Effects of artificial light with different spectral compositions on refractive development and matrix metalloproteinase 2 and tissue inhibitor of metalloproteinases 2 expression in the sclerae of juvenile guinea pigs

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ABSTRACT

Artificial light can affect eyeball development and increase myopia rate. Matrix metalloproteinase 2 (MMP-2) degrades the extracellular matrix, and induces its remodeling, while tissue inhibitor of matrix MMP-2 (TIMP-2) inhibits active MMP-2. The present study aimed to look into how refractive development and the expression of MMP-2 and TIMP-2 in the guinea pigs' remodeled sclerae are affected by artificial light with varying spectral compositions. Three weeks old guinea pigs were randomly assigned to groups exposed to five different types of light: natural light, LED light with a low color temperature, three full spectrum artificial lights, *i.e.* E light (continuous spectrum in the range of ~390-780 nm), G light (a blue peak at 450 nm and a small valley 480 nm) and F light (continuous spectrum and wavelength of 400 nm below filtered). A-scan ultrasonography was used to measure the axial lengths of their eyes, every two weeks throughout the experiment. Following twelve weeks of exposure to light, the sclerae were observed by optical and transmission electron microscopy. Immunohistochemistry, Western blot and RT-qPCR were used to detect the MMP-2 and TIMP-2 protein and mRNA expression levels in the sclerae. After four, six, eight, ten, and twelve weeks of illumination, the guinea pigs in the LED and G light groups had axial lengths that were considerably longer than the animals in the natural light group while the guinea pigs in the E and F light groups had considerably shorter axial lengths than those in the LED group. Following twelve weeks of exposure to light, the expression of the scleral MMP-2 protein and mRNA were, from low to high, N group, E group, F group, G group, LED group; however, the expression of the scleral TIMP-2 protein and mRNA were, from high to low, N group, E group, F group, G group, LED group. The comparison between groups was statistically significant ($p<0.01$). Continuous, peaks-free or valleys-free artificial light with full-spectrum preserves remodeling of scleral extracellular matrix in guinea pigs by downregulating MMP-2 and upregulating TIMP-2, controlling eye axis elongation, and inhibiting the onset and progression of myopia.

Key words: artificial light; axial length; guinea pig; matrix metalloproteinase 2; spectral composition; myopia.

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Introduction

Myopia is a major eyesight/vision health issue in childhood (0 year to <19 years). It is estimated that by 2025, 324 million young persons will be myopic worldwide.[1] Continuous myopia progression increases the risk of certain eye disorders such as retinal detachment.[2] The ambient light environment is correlated with myopia severity.[3-4] The observed global increase in the myopia rate may be associated with an increase in exposure to artificial light sources.[5] Variation in monochromatic light is closely related to dioptric development. Kroger *et al.* found that fisheye axes grew faster in the presence of long-wavelength than short-wavelength monochromatic lighting.[6] The axial lengths of guinea pigs were greater under 530 nm green light than they were under 480 nm blue light, and myopia was more evident in the former than in the latter case.[7] Chicks reared in red light developed a low degree of myopia while blue light rearing induced a low degree of hyperopia.[8] Guinea pigs became more myopic when they were reared under red (769 nm) or green light (530 nm), compared to those raised under white light or blue light (430 nm).[9] In contrast, rhesus monkeys and tree shrews are usually hyperopic under long-wavelength and myopic under short-wavelength light.[10-12] Engaging in outdoor activities can effectively prevent the development of myopia.[13-16] The risk of myopia onset decreases with increasing amount of time spent outdoors.[17-19] There are differences between artificial and natural light in terms of spectrum, stroboscopic effect, and rhythm of lighting.[20] Exposure to artificial light is an important cause of myopia as it affects eyeball development.[21] Artificial light encompasses all light shaped by human action, which may include other ranges of the light spectrum. The visible spectrum of artificial light consists mainly of combinations of various monochromatic long, medium, and short wavelengths.[22] The differences among light sources were discussed in our previous report.[23]

