Influence of mice microvascular induced by LED with different wavelengths

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Although a variety of experiments on light exposure stress to animals significantly affect the retina and vision neurons, it is still unknown whether light of different wavelengths can cause different extent of damage to micro vascular and circulation system. This study of light pollution on organisms is aimed to investigate the effects of four kinds of LED light radiation to mice. The results show that micro blood vessels are expanded and the flow is firstly accelerated and then slowed down. The extents of these variations are relatively different as the exposure time and wavelength changes. However, all of them cause damage to mice physiological conditions, producing some degree of light pollution. Experimental foundation to guarantee the safe wavelength and proper time of radiation is provided by the diversity of blood vessel condition. The research findings supply the guideline for the effective prevention of the impact on organism by light pollution from the view of science of optical life science.

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1. Introduction

The light pollution is coming as a new kind of environmental pollution after pollutions of waste gas, waste water, waste residue and noise, etc. mainly including bright pollution, artificial daytime and colour light pollution [1~4]. Light pollution has many negative influences, such as hurting eye and skin, arousing traffic accident, upsetting regular life cycle of animals and plants and so on. "Light and Health" becomes a research hotspot in the recent years becoming an important part of life science [5]. Researchers have paid more attention to the relations between light radiation and damage of the organisms [6, 7]. They focused on the amount of light which was able to cause damage, but the damaging extents of different wavelengths light were rarely mentioned. A series of practical data and inspiring results are collected by experimental simulation. Relation between the recorded damage and amount of light acting on the beings are studied, too. We will focus on the influence of the light produced by LED light source of different wavelength, on microcirculation in human beings.

Previous experimental researches using light radiation of animals have shown that light stress causes visual cell damage or even health problems [8-11]. Light stimulation on the rod and cone photoreceptors is known to increase retina blood flow in micro vessels. The elevated blood velocity and the increased retina vascular diameter have been measured by German scientists in 2010 [12]. The primary aim of micro vascular diameter expansion and blood flow regulation is to provide a stable oxygen supply to the retina. This is confirmed by Roy and Sherrington [13, 14] in their study on brain showing that an enhanced local blood flow may compensate for the increased oxygen demand in stimulated neural tissue. Bill and Sperber [15] have reported that a flicker light induced retinal blood flow increase in monkeys. Intense light exposure causes unrecoverable damage and may affect the microcirculation [16-19]. By its action on the photoreceptor cells, light radiation leads to expansion of micro vascular and accelerates the blood flow. However, there is few report of the extent of light induced damage on animal micro vessels. In this study, albino mice are used to measure the diameter of their micro vascular tissue. Light-induced damage in the albino mice is a suitable model system to determine the extent of light pollution of animal micro vascular system. The present study is designed to find the impact of different light radiation on animals.

2. Materials and methods

2.1 Experimental preparation

Some ICR (Institute of Cancer Research) male mice (adult, 18~22 g) were housed in individual polypropylene cages and fed with commercial pellet rat food and tap water. The mice were allowed to move freely in cages at room temperature of 26±2 ℃ under normal light and dark cycle. The mice were divided into 4 groups (group a to d). All the experiments were carried out in the darkroom. The mice were put on the experimental devices with head fixed. About ten minutes later, the animals were adapted to the experimental conditions and calm down. We recorded microscope images of auricle microcirculation of the mice under normal physiological conditions and have measured

the diameter of the micro vessels. These data were accepted as controls.

