pre-pubertal, young adult, middle-aged mice micro-circulation decrease stepwise, but senescent mice perfusion reduced more significant, which means micro-circulation is in direct proportion to senility [20, 21].

Micro-circulation has been progressively emphasized during the past twenty years in medical and drug research [18], but using the Laser Doppler Imager as a means to construct skin micro-circulation and skin senility relation in cosmetic area is the first attempt. Moreover, there is still a lot of unknown area to be explored. For example, establishing a correlation analysis of skin micro-circulation and skin evaluation index (such as skin moisture content, water de-absorbtion etc.).

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