Myopia may be related to scleral remodeling.[24] In chickens, tree shrews, and monkeys, remodeling of the scleral extracellular matrix (ECM) rapidly alters the biomechanical properties of the sclera and changes the axial length.[25-28] Matrix metalloproteinase 2 (MMP-2) degrades the ECM, induces remodeling,[29] reduces the collagen content, and weakens the biomechanical function of the sclera, thereby rendering it susceptible to deformation. After myopia is induced, MMP-2 is upregulated in the sclera and significantly increases with myopia severity in a time-dependent manner.[30] In contrast, MMP-2 is downregulated when myopia and axial elongation are delayed.[31] The tissue inhibitor of matrix MMP-2 (TIMP-2) secreted by scleral fibroblasts inhibits active MMPs affects scleral matrix degradation, and remodels the sclera. The degree of ECM protein breakdown and tissue remodeling is typically determined by the harmony of MMP-2 and TIMP-2 for most cases.[32]

The natural light spectrum is continuous and has no peaks or valleys, whereas light-emitting diode (LED) lamps emit 430-460 nm for a blue peak and 480 nm for a blue valley. Blue light-filtering intraocular lenses are beneficial for eye health.[33] In a prior simulation work on white New Zealand rabbits, we used artificial light with full-spectrum that closely resembled the nature of natural light's composition and observed that artificial light with full-spectrum inhibits elongation of the eye axis and mitigates degradation of the retinal structure.[23] We then subjected guinea pigs to artificial light with various spectral compositions and found that artificial light with continuous full-spectrum inhibits the elongation of the eye axis *via* the retinal dopaminergic and melanopsin systems.[22] As far as we are aware, few previous reports have discussed the impacts of mixed light with different spectral compositions on refractive eyeball development from the scleral tissue. Here, we applied LED lights with low color temperature and three other artificial light sources with the same intensity to determine the effect of artificial light with various spectral compositions on the development of the eye axis from the clera in guinea pigs. We also investigated whether full-spectrum artificial light may be protective in refractive development.

Materials and Methods

Laboratory animals

Thirty (15 males and 15 females) healthy three-week-old guinea pigs were purchased from Yizheng Anlimao Biotechnology Co. Ltd. (Jiangsu, China), kept indoors at 18-24°C with a relative humidity (RH) of 40-70%, and administered fresh food and water daily. The Statement for the Use of Animals in Ophthalmic and Vision Research was followed in the handling and care of the animals. The Animal Care and Use Committee of Affiliated Hospital of Nanjing University of Chinese Medicine accepted the research procedure (approval no. 2022DW-49-01).

Experimental design

Four distinct kinds of artificial light sources with various spectra and the same light intensity were given to the guinea pigs to determine whether LED based on the natural light (N light) spectrum protects axial development. An HR2000 fluorospectrophotometer (Oceatics Inc., Osaka, Japan) with a detection limit range of 200-1100 nm was utilized to quantify optical characteristics such as frequency of flickering, illuminance, irradiance and spectrum composition of light. The animals were randomly assigned to five groups, 6 for each group. The groups were exposed to five different types of light: N light (spectrum is continuous and has no peaks or valleys), LED light (L light) with a low color temperature (a blue peak at 430-460 nm and a blue valley at 480 nm), and three kinds of continuous spectrum based artificial light, *i.e.* E light: spectrum is continuous in the range of ~390–780 nm; Gulia light (G light): a blue peak at 450 nm and a small valley 480 nm; 3) Fulia light (F light): spectrum is continuous and wavelength of 400 nm below filtered. The spectra of the five light groups which refer to the figure published by Xu *et al.*[22] are shown in Figure 1. All

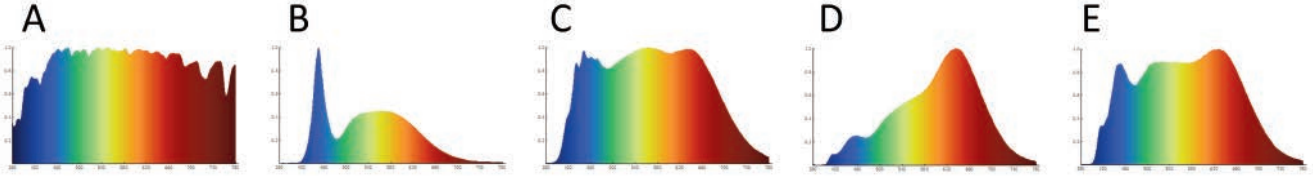

Figure 1. Spectra of natural and four artificial light sources. **A**) N light. **B**) L light. **C**) E light. **D**) F light. **E**) G light.