2.2 Light source selection

The first priority in our experiment was to select some appropriate artificial light sources in daily life as light pollution sources. LED technology as well as its applications was growing very fast. Powerful and high brightness products were continued to be brought to the marketplace, which deserves more attention to the hazard of LED radiation. The enlargement of power and external quantum efficiency of LED chips, the wide applications of different wavelength LED chips, the multi-chips integration technologies have made LED radiation more harmful nowadays. Spectrum of the chosen light sources must be tested to find monochromatic light. We focused on the effect of visible light pollution to animals. 4 LED light of different wavelengths were selected as experimental light sources. The wavelengths were 464.7 nm, 512.6 nm, 595.0 nm, and 637.3 nm. We measured the emission spectra of these typical light sources as follows (Fig.1):

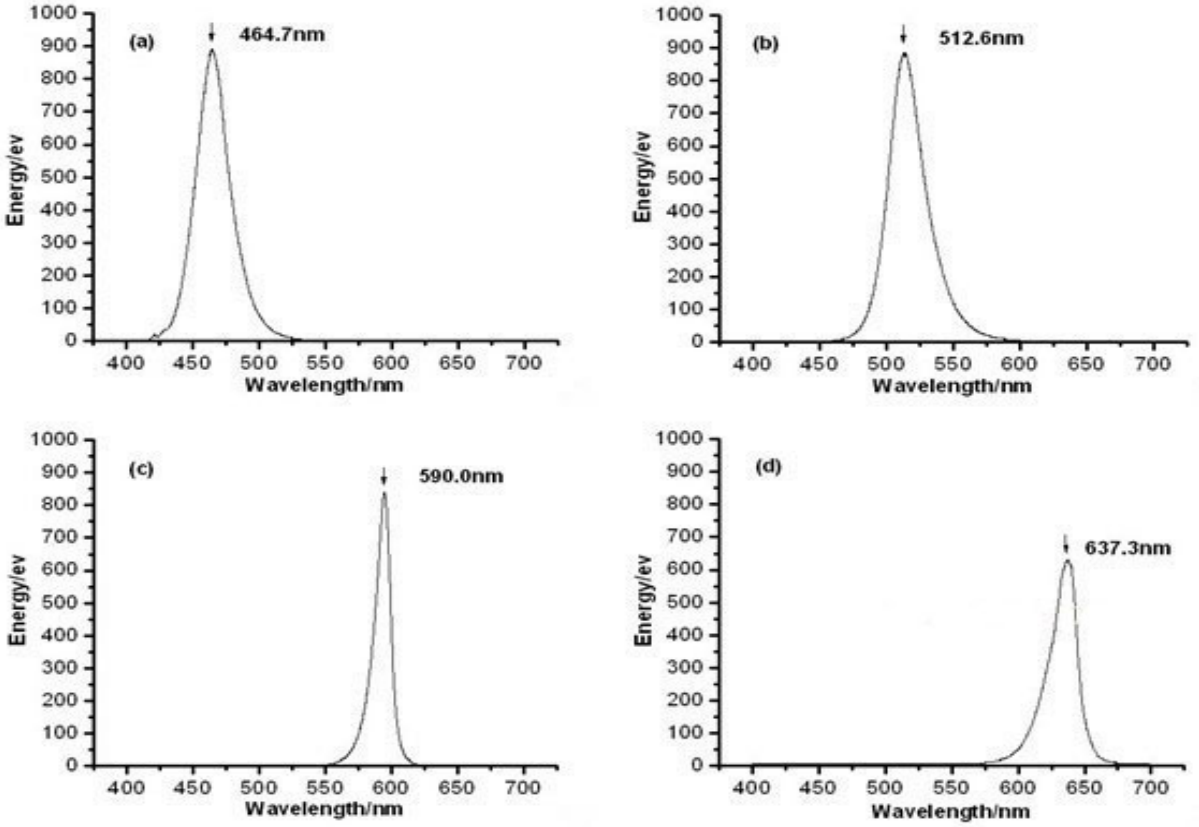

Fig. 1. Spectrum of LED light sources with different wavelengths.

2.3 Experimental system and methods

The main experiments were using the LED light radiation to irradiate the animals. The 4 LED light sources were of the same power, 1.0 W. All the 4 groups of animals were fixed on the experimental table. The part of the radiation was directed towards the mice eyes and the exposure time was 40 minutes. The distance between the light source and animal eyes was set as 5 centimetres. We used optical fibber to couple the LED light to make sure that the mice receive the same amount of light radiation. Groups (a) to (d) were irradiated with LED light of wavelengths 464.7 nm, 512.6nm, 590.0 nm and 637.3 nm. The auricle microcirculation images were recorded and manipulated while the blood flow volumes were preserved during the radiation procedure. We observed the phenomenon in micro vascular change and made comparisons of them before and after the radiation.

