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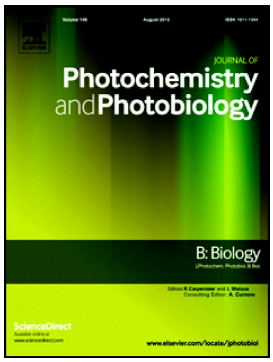

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The effects of low-color-temperature dual-primary-color light-emitting diodes on three kinds of retinal cells

Ming Jin[a#], Xiongfeng Li[a#], Feng Yan[a#], Weixin Chen[a], Lei Jiang[b], Xu Zhang[a]

[a]Affiliated Eye Hospital of Nanchang University, Jiangxi Research Institute of Ophthalmology and Visual Science, Jiangxi Clinical Research Center for Ophthalmic Disease，463 Bayi Road, Nanchang, Jiangxi, 330006, China.

[b]Queen Mary School of Nanchang University, 461 Bayi Road, Nanchang, Jiangxi, 330046，China

#contributed equally to this work

***Corresponding author:**

Prof. Xu Zhang, MD, PhD. Affiliated Eye Hospital of Nanchang University. 463 Bayi Road, Nanchang, Jiangxi, 330006, China. Phone: +86-791-86318907; Email: xuzhang19@163.com

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ABSTRACT

Long-term illumination of the retina with blue-light-excited phosphor-converted light-emitting diodes (LEDs) may result in decreased retinal function, even if the levels of blue light emitted are low. New low-color-temperature dual-primary-color LEDs have been developed that are composed of only two LED chips: a red chip and a yellow chip. These LEDs are expected to become a new type of healthy lighting source because they do not emit blue light, they lack phosphor, and they solve the problem of low efficiency encountered with phosphor-converted low-color-temperature LEDs. Many studies have indicated that these new low-color-temperature LEDs are likely to have therapeutic effects. However, the biological safety of these LEDs needs to be explored before the therapeutic effects are explored. Therefore, this experiment was conducted to investigate the effects of the new low-color-temperature LEDs and fluorescent white LEDs on three types of

retinal cells. We observed that the viability and numbers of retinal cells decreased gradually with increasing LED color temperature. The new low-color-temperature LEDs caused less death and adverse effects on proliferation than the fluorescent white LEDs. After irradiation with high-color-temperature LEDs, the expression of Zonula Occludens-1 (ZO-1) was decreased and discontinuous in ARPE-19 cells; the stress protein hemeoxygenase-1 (HO-1) was upregulated in R28 cells; and glial fibrillary acidic protein (GFAP) and vimentin were upregulated in rMC-1 cells. We therefore conclude that the new white LEDs cause almost no damage to retinal cells and reduce the potential human health risks of chronic exposure to fluorescent white LEDs.

Keywords: Low color temperature; Multi-primary color; White LED; Retinal cell; Biosafety

1. Introduction

At present, light-emitting diodes (LEDs) have replaced traditional light sources and are commonly used for applications such as display and lighting. Fluorescent white LEDs use blue light to excite phosphor powders (such as red powder, yellow powder, green powder, etc.) in order to obtain white light; these LEDs thus emit different amounts of blue light. Numerous experiments have shown that long-term blue-rich radiation is harmful to human health[1-9]. Therefore, reducing the blue-light content in the LED spectral output seems to be particularly important.

In recent years, the luminous efficiency of yellow-light LEDs has gradually improved. White light synthesis by multi-primary-color LEDs can enhance both index and efficiency values, reduce blue-light emission, meet needs for low color temperatures, save rare earth resources, and enable intelligent scene lighting and visible light communication technology. White LEDs are often measured in terms of color temperature; a higher color temperature of a white LED is associated with greater blue-light emission. The dual-primary-color LEDs used in this experiment match the power ratios of two-color LEDs with red light (wavelength 630 nm) and yellow light

(wavelength 560 nm) to act as low-color-temperature light sources without blue-light components. The color temperature can be continuously adjusted between 1500-2200 K to meet the lighting needs of low-color-temperature scenes. Many studies have affirmed the therapeutic effects of red light and yellow light[10-15], so we speculate that 1900 K low-color-temperature lamps without blue light are likely to have the same therapeutic effects as red light and yellow light. In addition, low-color-temperature lamps are dual-primary-color light sources, so their protective effects on biological tissue may be greater than those of monochromatic light sources (emitting red or yellow light). In addition, we have previously proven that 1900 K low-color-temperature LEDs can not only improve wound healing and hair growth but also regulate melatonin secretion, alleviate insomnia and promote the maintenance of normal biological rhythms. We have also found that these lamps can protect the ocular surface and reduce ocular surface health problems caused by blue light-emitting LEDs[16].

Thus far, these new low-color-temperature LEDs have been applied for civil lighting. To further prove the effects of the new LEDs on eye health, especially retinal health, three types of retinal cells commonly used in ophthalmology research were selected for this study: rMC-1 cells, ARPE-19 cells, and R28 cells. By analyzing cell viability, cell death, cell proliferation and the expression of various proteins, we compared the effects of fluorescent white LEDs at different color temperatures and the new low-color-temperature LEDs on retinal cells. Our findings provide necessary evidence that the new low-color-temperature LEDs are healthy light sources that can be used for noninvasive light treatment.

2. Materials and Methods

2.1. Cell culture

ARPE-19 cells (ATCC, Rockville, MD; kindly donated by Dr. Jing Yu, Shanghai Tenth People's Hospital, Tongji University, Shanghai, China), R28 cells (kindly

provided by Dr. Guotong Xu, Tongji University School of Medicine , Shanghai, China) and rMC-1 cells (derived from lines at Northwestern University Medical School) were maintained in media (Dulbecco's modified Eagle medium (DMEM)/F-12, HyClone, Beijing, China), low-glucose DMEM (BI, Israel), and high-glucose DMEM (HyClone, Australia), respectively , containing 10% fetal bovine serum (FBS, BI, Israel), 100 U/mL penicillin and 100 μg/mL streptomycin (Solarbio, Beijing, China). The cells were passaged by trypsinization every 3 to 4 days. All three kinds of cells were incubated in a humidified atmosphere of 5% CO_2 at 37 °C.