guinea pigs except those in the N group were housed in cages with overhead lighting inside and black cloth coverings. Each cage exterior had a device that automatically regulated the light intensity and synchronized the cage's lighting pattern with that of the surrounding natural light. The cage of the N light group was close to the south window (quartz glass with sunlight), and the top of the cage only was not covered. The average light illuminance and irradiance of each group were regulated to 350 lux and 5w/m²respectively, and the distance between cage and window was adjusted in order to reach such illuminance and irradiance value in consideration of the weather. The details were also described in our previous study.[22]

Ocular biometry

Before the start of the experiment, and every two weeks thereafter, a 10-MHz probe-equipped A-scan ultrasonograph (KN-1800; Kangning Medical Device Co. Ltd., Wuxi, China) was used to measure the guinea pigs' respective axial lengths. One drop of 0.4% oxybuprocaine hydrochloride eye drops (Sensei Corporation, Hyogo Prefecture, Japan) was placed thrice in each eye at 5-min intervals before the axial length measurements. Every corneal apex was directly touched by the ultrasonic probe, and the ultrasound probe was vertical relative to each corneal surface. Each eye was measured 10 times, figures with clear, stable waveforms were read, the standard deviation (SD) was <0.05 mm, and the mean eye axis lengths were calculated.

Scleral histopathology

All guinea pigs were euthanized by intraperitoneal injection of excess sodium pentobarbital after 12 weeks light exposure, their eyeballs were excised, and their sclerae were isolated, fixed with 4% paraformaldehyde (PFA) for more than 24 h. After fixation, phosphate buffer saline (PBS) was used to rinse the scleral tissue, then dehydration was performed with 80%, 90%, 95% and 100% ethanol at room temperature for 2 h per step, embedded in paraffin at 65°C for 3 h and cut into 3-μm serial slices, then stained with hematoxylin and eosin (H&E) after deparaffinization and rehydration. The stained sections were then dehydrated and sealed with neutral gum, and observed for pathological changes in the sclerae using a light microscope (Nikon Eclipse E100; Nikon Corporation., Tokyo, Japan).

Sclera examination by transmission electron microscopy

After washing once in PBS, the scleral tissue was fixed for 15 min with 4% PFA and then with 2.5% glutaraldehyde for 2 h at 4°C. After that, the sclerae were rinsed with PBS (three changes, for 15 min each) and fixed in 1% osmic acid for 2 h at 4°C, then gradually dehydrated and embedded in epoxy resin; 70 nm-thick serial sections were cut, stained with 7.3% uranyl acetate plus lead citrate, and observed and photographed under a transmission electron microscope (TEM) (Hitachi HT-7700; Hitachi Ltd., Tokyo, Japan).

Immunohistochemistry

Immunohistochemistry (IHC) was used to detect the expression of scleral MMP-2 and TIMP-2. The guinea pigs were euthanized, their eyeballs were rapidly excised and placed on ice, and their sclerae were immobilized, fixed with 4% PFA, embedded in paraffin and sectioned. After deparaffinization and rehydration, the tissue sections were placed in citric acid antigen retrieval buffer, then treated with 0.3% hydrogen peroxide for 30 min (to inhibit endogenous peroxidase activity), and blocked with 5% bovine serum albumin (BSA) for 1 h. The sections were incubated with anti-MMP-2 (1:200; No. 10373-2-AP; Proteintech Group, Rosemont, IL, USA) or anti-TIMP-2 (1:200; No. 17353-1-AP; Proteintech Group) overnight at 4°C. Next, they were incubated with goat anti-rabbit immunoglobulin G that had been tagged with horseradish peroxidase (HRP) (IgG; 1:200; No. SA00001-2; Proteintech Group) at room temperature for 2 h. After incubation with diaminobenzidine (DAB), the sections were counterstain with hematoxylin, subjected to 1% hydrochloric acid in 70% alcohol for 10 s, and sealed with neutral gum. The pre-test of positive and negative controls were performed before the experiment, and no false positives and false negatives were found. To evaluate the expression levels of scleral MMP-2 and TIMP-2 protein, the Image-Plus Pro software (Media Cybernetics Inc., Rockville, MD, USA was used to measure the average optical densities (OD) of five randomly selected visual fields taken with an Axioplan 2 light microscope (Carl Zeiss AG, Oberkochen, Germany; objective 40x).