The optical pathway is shown in figure 2. The whole system is consisting in optical microscope, image acquisition system, real-time monitoring system, and data processing system. Light source 1 is a mercury lamp and has strong radiation capacity. We are able to easily uptake the image. Light source 2 is the LED we selected as the excitation source. The filter is used to filter out light of other wavelengths while the cover sheet can block the light from source 1. All of the operations were carried out in this experimental system.

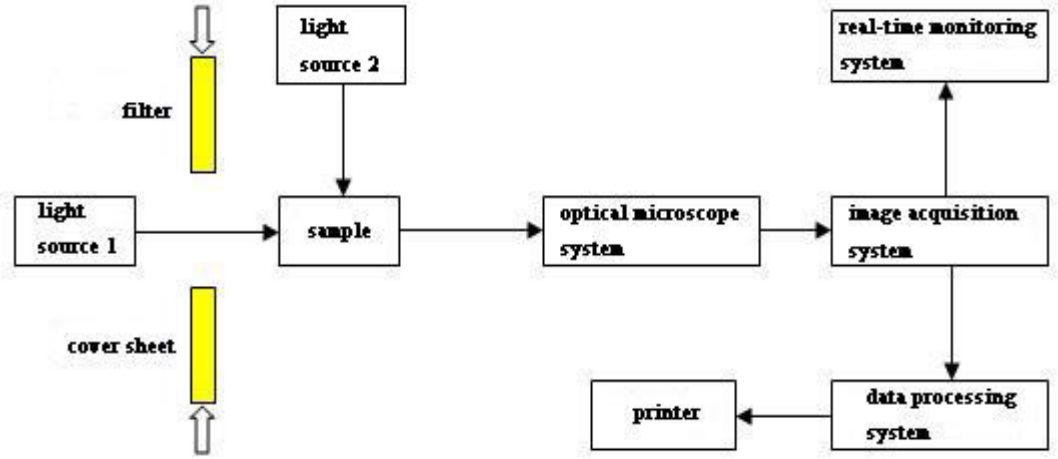

Fig. 2. The optical pathway and the experimental system

3. Results and discussion

We studied the impact on the microscopic images of biological tissue through LED radiation to animal eyes. The micro blood vessel change and the flow variation were observed. Four coloured micro images recording the changes of micro vascular are shown as follows:

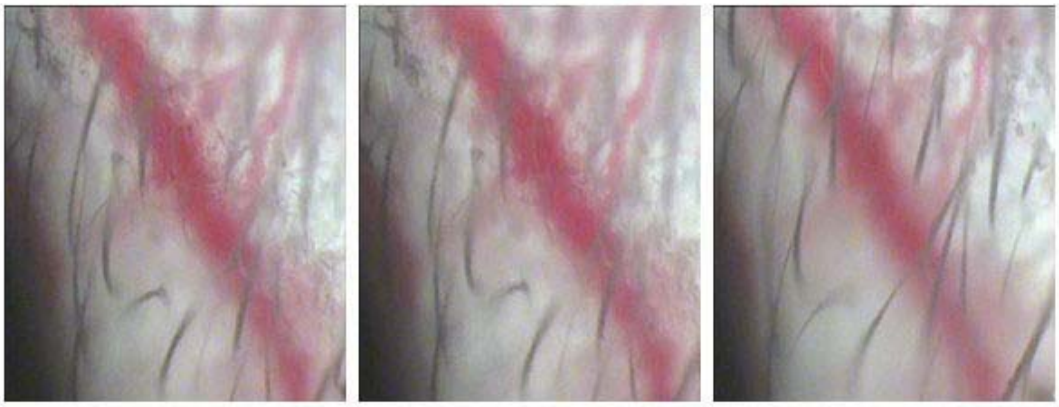

Fig. 3. Micro images of mice auricle blood vessel: (a) Before radiation, (b) 3 minutes after the radiation, (c) 18 minutes after the radiation.