2.2. Lighting treatment

To test the responses of the three types of retinal cells to LEDs with different color temperatures and to blue light, we subjected the cells to six different light treatments, including dark treatment, 1900 K-LED treatment, 3000 K-LED treatment, 4000 K-LED treatment, 6600 K-LED treatment and blue-light (470 nm) treatment. The light sources were provided by National Engineering Technology Research Center for LED on Silicon Substrate, Nanchang, university. As shown in **Fig. 1**, the peaks of the 1900 K LED were concentrated between 500 nm and 650 nm; thus, the emitted light lacked blue light and consisted of red and yellow light (**Fig. 1a**). In addition, the 1900 K LED has a color rendering index of 80 and a luminous efficiency of 66.73 lm/W, indicating that it is a suitable light source. The artificial fluorescent white LEDs include 3000 K, 4000 K, and 6600 K LEDs (**Fig. 1b-d**), whose peak ranges were between 300 nm and 780 nm. The peak of blue light is 470 nm (**Fig. 1e**). We found that blue light not only existed in traditional white light sources such as the 3000 K, 4000 K, and 6600 K LEDs but also tended to increase with increasing color temperature. We created a light box divided into six independent areas so that the different groups of cells were not affected by the light administered to the other groups. Lamp beads were placed on the inner surface of the light box lid, and the cells could be irradiated by closing the lid (**Fig. 1B**). The cells were seeded into 96-well plates at a density of 3×10^3 cells per well and into six-well plates at a density of 3 ×

10^4 cells per well and then incubated at 37 °C under 5% CO_2 for 24 hours. Subsequently, the cells were exposed to the different light sources for 24 hours. Cells grown in the dark were used as controls. To control the variables, we kept the irradiated cells directly under the lamp beads and kept the intensity consistent among the five kinds of light (10 W/m^2) during the experiment. Our cell incubator has two doors, the outermost is an opaque thick door, and the inner is a transparent glass door. The six independent spaces in the light box have small holes on the non-adjacent box surface to let light out. After retinal cells received light, we will close the glass door to observe the light. Afterwards, during the whole irradiation process, we will open the outer door of the cell incubator from time to time to check whether the light treatment is still continuing. After the light is over, we turn off the light device and take out the cells for future experiments.

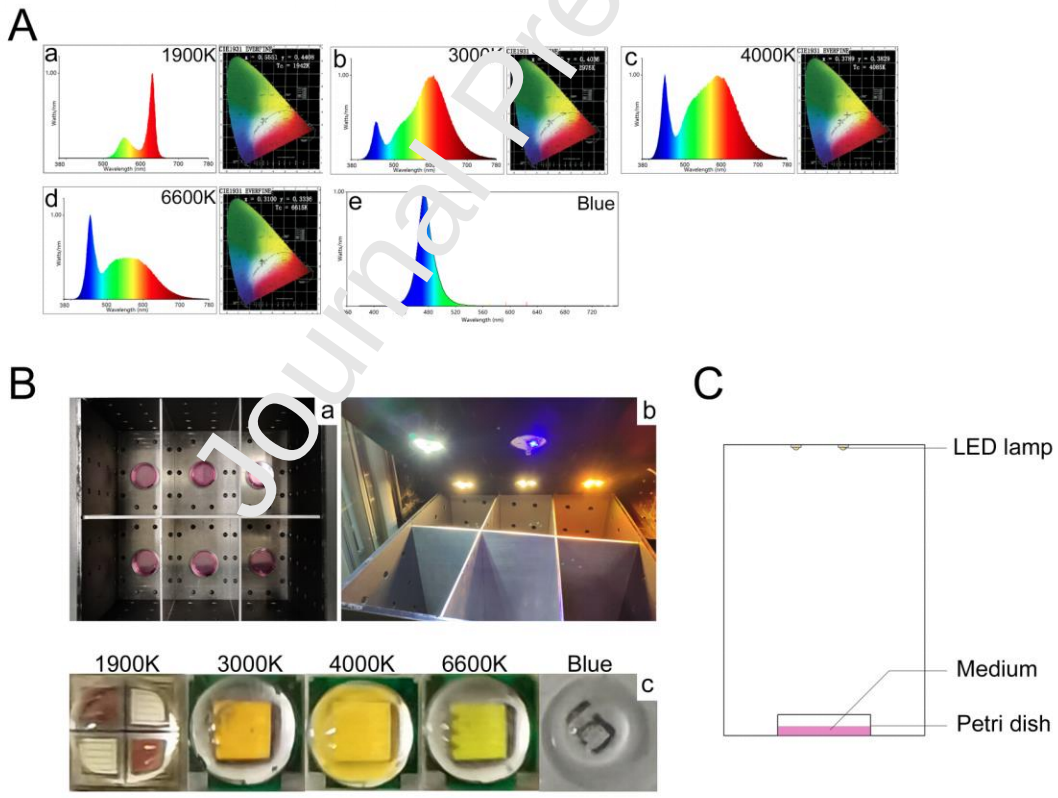

Fig. 1: Illuminating device. (A) The electroluminance spectra of lights with different color

temperatures. (a-e) The electroluminance spectra and CIE1931 chromaticity diagrams of four light sources with different color temperatures (1900 K, 3000 K, 4000 K, and 6600 K) and blue light were characterized by integration of spheres. (B) Diagram of the illuminating device. (a) The light box. The box body is also divided into six areas. (b) The lamp cover. Different lamp beads were installed on the lamp cover. (c) LED chips with different color temperature (C) lighting diagram.

2.3. Cell viability assay

Cell viability was measured by Cell Counting Kit-8 (CCK-8) analysis according to the kit instructions. The three kinds of cells were seeded into 96-well plates at a density of 3×10^3 cells per well and then cultured in a humidified atmosphere of 5% CO2 at 37 °C for 24 hours. The cells were then exposed to the five light sources for 24 hours as previously described. Next, the cells were cultured in medium containing 10% WST-8 (BB-4202-1, Bestbio, Shanghai, China) for 2 hours. The absorbance of each group was measured using a microplate reader (Multiskan Mk3, Thermo Scientific) to determine the cell viability in each experimental group.

2.4. Cell death and proliferation experiments

LIVE/DEAD viability assay. After culture in an incubator for 24 hours, the medium of ARPE-19 cells was replaced with medium containing no FBS, and the cells were exposed to different light conditions (control (con), 1900 K, 4000 K, blue) for 24 hours. At the end of the treatment, calcein-AM and propidium iodide (PI) (Bestbio, BB-4126-1, China) were added to the culture medium at final concentrations of 8.1 mM and 1.5 mM, respectively, and the cells were incubated for 15 minutes. Images were obtained using a confocal microscope (ZEISS, LSM800, Göttingen, Germany). The dead cells and total cells were counted in five fields of view, and the percentages of PI-positive cells were calculated.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay: Twenty-four hours after exposure to the different LEDs, R28 cells were fixed with 4%

paraformaldehyde and then incubated with TUNEL reagent (In Situ Cell Death Detection with Fluorescein; Lot No. 26467700， Roche Biochemicals, Mannheim, Germany) for 1 hour in a 37 °C dark incubator. The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, Boster, Wuhan, China), and the apoptotic cells and total cells were counted in five randomly selected fields of view under a confocal microscope.

Bromodeoxyuridine (BrdU) treatment: After rMC-1 cells were treated with the different light sources (con, 1900 K, 4000 K, blue) for 24 hours, 10 μM BrdU (B5002, Sigma-Aldrich,Hamburg, Germany) was added to each six-well plate, and the cells were incubated for 4 hours, as described previously[17]. Afterwards, the rMC-1 cells were fixed with 4% paraformaldehyde and then incubated with a primary anti-BrdU antibody at 4 °C overnight. The cells were incubated with a secondary antibody for 1 hour the next day. After 15 minutes of DAPI staining, the proliferating cells and the total cells were counted in five randomly selected fields of view under confocal microscopy.