Before the LED light irradiation of the animal eyes, the mouse ear microcirculation was normal and its image clear which is shown in Figure 3 (a). There were many capillaries, blood vessels branches and less direct vessels. Blood vessel seems to be reticular and uniform. The blood flow was smooth and showed line-particle flow, particle-line flow or particle flow. No significant red blood cell aggregation and micro-thrombosis were observed in the viewing region. In our experimental conditions, the mouse ear microcirculation changed obviously when they were irradiated with LED lights. The blood flow became slightly greater and the microcirculation image clearer, as was shown in Figure 3 (b). A few minutes after the irradiation, the blood flow became slow and micro vascular diameter expanded a little. This phenomenon is illustrated in Figure 3 (c). When the time of exposure to LED light has reached 15-20 minutes, microcirculation imaging became fuzzy, like Figure 3 (d). The blood flow velocity achieved a maximum value. Leukocytes can be seen rolling adhering to the wall. Mild aggregation of erythrocytes appeared in some micro vessels. There was no micro-thrombosis but the diameter dilated significantly. The gap between blood vessels and surrounding tissue was slightly blurred, while the colour of perivascular cells becomes reddish. About 5-10 minutes later, micro circulation gradually returned to normal and the flow rate slowed down. The micro vascular diameter decreased a little, but it was still higher than that of the normal

conditions. The micro circulation images became clearer and the colour of blood vessel surrounding tissues became lighter.

Suppose the input digital image is described by a two dimensional function, $I(x,y)$. It can be considered as a 3-dimensional surface, which was:

$$\{(x,y,z) \mid z = I(x,y)\} \tag{1}$$

Here $I(x,y)$ represents the gray value in *x*-*y* coordinate system. The curvature of this surface could be defined with Hessian matrix:

$$H(P) = \begin{bmatrix} I_{xx}(x,y) & I_{xy}(x,y) \\ I_{yx}(x,y) & I_{yy}(x,y) \end{bmatrix} \tag{2}$$

where $I_{xx}(x,y)$, $I_{xy}(x,y)$, $I_{yx}(x,y)$ and $I_{yy}(x,y)$ represent the second-order differentials of the image. In order to easily calculate, we extracted the gray level information of the micro vessel, since the gray value of the vessel cross-section presents a Gaussian distribution. We used Gaussian function to do convolution with the second derivative of the image, which was:

$$I_{ab}(x,y) = h_{ab}(x,y)I \tag{3}$$

Here a,b indicated a particular value of x,y. The second order Taylor expansion at point P was:

$$I(P+\Delta P) \approx I(P) + \Delta P^T \nabla I(P) + \Delta P^T H(P) \Delta P \tag{4}$$

Here $\nabla I(P)$ represented the gradient vector at point P. *H(P)* was the Hessian matrix at point P. For the point on the centreline of blood vessel, eigenvector corresponding to smaller absolute eigenvalue represented the smaller direction of the surface curvature. Eigenvector corresponding to larger absolute eigenvalue represented the larger direction of the surface curvature. The two eigenvectors were orthogonal.

We used the Hessian matrix to track the 2-dimensional micro vascular image centreline, including the following steps:

For a random point, P, on the micro vascular tree, its coordinate was *(x,y)*. We arranged the two eigenvlaue of its Hessian matrix from small to large order, which was $\lambda_1 < \lambda_2$. The corresponding eigenvectors were v_1, v_2. These two eigenvectors determined the direction of the cross-sectional vertical to the centreline.

The point, P, was on the centreline of the micro vascular bed, when subjected to the following conditions:

(1) $(v_1 \bullet \nabla I)^2 + (v_2 \bullet \nabla I)^2 < 0.001$, ∇I was the gradient of I at point P;

(2) $\lambda_1 < \lambda_2 < 0$

To be accurate, the first condition should be $v_i \bullet \nabla I = 0$ (i =1,2). However, the inner product of two vectors could not be fully equal to zero. We used a minimal positive number instead and the sum of squares was less than it. The centreline of micro vascular bed could be obtained after those steps.

Suppose (x_i, y_i) was a random point , P_i (i = 1, 2,…) on the centreline. The slope of the line could be obtained with multi-point fitting of the centreline. As a result, the vertical line equation of it was:

$$-\frac{1}{k_i}x - y + \frac{1}{k_i}x_i + y_i = 0 \tag{5}$$

We searched the intersection of the centreline perpendicular with the border in both directions from the point on centreline. When the distance between the point, P, to the perpendicular was subjected to:

$$d = \frac{\left| -a - b + \frac{1}{k_i}x_i + y_i \right|}{\sqrt{\frac{1}{k_i^2} + 1}} \times \frac{\sqrt{2}}{2} \tag{6}$$

We got two intersection points $D(x_1,y_1)$ and $G(x_2,y_2)$. The diameter of micro vascular vessel in point $C(x_i,y_i)$ was:

$$d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2} \tag{7}$$

By repeating these steps, the diameter of mice auricle micro vessel in different positions along the direction of blood flow was calculated by computer simulation. The position was shown in Fig. 4.

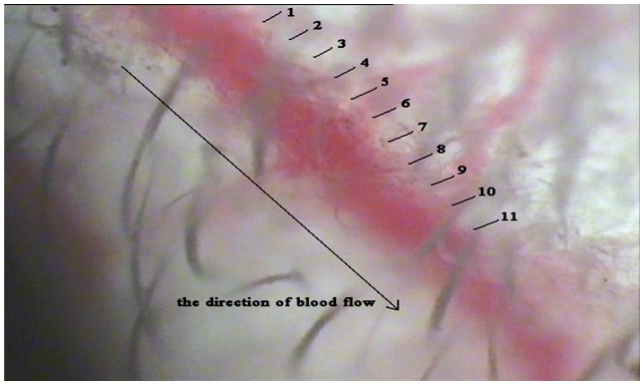

Fig. 4. Selection of positions along the micro blood vascular.

The relation between wavelength and micro vascular diameter was studied. The change curves of the relationship between time and blood flow volume were graphed. The results are shown in Fig. 5 and Fig. 6.

Fig. 5 shows that the average diameter of mice auricle micro vascular tree in four groups expanded after the irradiation. The variations of the average diameter were 2.61 μm, 1.67 μm, 1.09 μm and 1.18μm respectively. Figure 6 also suggested that 464.7 nm LED radiation presents the greatest impact on mice. The change rate of blood flow has firstly reached its maximum. The variation was also the largest one.