2.5. Immunofluorescence staining

The three kinds of cells were seeded into six-well plates at a density of 3×10^4 cells per well. The rMC-1 and R28 cells were incubated for 24 hours. The R28 cells were then placed in low-glucose DMEM containing 1% FBS. The medium of ARPE-19 cells was also changed to DMEM/F-12 containing 1% FBS, and the cells were incubated for 7 days. The three kinds of cells were then exposed to 10 W/m2 white LEDs (1900 K and 4000 K) and blue-light LEDs for 24 hours. Then, the cells were fixed with 4% paraformaldehyde for 15 minutes, blocked in 3% horse serum for 1 hour, and incubated with primary antibodies (against ZO-1, HO-1, vimentin, and GFAP) overnight at 4 °C. Table 1 lists all the information about the primary antibodies. The secondary antibodies included donkey anti-rabbit Alexa Fluor® 488 and donkey anti-mouse Alexa Fluor® 594 (Abcam, Cambridge, MA, USA). The cells were incubated with the secondary antibodies at dilutions of 1:200 and with 0.2% Triton X-100 for 1 hour at room temperature. Subsequently, the cells were

counterstained with DAPI, mounted and examined under a Zeiss confocal microscope to obtain digital images.

All these experiments were performed in at least three times.

Table 1 | Primary antibodies used in the study.

Antibody	source	Catolog.No	Type of Ab	Dilution	MW
GFAP	Sigma	G3893	Mouse mAb	1:100	50
Vimentin	CST	#5741	Rabbit mAb	1:100	57
HO-1	Abcam	ab13248	Mouse mAb	1:100	34.6
ZO-1	CST	13663S	Rabbit mAb	1:100	220
BrdU	SIGMA	B8434	Mouse mAb	1:100	

2.6. Data Analysis

All quantified data represents an average of at least three samples. Graph Pad Prism 6.0 software were used for statistical analysis. All data were analyzed by one-way ANOVA and Post Hoc Tukey Test to determine the significance of the response. $P < 0.05$ was considered significant.

3. Results

3.1. Cell viability decreases as color temperature increases.

We found that the overall viability of the three kinds of cells showed a downward trend as the color temperature increased (**Fig. 2A-C**). The cell viability of the control group (the dark group) was similar to that of the 1900 K group. For all three types of retinal cells, cell viability was comparable among the 3000 K, 4000 K, and 6600 K groups. In general, the cell viability of the 3000 K, 4000 K and 6600 K groups was

lower than that of the control group and the 1900 K group. Blue light was the type of light that damaged the cells the most. Considering that the results for the 3000 K, 4000 K, and 6600 K groups were relatively similar, we chose the 4000 K LED and the blue light as the experimental light sources for subsequent comparison with the 1900 K low-color-temperature LED. We found that the brightfield light results corresponded to the CCK-8 results: as the amount of blue light emitted by the LEDs increased, the number of retinal cells decreased (**Fig. 2D**). In addition, for rMC-1 and R28 cells, the edge refraction of most cells in the blue-light group was elevated, and the cells became round and lost their normal cell morphology (**Fig. 2D-e - Fig. 2D-l**).

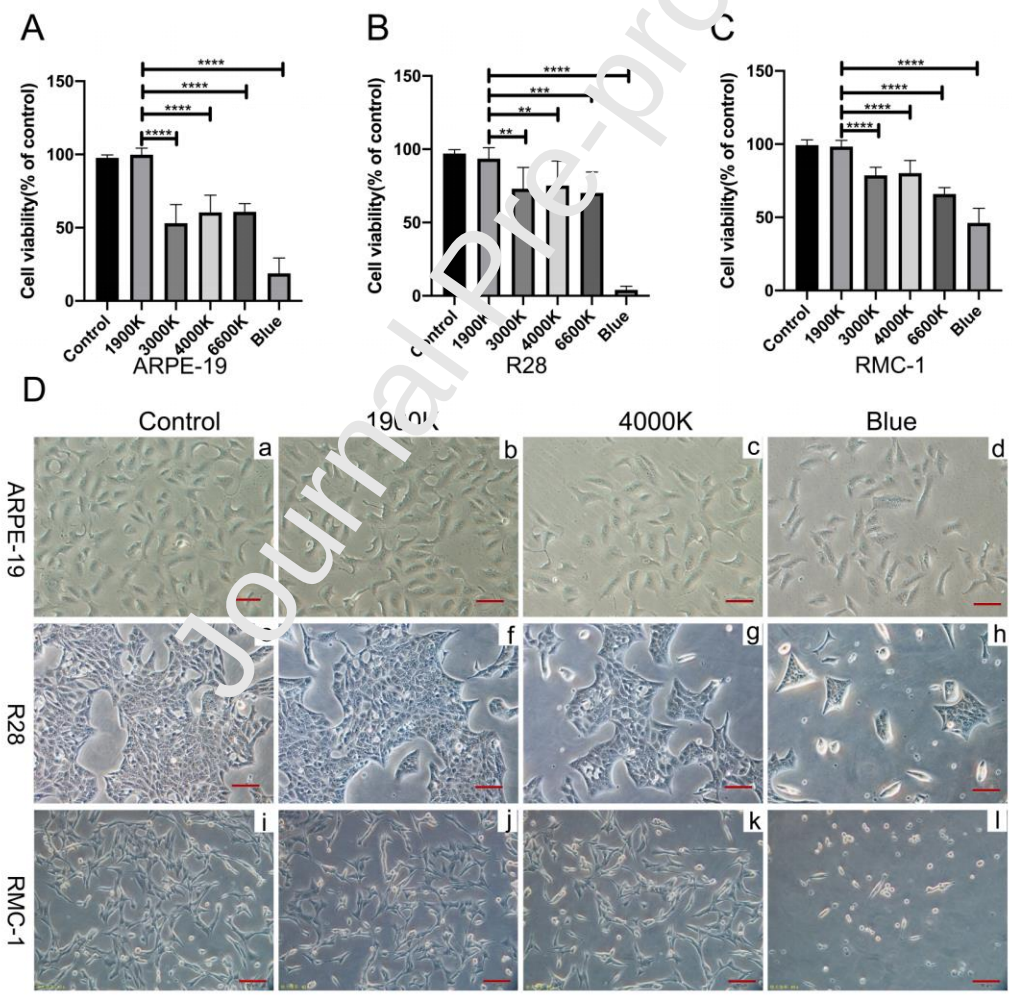

Fig. 2: Effects of light of different color temperatures on retinal cell viability and morphology. (A-C) Cell viability decreased as color temperature increased. (D) As the color

temperature of the LEDs increased, the numbers of the three kinds of cells decreased. The results are expressed as the mean values ±SEMs, n = 8. One-way ANOVA, post hoc Tukey's test (*p < 0.05, **p < 0.01, ***p < 0.001). Scale bars=50 μm.

3.2. Low-color-temperature LEDs rarely cause ARPE-19 and R28 cell death. With increasing blue-light dose, the proliferation ability of rMC-1 cells decreases.

As shown in **Fig. 3a-d** and **Fig. 3B**, there were significantly more dead cells in the blue-light group than in the 1900 K group. Although there were no statistically significant differences among the other groups, we could deduce some discrepancies from the pictures: the 4000 K group had slightly more dead cells than the 1900 K and control groups, and the blue-light group had more dead cells than the 4000 K group. A TUNEL experiment was used to detect early apoptosis, and the TUNEL results for R28 cells were the same as the calcein-AM/PI staining results for ARPE-19 cells (**Fig. 3e-h and Fig. 3C**). Among rMC-1 cells, there were fewer BrdU-positive cells in the 4000 K group than in the 1900 K group, and there were fewer BrdU-positive cells in the blue-light group than in the other three groups (**Fig. 3i-1 and Fig. 3D**). These findings indicate that LEDs emitting higher levels of blue light have greater negative effects on rMC-1 cell proliferation than those emitting less blue light.

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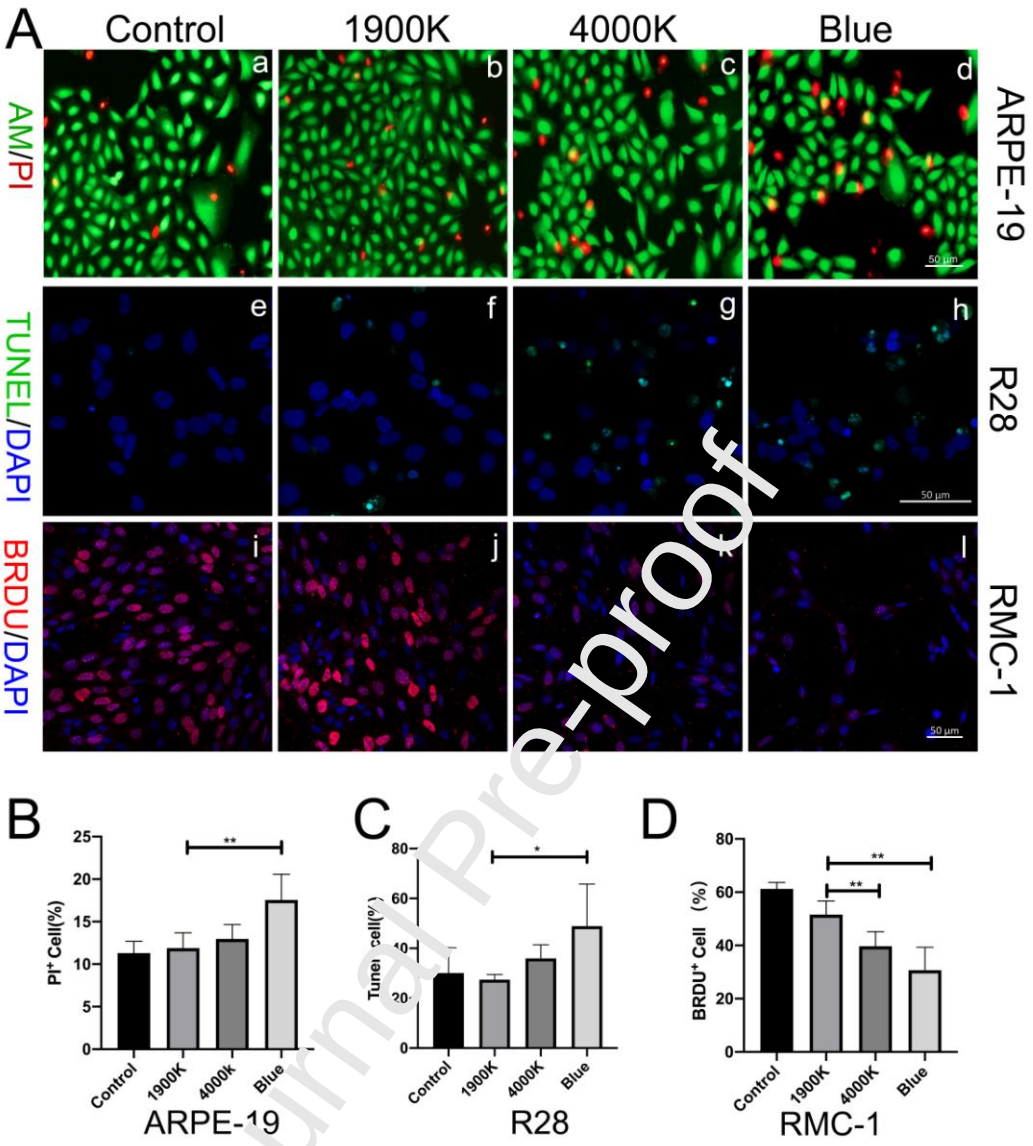

Fig. 3: Low-color-temperature LEDs rarely cause ARPE-19 and R28 cell death. With increasing blue light dose, the proliferation ability of rMC-1 cells decreases. (A) Immunofluorescence results for the three kinds of cells after different death and proliferation experiments. (a-d) ARPE-19 cells stained with calcein-AM/PI. The orange cells are dead cells stained by PI. And the green cells are live cells stained by calcein-AM. (e-h) R28 cells stained with TUNEL and DAPI. The cyan cells are $TUNEL^{+}$ cells. (i-l) rMC-1 cells stained with BrdU and DAPI. The purple cells are proliferating cells. (B-D) Quantitative analysis of the fluorescent staining-positive cells. The results are expressed as the mean values ±SEMs, n = 5. One-way ANOVA, post hoc Tukey's test (*p < 0.05, **p < 0.01, ***p <

0.001). DAPI was used to stain the nuclei (blue). Scale bars=50 μm. PI: propidium iodide; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling; DAPI: 4',6-diamidino-2-phenylindole; BrdU : Bromodeoxyuridine.

3.3. The expression of ZO-1 in ARPE-19 cells changes after exposure to LEDs emitting high levels of blue light.

As shown in Fig. 4, ZO-1 was mainly expressed on the cell membrane. Although we did not attempt to quantify ZO-1 in ARPE-19 cells, ZO-1 staining clearly occurred in a continuous line surrounding the nucleus in the control and 1900 K group. In the 4000 K group ZO-1 was still expressed, but the expression was discontinuous, suggesting that the amount of ZO-1 protein on the cell membrane may have been decreased or that the proteins were no longer aligned normally. The situation in the blue-light group was even worse; the ZO-1 arrangement on the cell membrane was intermittent, and the ZO-1 expression level was much lower than that in the other three groups.

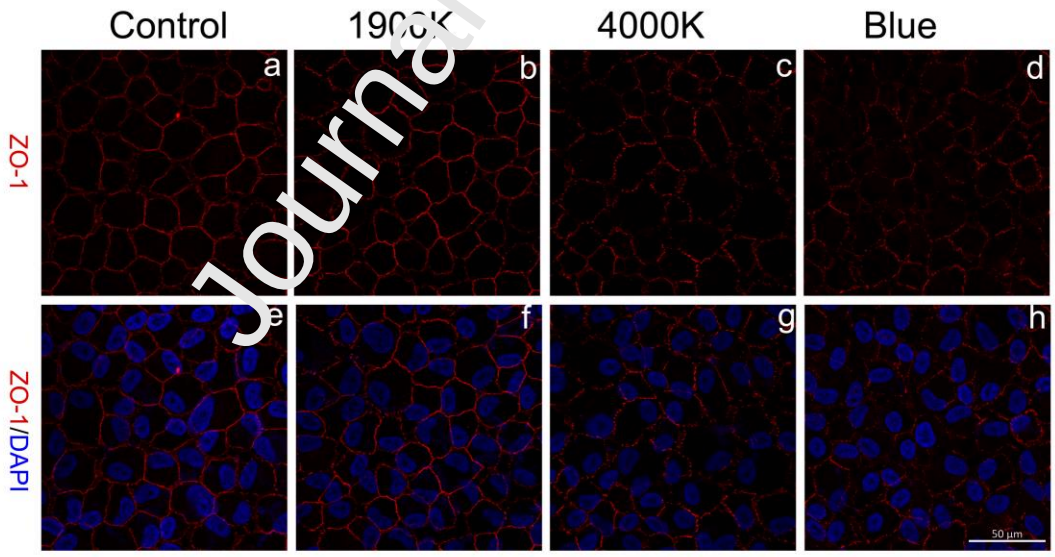

Fig. 4: The expression of ZO-1 in ARPE-19 cells changes after exposure to LEDs emitting high levels of blue light. (a-d) Immunofluorescence results for ZO-1 in ARPE-19 cells. (e-h) Synthetic diagram of ZO-1 and DAPI. DAPI was used to stain the nuclei (blue). Scale bars=50 μm. ZO-1: Zonula Occludens-1; DAPI:

4',6-diamidino-2-phenylindole.