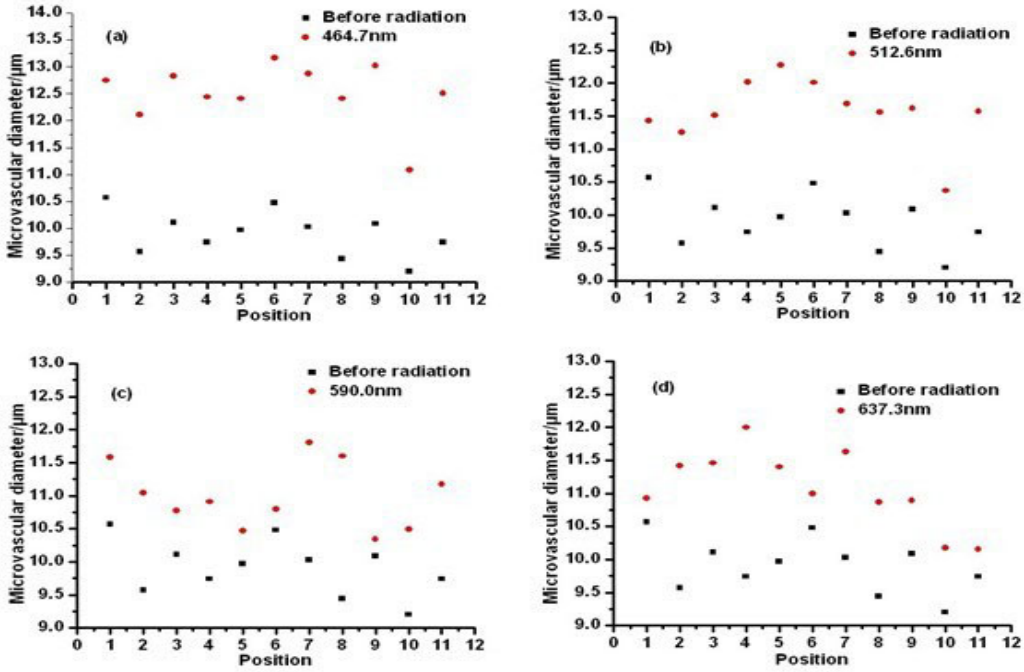

Fig. 5 Comparison of micro vascular diameter before and after four LED radiations.

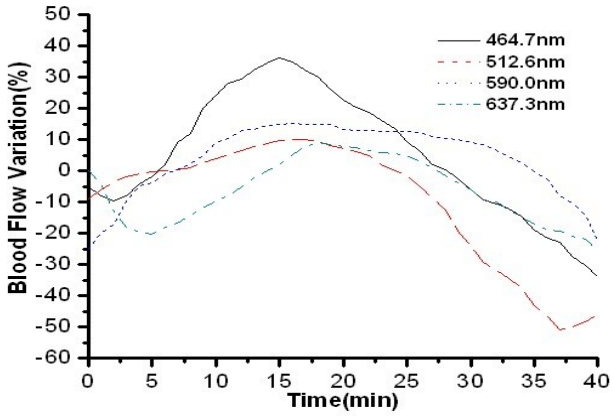

Fig. 6 Changes of blood flow volume and radiation time under the irradiation of four wavelengths LED

The percentage became 135 % of that in normal conditions. After 40 minutes of irradiation, the blood flow decreased to 55 % of that before irradiation. These significant increases in vascular diameter and blood flow can be considered as an acute response to light induced damage. These four LED light sources have all created light pollution to animals, especially the 464.7 nm LED light. It made the changes of mice micro vascular circulation system beyond the range of normal, the vascular diameter variation being the greatest. Long time exposure to intense light would result in the mice micro circulation system disorders, thereby affecting their physiological state. The harmful effect produced by 512.6 nm LED light was only second to 464.7 nm light. The diameter change was 1.67μm and the blood flow change rate altered from minus 50 % to 10 %. It can be concluded (figure 6) that blood flow increases slowly during the first

18 minutes of light exposure. Up to 18 minute to 40 minutes, the blood flow decreasing trend was accelerated as the irradiation time increases. Other two wavelengths of LED light sources presented a similar influence on the organisms, and the impacts were both less than that of 512.6 nm LED light.

Experimental data indicate that long time exposure to intense light will cause damage in micro circulation system. There are many differences between biological tissue and conventional optical media [20]. Biological tissue has complex structures and is consisting of large amount of cells of different volume and species [21, 22]. These cells vary in light refraction, reflection and absorption caused by the differences in their cell membrane, cytoplasm and nucleus. Light transmission in biological tissues is mainly affected by absorption [23]. The light energy can be transformed into other forms of energy, such as molecular kinetic energy, other in fluorescence radiation energy, etc. Thus, the physiological state of the organisms will be changed as the blood cell and vascular cell are affected by the energy transformation.