3.4. The expression of HO-1 in R28 cells increases after exposure to LEDs emitting high levels of blue light.

The R28 cells in the control and 1900 K groups did not express HO-1, but a few R28 cells in the 4000 K group expressed HO-1. In contrast with the 4000 K group, the blue-light group exhibited HO-1 expression in almost all cells in the entire field (**Fig. 5)**.

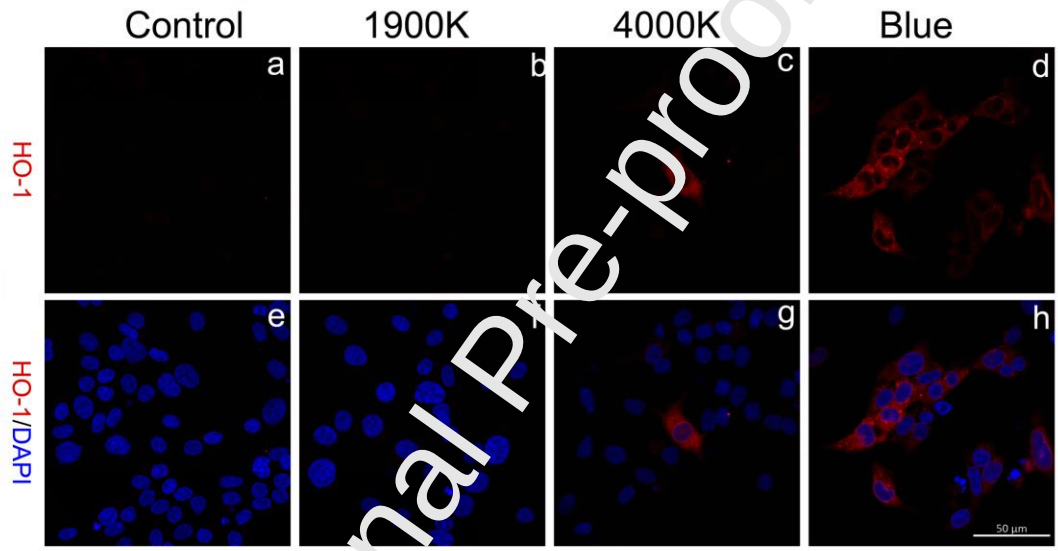

Fig. 5: The expression of HO-1 in R28 cells increases after exposure to LEDs emitting high levels of blue light. (a-d) HO-1 immunoreactivity in R28 cells. (e-h) Synthetic diagram of HO-1 and DAPI. DAPI was used to stain the nuclei (blue). Scale bars=50 μm. HO-1: hemeoxygenase-1; DAPI: 4',6-diamidino-2-phenylindole.

3.5. The new low-color-temperature LEDs do not induce or downregulate the expression of GFAP or vimentin in rMC-1 cells.

We found that the expression of GFAP in the blue-light group was higher than that in the 4000 K group, and the red fluorescence of GFAP in the 4000 K group was slightly stronger than that in the control group. However, the fluorescence intensity of GFAP in the 1900 K group was significantly weaker than that in the control group (**Fig.**

6a-d). The results for vimentin were similar to those for GFAP. Similar levels of vimentin expression were observed in the control and 1900 K groups. However, the cells in the 4000 K group expressed slightly more vimentin than those in the 1900 K group, and the fluorescence intensity of vimentin was strongest and most obvious in the blue-light group (**Fig. 6e-h**).

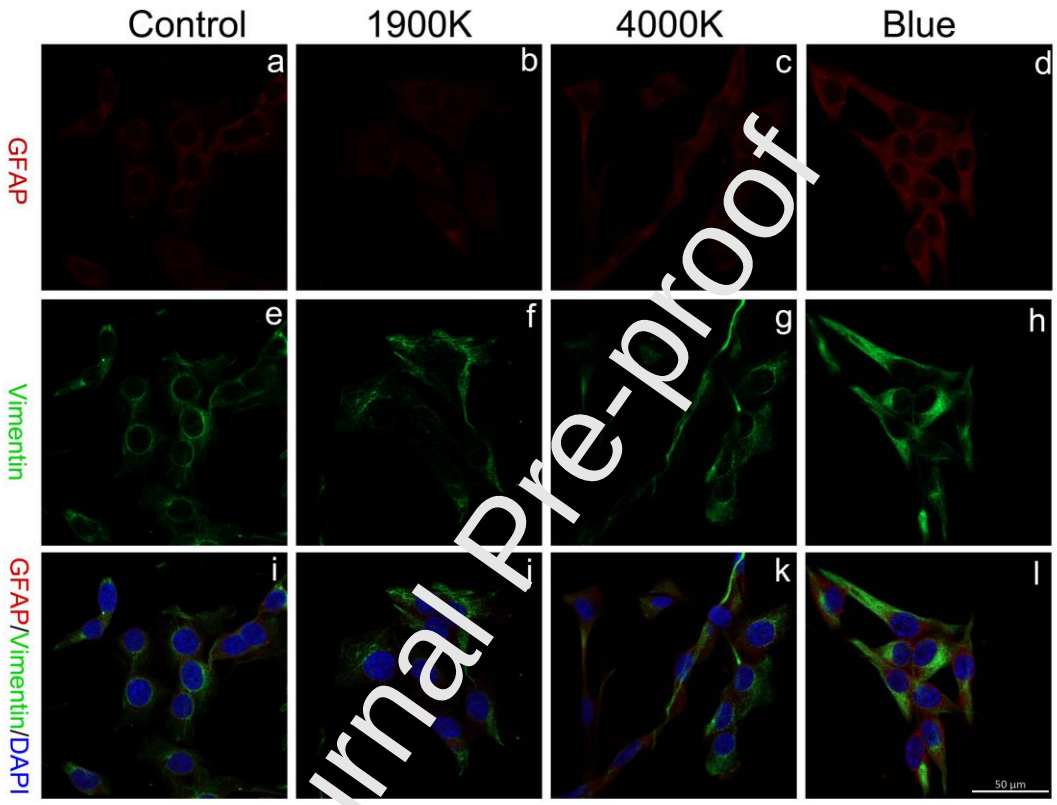

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Fig. 6: The new low color-temperature LEDs do not induce or downregulate the expression of GFAP or vimentin in rMC-1 cells. (a-d) Immunostaining of GFAP in the control, 1900 K, 4000 K and blue-light groups. (e-h) Immunostaining of vimentin in the four groups. (i-l) Synthetic diagram of GFAP, vimentin and DAPI. DAPI was used to stain the nuclei (blue). Scale bars=50 μm. GFAP: glial fibrillary acidic protein. DAPI: 4',6-diamidino-2-phenylindole.

4. Discussion

People are unduly exposed to blue light emitted by LEDs in modern society. Excessive and inappropriate blue-light exposure can cause circadian clock disorders and even cause certain diseases, such as eye diseases, depression, anxiety, attention deficit disorder, gastrointestinal diseases, cardiovascular diseases, and breast cancer[4, 18-23]. Researchers are gradually realizing that daytime and nighttime exposure to white LEDs also poses certain health threats. Although the white light emitted from these LEDs looks like normal light to human vision, it also contains blue light with wavelengths in the range of 460 to 500 nm. This blue light corresponds to the known retinal hazard spectrum[24]. Abdel-Rahman F et al.[25] used *Caenorhabditis elegans* as a model to study the biological effects of domestic LED lighting and discovered that the blue-light component of white LEDs may cause health problems. Thus, further research is needed to test commercial white LEDs that emit different amounts of blue light. Studies have shown that long-term exposure to even compact fluorescent lamps with low blue-light emission can cause retinal function decline, and fluorescent white LEDs can release more blue light as the usage time increases[7]. Therefore, it is very important to minimize the blue light in the spectral output of light sources. A randomized controlled trial conducted by Shechter et al.[21] revealed that wearing amber blue-light-blocking glasses before bedtime can improve sleep in patients with insomnia. In another study, a filter that can remove 94% of blue light was used to protect the retina from high-intensity white-light damage[26]. The findings of the above studies directly confirm our results, showing that the removal of blue light can protect the retina. The new low-color-temperature LEDs (1900 K) used in this study are more convenient to use than light-blocking elements. The most important property of these LEDs is their potential to exert protective effects. We explored the effects of the LEDs on retinal cells and found that the new low-color-temperature LEDs had almost no effects on the cell viability of three kinds of retinal cells. The brightfield light results corresponded to the CCK-8 assay results. With increasing blue-light doses, the numbers of retinal cells decreased. The new LEDs also rarely caused the death of ARPE-19 and R28 cells. In addition, the proliferation ability of rMC-1 cells decreased with increasing blue-light emission of

LEDs. Notably, there was no significant difference in the expression of ZO-1 between the control and 1900 K groups of ARPE-19 cells. ZO-1 was still expressed in the 4000 K group, but the expression was not continuous. The situation in the blue-light group was even worse; the ZO-1 arrangement on the cell membrane was intermittent, and the expression level of ZO-1 was much lower than that in the other three groups. The R28 cells in the control and 1900 K groups did not express HO-1, but a few R28 cells in the 4000 K group expressed HO-1. Most cells in the blue-light group expressed HO-1. The expression of GFAP in the blue-light group was higher than that in the 4000 K group. The red fluorescence of GFAP in the 4000 K group was slightly stronger than that in the control group, but the fluorescence intensity of GFAP in the 1900 K group was significantly weaker than that in the control group. The results for vimentin were similar to those for GFAP. Overall, the results obtained in this study suggest, for the first time, that the new LEDs cause less damage to retinal cells than other types of LEDs and can reduce the potential human health risks of fluorescent white LED exposure.

We found that the overall viability of retinal cells after 24 hours of irradiation showed a downward trend as the color temperature increased. The brightfield light results for the control, 1900 K, 4000 K and blue-light groups were also consistent with the CCK-8 assay results. We ensured that the different light sources had the same irradiance levels; thus, our findings indicate that LEDs that emit high levels of blue light (that is, LEDs with high color temperatures) have greater negative impacts on retinal cells than LEDs that emit lower levels of blue light. In a previous study, we found that the noninvasive tear film breakup time (NITBUT) values of volunteers who read books under 1900 K LED increased, while those of volunteers in three other groups decreased. The findings indicated that the tear film stability of volunteers was better in the 1900 K group than in the other groups. In addition, the volunteers in the 1900 K group had the lowest red-eye index values, showing that the new dual-primary low-color-temperature LEDs (1900 K) can reduce dry-eye or red-eye symptoms[16]. Dry-eye patients may be more sensitive than other individuals to the ocular surface

toxicity of blue light, so exposure of people with ocular surface diseases to fluorescent white LEDs emitting high levels of blue light may cause a vicious cycle[1]. Notably, red light has been shown to accelerate the repair of the corneal epithelium[12]; thus, irradiation with 1900 K LEDs may be a noninvasive way to treat ocular surface diseases with great potential. In addition to ocular-surface issues, excessive blue-light exposure can cause cataracts and refractive errors[6, 27]. Xie C et al.[6, 28] explored the effects of different color temperatures on lens epithelial cells and primary retinal pigment epithelium (RPE) cells and found that white LEDs with high color temperatures may cause serious photobiological damage to these cells. The researchers used fluorescent white LEDs with a minimum color temperature of 2954 K in the experimental group. We used a similar color temperature of 3000 K for our experimental group. The wavelengths on the visible electromagnetic spectrum that can reach the retina are between 400 and 780 nm or even 800 nm. The wavelength of blue light is within this region. Blue light is characterized by a short wavelength, high energy and relatively strong penetrating power, which enable it to cause mitochondrial dysfunction; blue light is the component of the visible spectrum that causes the most damage to the retina. Studies have further shown that the blue light emitted by LEDs in devices such as mobile phones and computers can damage cells in the brain and retina[29]. Retinal diseases are more difficult and expensive to treat than other ophthalmic diseases, which is why we chose to explore the effects of low-color-temperature dual-primary-color LEDs and fluorescent white LEDs on the retina. Moreover, we have previously discovered that the new low-color-temperature LEDs can protect the ocular surface, so we deemed it necessary to continue to explore the therapeutic effects of these LEDs on the retina. Certainly, the biological safety of these new low-color-temperature dual-primary-color LEDs need to be explored before subsequent experiments are conducted to explore their protective roles. And this study is the first to describe the biological safety effects of low-color-temperature dual-primary-color LEDs on retinal cells.