The process of light absorption of eyeball depends on visual stimulation [24-26]. The capillaries of the eye can only be affected by interference with normal visual environment. As a result, the exposure of animal eyes to light will lead to a great change in the optic nerves because the radiation is mainly absorbed by the sensory nerve. Photoreceptor is the first part that perceives the change in visual environment. Intensity of the spectral signals received by various photoreceptors will change with the visual environment. It is especially true for monochromatic light of different wavelengths. Cone cells on the retina of ICR mice are sensitive to the light of 464.7 nm and 512.6 nm wavelengths, because there are two kinds of opsin. They have different spectral absorption characteristics [27, 28]. The characteristics of cone cells are determined by the opsin. From the perspective of molecular genetics, all the abnormalities in colour vision can be considered as caused by the lack of corresponding photo pigment and visual acuity protein in cone cells. Retina cells contain an abnormal retinaldehyde called A2E. A2E has two absorption peaks, one 335 nm in the ultraviolet region and another 435 nm in the blue-green area. The toxicity created by A2E on retinal pigment epithelium greatly increased under light radiation. The numbers of photons captured by photoreceptors are all equal. Thus, the light stimulations are all the same. Therefore, the irradiation experiments have comparability with each other.

The auricle micro vascular diameter expanded significantly after the blue LED light irradiation. We believe that this change can be explained as that the wavelength of blue LED light is relatively short. 464.7nm radiation mainly takes effect inside the molecules. Strong stimulus was imposed on the vision system of the mice. The oxidation of retinal cells in the macular region was accelerated. Free radicals were released from the mitochondria in cells. This would cause cell swelling, necrosis, and rupture [29]. Large amounts of white blood cells were released. The blood flow volume and flow rate were increased to ensure that the damaged cells could be repaired as far as possible. The most important light absorber *in vivo* is hemoglobin. Its light absorption degree strongly depends on the oxygen saturation. The oxygen saturation changes with the congestion of visual tissues under intense light stimulation, affecting the absorption characteristics of blood cells and surrounding tissue [30]. Red blood cells in mice retina and head skin blood absorbed a large number of photons. Tissue repair and micro-metabolism required a large amount of blood. The micro vessels expanded accordingly.

It has been shown in the figures that the micro vascular system in mice auricle also expands after the 512.6 nm, 590.0 nm and 637.3 nm wavelength LED light irradiation. However, the variations are less than the changes caused by the LED light of 464.7 nm. We can explain this as these wavelengths belong to visible light. In this wavelength band, most of the tissues present to be turbid. Lens and the cornea are transparent in this band, but the scattering accounts for the major part of interaction between light and biological tissues. Therefore, the absorptions of these three kinds of light are much less than that of blue light. The main damage to animals of 512.6nm, 590 nm and 637.3 nm LED light is the thermal accumulation during radiation time. Figure 4 shows that the blood flow decreased significantly when the exposure time has reached 25 minutes. When the organism self-regulation has reached its limits, confusion will be caused in local microcirculation. Thermal accumulation leads to temperature increase. These thermal effects will lead to metabolic disorder of eye enzyme systems and overheat of aqueous humor. Cell damage is the main reason of micro vascular expansion under short wavelength light radiation. The micro vascular system expands to increase blood flow to bring off the accumulated heat. These changes can be considered as a stress response and regulation of temperature. All of these light radiations produced light pollution to animals with different severity degrees. We can conclude that 464.7 nm LED light performed the greatest damage to animals, followed by the 512.6 nm light. The impact of 590 nm light and 637.3 nm light to organism are almost the same.

4. Conclusion

By comparison of the changes before and after four different LED light exposure of mice eyes, it has been discovered that the micro vascular diameter variation caused by 464.7 nm LED light is relatively larger than that provoked by other wavelength LED light under the same time of irradiation. The blood flow change also proves this conclusion. Influence of 512.6 nm LED light is comparatively larger than that of 590 nm and 637.3 nm light. These changes indicate the different severity of the

light induced damage to organisms and tissues. It also shows that long time exposure to intense light will exert a bad influence on the microcirculation. From the analysis of the experimental data it can be drawn the conclusion that cell damage and thermal accumulation have induced a local temperature increase, this being the main causes of the variation in the micro vascular diameter and blood flow. Blood vessel expands in order to increase the blood flow so as to enhance the immune ability and bring off the excess energy. The confusion of micro circulation is also one of the influences created by the light stimulus. These will cause damage to local blood circulation and mice physiological conditions, resulting in different degree of light pollution.

Acknowledgments

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