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Next, we used different experiments to prove the beneficial effects of the new low-color-temperature dual-primary-color LEDs on cell death and proliferation. We found that the results for the control groups and the 1900 K groups of ARPE-19 and R28 cells were the same. Greater emission of blue light by LEDs was associated with more cell death among ARPE-19 cells, as revealed by PI staining; more apoptosis among R28 cells, as revealed by green early apoptotic cell staining; and greater negative effects on the proliferation of rMC-1 cells. These results explain why the 1900 K low-color-temperature dual-primary-color LEDs caused little (almost negligible) damage to retinal cells. The three kinds of retinal cells used in this study are closely related to fundus diseases; thus, the observed effects on these cells can mimic the effects of the low-color-temperature dual-primary-color LEDs on fundus diseases to a certain extent. The RPE performs many functions necessary for normal retinal health and function. For example, it acts as a physiological barrier between photoreceptor cells and the choroidal blood supply, restricts infectious or other harmful substances from entering the retina, and protects the retina from systemic damage by regulating the immune response. In addition, the RPE is important for the transport of nutrients and ions extracted from choroidal blood to the photoreceptors and enables the reverse movement of waste produced by the photoreceptors into the choroidal circulation. It can also synthesize and release growth factors to build and maintain choroids and photoreceptors and participate in the visual cycle[30-34]. RPE dysfunction is directly involved in the occurrence of irreversible blinding diseases of the retina, including age-related macular degeneration (AMD), Stargardt disease and choroiditis[30, 35-39]. Blue light is an important factor in the pathogenesis of AMD[40]. After blue-light irradiation, degradation of the RPE precedes the degradation of photoreceptors. RPE cells thus become damaged and exhibit reduced phagocytotic activity. Subsequently, fatty acid peroxides accumulate around the inner segment (IS)/outer segment (OS) junction, which affects the material exchange between the photoreceptor cells and the choroid. The accumulation of fatty acid peroxides can also cause macrophages to infiltrate the retina, inducing the release of inflammatory factors, which accelerates retinal cell death[41-43]. Therefore, we chose

ARPE-19 as the experimental cell line in this study. We preliminarily simulated the impacts of the new low-color-temperature dual-primary-color LEDs on the pathogenesis of AMD by exploring the damaging effects of these LEDs on ARPE-19 cells. These 1900 K LEDs do not emit blue light. The PI results for the 1900 K group were the same as those for the control group, indicating that 1900 K LEDs cause minimal damage to the RPE. Photobiomodulation or near-infrared light therapy is a method of noninvasive irradiation of tissue in the far-infrared to near-infrared spectrum (630-1000 nm) used to achieve therapeutic effects. This intervention specifically targets the key mechanism of RPE dysfunction that is involved in the pathogenesis of AMD[44]. There is not much evidence on the efficacy of photobiomodulation in AMD treatment, but its safety and proposed protective mechanism have stimulated further research into this method as a new therapy for AMD. The light emitted by our new low-color-temperature dual-primary-color LEDs is composed of yellow and red light with a wavelength of 630 nm, which is exactly in the red wavelength range required for photobiomodulation. Therefore, we hypothesize that the new low-color-temperature dual-primary-color LEDs can protect RPE cells. However, more experiments are needed to test this hypothesis. Blue light damages not only the RPE but also photoreceptor cells. Kuse et al.[45] exposed cultured mouse cone cells to blue-light LEDs (464 nm), white-light LEDs (456 nm, 553 nm), and green-light LEDs (522 nm) with a light intensity of 0.38 mW/cm2 for 24 hours. They found that the active oxygen products increased by 1.4 times, 1.2 times, and 1.0 times in the blue-light LED group, the white-light LED group, and the green-light LED group, respectively. The blue-light LED group had the most severe cell damage. Although the green-light LED group exhibited increased levels of active oxygen products, no significant cell damage was found. The setup of this study was very similar to that of our experiment, and the trends in their results are also consistent with the trends in ours. However, Kuse et al. studied the effects of green light, blue light and composite white light on cone cells, and we studied the effects of composite white light and blue light on retinal cells. The wavelengths of the blue light used in the two experiments are the same, but the wavelength of the red and yellow light in

the dual-primary-color LEDs is longer than that of green light, so the damage to cells should be milder than that caused by green light. Müller glial cells, the major glial cells in the retina, support the retina, recover neurotransmitters, remove waste generated by the retina, regulate potassium levels, store glycogen, electrically insulate nerve cells, and maintain a stable retinal environment[46-48]. Therefore, rMC-1 cells are often used in experimental cell models of retinal injury. Furthermore, in the context of glaucoma, Müller cells are reactivated, and glia proliferate. Reactivated Müller cells produce cytotoxic factors such as nitric oxide (NO), tumor necrosis factor (TNF), reactive oxygen species (ROS), and prostaglandin (PGE2), which induce apoptosis of retinal ganglion cells (RGCs)[49]. However, after irradiation with blue light, Müller cells become hypertrophic, "glialization" occurs, and the expression of aquaporin-4 protein is upregulated[50]. Therefore, blue light can accelerate the progression of glaucoma. Some previous studies have shown that 670 nm red light can reduce the adverse effects of Müller cell activation after photodamage, downregulate Müller-related inflammatory factors, and prevent Müller cells from producing glial scars[51, 52]. Therefore, we hypothesize that the new low-color-temperature LEDs can also reduce the glialization of Müller cells in glaucoma by improving Müller cell mitochondrial function, which can delay RGC death and thus slow the progression of glaucoma. Through related research, scientists have recently discovered that blue light can aggravate glaucoma by directly affecting RGC mitochondrial function, inducing photooxidative stress and activating apoptosis[53, 54]. Other studies have shown that RGCs are more sensitive to blue light than glial cells because RGCs are less capable of repairing DNA than glial cells[55]. Red and near-red light can protect RGCs by improving mitochondrial function, so low-color-temperature LEDs are likely to protect the optic nerve and thereby delay the progression of glaucoma by exerting the same effect[56]. The use of RGCs in this experiment would have helped elucidate the effects of the new low-color-temperature dual-primary-color LEDs on ganglion cells. However, RGCs are difficult to obtain, so R28 cells were used as cell models for glaucoma-related eye diseases in this experiment. Although R28 cells are retinal precursor cells, they are highly

heterogeneous, can differentiate into different cell types under different cell culture conditions, and can more accurately simulate the complex situation of the retina than RGCs. They are often used in in vitro toxicity and neuroprotection studies. In vitro experimental models for glaucoma research have also been described previously[57].

We performed immunofluorescence experiments on different cell types to detect the production of landmark proteins after exposure to light. After irradiation of ARPE-19 cells with 4000 K and blue-light LEDs, the expression of ZO-1 on the cell membrane was downregulated and discontinuous. The ZO-1 protein is an important constituent of tight junctions and plays a significant role in maintaining the mechanical barrier function and permeability of the mucosal epithelium. Studies by Iriyama[58] have revealed that the nucleic acid and protein levels of ZO-1 in ARPE-19 cells are decreased and that RPE cell phagocytosis is relatively suppressed after fluorescent white LED irradiation. In addition, long-wavelength visible red light (600-1000 nm) has no negative effects on cultured cells but can attenuate the negative effects of blue light on cells[59]. Many studies have revealed the protective mechanism of red light: red light is absorbed by mitochondrial COX in a process that may involve photodissociation of inhibitory NO, which binds to the copper and heme centers of COX, preventing oxygen from binding to active sites[60, 61]. Oxygen consumption and ATP production increase after NO photodissociation, and mitochondrial membrane potential subsequently increases[62], which leads to many beneficial effects, including reductions in oxidative stress, inflammation, and cell death[63, 64]. The above findings reaffirm that long-wavelength (red to infrared) light exposure may be a noninvasive treatment method for retinal mitochondria-related diseases.

HO-1 is an acutely reactive heat shock and stress protein that can be induced under certain cellular stress conditions, including heat shock[65], ferroptosis, oxidative damage, and ischemia-reperfusion injury[66, 67]. Previous studies have shown that induction of HO-1 by gene transfer or pharmacological manipulation can inhibit apoptosis and provide cytoprotection after injury[68, 69]. We observed that higher color temperatures of LEDs elicited stronger fluorescence of HO-1 in irradiated R28

cells. Consistent with other results in our experiment, the control and 1900 K groups did not express HO-1 because the 1900 K LEDs did not cause damage to cells.

For rMC-1 cells, the expression of GFAP and vimentin was upregulated as the blue light emitted by the LEDs increased. Müller cells upregulate the expression of GFAP and vimentin after encountering adverse stress conditions[70, 71]. Blue light can also cause retinal Müller cell hypertrophy and upregulation of GFAP in rats[72]. In our experimental results, the fluorescence intensity of GFAP in the 1900 K group was even lower than that in the control group. Generally, control groups are expected to have the least damage. However, ordinary cell culture and preservation techniques can cause some damage to cell mitochondria. Given our findings, the new LEDs are likely to improve mitochondrial function, reduce the production of intracellular oxidation products, and thereby reduce the expression of GFAP. Therefore, there is additional evidence that the new low-color-temperature LEDs may have therapeutic effects.

One previous experiment explored the changes caused by long-term low-intensity irradiation of rat retinas with LEDs and revealed that long-term exposure to blue light-rich LEDs had greater adverse effects on the retina than exposure to LEDs emitting low blue-light levels. However, the results also showed that the b-wave amplitude in the electroretinogram (ERG) of the yellow compact fluorescent lamp group did not decrease significantly by the 9th day but decreased by 21% by day 28. This finding indicates that long-term exposure to even a 3000 K compact fluorescent lamp will cause a decline in retinal function. Notably, fluorescent white LEDs release more blue light as the usage time increases[7]. Kuse et al.[45] reported that 661W cells exposed to blue-light LEDs (wavelength of 464 nm) are more sensitive to light-induced damage than cells exposed to green-light (522 nm) or white-light (456 and 553 nm) LEDs. Similar results have been observed in primary retinal cells. These data support the idea that long-term exposure to blue light in the 400-470 nm range, even at low doses, may damage photoreceptors and RPE cells. Although most studies have focused on the acute effects of light, some have also studied the cumulative effects of light. For example, Noell et al.[73] reported that a single 5-minute exposure

does not cause significant damage to photoreceptor cells, while multiple consecutive 5-minute exposures cause significant photoreceptor cell damage. In addition, the time between exposures affects the cumulative effects of light[74, 75]. In some cases, intermittent exposures can cause more damage than a single exposure to an equivalent amount of light[76]. Therefore, long-term intermittent exposure to traditional white LEDs may pose a threat to health. In addition to the duration of exposure, the time of exposure is also an influencing factor. Some studies have shown that under the same light conditions, the loss rate of photoreceptor cells at night is significantly higher than that during the day. Furthermore, the increased levels of melatonin at night increase not only the susceptibility of the retina to photoinduced damage but also the risks of gastrointestinal, cardiovascular, and breast cancer in women[19, 77]. However, in modern society, it is difficult to avoid using white-light LEDs emitting blue light at night, and a large proportion of people continue to use electronic display terminals such as mobile phones and computers after turning off the lights. Exposure from such devices can greatly impact human health. Therefore, it is of great health significance to find a healthy blue-light-free light source that can replace traditional white LEDs at night. Additionally, the protective mechanism of the retina is important in preventing light-induced damage. Lutein or blue-blocking pigments can prevent light damage to the retina[78]. With increasing age, the mitochondrial function of retinal cells gradually declines[79], the retina becomes zinc-deficient, superoxide dismutase 1 (SOD1) and protective enzymes fail to function properly, self-protection of the retina weakens, and accumulation of harmful products increases. The protective mechanism against optical radiation damage is correspondingly weakened[80, 81]. Therefore, patients with optic neuropathies (such as glaucoma and Leber's disease) or other underlying retinal diseases, elderly patients, and diabetic patients are more likely than other healthy people to exhibit blue-light photochemical damage. Our new low-color-temperature LEDs that do not emit blue light and may have therapeutic effects are undoubtedly the best choices for home lamps for these patients. At present, low-level laser treatments generally use red light with a wavelength above 670 nm. Red light has disadvantages in illumination and a low utilization rate. Although the

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wavelength of red light in the 1900 K low-color-temperature dual-primary-color LEDs in this experiment did not reach 670 nm, we can try to adjust the wavelength of red light in new white LEDs to 670 nm or above to optimize the use of these LEDs for treatment and illumination before further examining their protective effects.

5. Conclusion

The CCK-8 assays, cell death/proliferation experiments and immunofluorescence staining experiments performed in this study yielded several findings. Fluorescent white LEDs, even those with color temperatures as low as 3000 K, cause certain damage to retinal cells. The new low-color-temperature dual-primary-color LEDs do not cause damage to three types of retinal cells because they do not emit blue light; rather, the light they emit is composed mainly of red and yellow dual-color light. These LEDs can reduce the potential human health risks of chronic exposure to fluorescent white LEDs. Our experimental results suggest that long-term use of low-color-temperature LEDs will not cause eye damage and lay a foundation for the therapeutic use of low-color-temperature LEDs in the future. On the basis of our results, we conclude that it is safe to explore the protective effects of low-color-temperature LEDs on the eyes. The results of our preliminary investigations with the new low-color-temperature LEDs show that these light sources may protect the ocular surface and ameliorate insomnia. Given the results of previous studies regarding the protective mechanisms of red and yellow light, these LEDs have great potential for the treatment of insomnia and for protection of the retina, ocular surface and even other tissues and organs. However, the potential ability of these light sources to treat diseases other than insomnia and eye disease requires further investigation. The new 1900 K low-color-temperature LEDs in this study may have value for both lighting and treatment applications and are very suitable as home light sources, especially for patients or elderly individuals who have trouble with eye diseases and insomnia. We believe these LEDs will be some of the most promising home health light sources in this century.

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Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

X.Z. developed the concept and coordinated the project. M.J. and XF.L. wrote the manuscript. M.J., XF.L. and F.Y. analyzed the data and interpreted the results. X.Z. revised the manuscript and obtained funding.

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Author Statement

Ming Jin: Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization

Xiongfeng Li: Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing

Feng Yan: Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Review & Editing

Weixin Chen: Data Curation, Validation, Investigation

Lei Jiang: Data Curation, Validation, Investigation

Xu Zhang: Conceptualization, Validation, Resources, Data Curation, Supervision, Project administration, Funding acquisition

Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors:

Ming Jina

Xiongfeng Li

Feng Yan

Weixin Chen

Lei Jiang

Haijian Hu

Guiping Chen

Xu Zhang

Affiliated Eye Hospital of Nanchang University, Jiangxi Research Institute of Ophthalmology and Visual Science, Jiangxi Clinical Research Center for Ophthalmic Disease，463 Bayi Road, Nanchang, Jiangxi, 330006, China.

Highlights

1) New low-color-temperature LEDs cause no damage to retinal cells
2) New white LEDs may reduce the health risks caused by long-term blue-light exposure
3) New white LEDs may be protective for users suffering eye diseases
4) New white LEDs may be useful for noninvasive phototherapy

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