Western blot

Using radioimmunoprecipitation assay (RIPA) lysate, total protein was extracted from fresh sclerae, and the bicinchoninic acid (BCA) test was used to measure the amount of protein. The protein samples were collected, separated with 10% gel, concentrated with 5% gel, subjected to gel electrophoresis, and transferred to polyvinylidene fluoride (PVDF) membranes that were blocked with 5% skim milk for 1 h, then washed with PBS, and incubated with anti-MMP-2 (1:3,000; No.ab92536; Abcam, Cambridge, MA, USA), anti-TIMP-2 (1:1,000; No. ab180630; Abcam), or anti-GAPDH (1:5,000; No.10494-1-AP; Proteintech Group) antibodies, overnight at 4°C. After being washed with Tris-buffered saline with Tween 20 (TBST), the samples were incubated for 1.5 h at room temperature with HRP-labeled goat anti-rabbit IgG (1:5,000; No. SA00001-2; Proteintech Group), and washed with TBST. The membranes were then exposed to HRP color-developing substrate and an automatic chemiluminescence analyzer was used to detect and image the signals. The latter were semi-quantitatively analyzed with the Image-Plus Pro software (Media Cybernetics Inc., Rockville, MD, USA. GAPDH was used as the internal reference protein.

RT-qPCR detection

Freshly separated sclerae were fragmented and homogenized, and 200 μL TRizol lysate (Thermo Fisher Scientific, Waltham, MA, USA) was added per 20 mg scleral tissue to extract total RNA. A TaKaRa Reverse Transcription Kit (JiangSu CoWin Biotech (CWBIO), Jiangsu, China) was used to reverse-transcribe one microgram of total RNA into cDNA that utilized as a model for the amplification of qPCR. The latter was performed with a SYBR Premix Ex Taq II Kit (No. RR820A; TaKaRa Bio Inc., Kusatsu, Shiga, Japan). The thermal program was as follows: 40 cycles of 5 min at 94°C for pre-denaturation, 10 s at 94°C for denaturation, 30 s for annealing at 60°C, and 10 min for stretching at 72°C. Three replicates per sample were made, and GAPDH was internal reference control; the $2^{-\Delta\Delta CT}$ method was used to calculate the relative levels of target gene mRNA expression. The sequences (5'-3') of the primers (Shenggong Bioengineering Co. Ltd., Shanghai, China) were as follows: MMP-2, GCTCTCTGCTTCCTGAGCTG and AACTTGATGATGGGCGAT; TIMP-2, GAGCGAGAAGGAGGTGGATTCCGGG and ATGTCAAGAAACTCCTGCTTCGGGGG; GAPDH, TCGCTCCTGGAAGATGGTG and TCATTGACCTCCAGTACATGG.

Statistical analysis

The SPSS v. 22.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Normality and mean square error tests were performed before selecting statistical tests. For every guinea pig, the results for the left and right eyes were averaged, these data are expressed as mean ± SD. The means of the two samples were

compared using independent sample *t*-tests. the variations among multiple groups were compared by one-way analysis of variance (ANOVA). A statistical significance of $p<0.05$ was indicated, and $p<0.01$ was considered as highly significant.

Results

The impact of varying light sources on axial lengths

Prior to the experiments, there was no considerable difference on the axial lengths among groups ($p>0.05$). During the research, the axial lengths of each group gradually increased with irradiation time (Table 1). After four weeks of irradiation, the guinea pigs in the L and G light groups had axial lengths that were considerably longer than the animals in the N light group while those in the E (simulated natural light spectral composition) and F light groups were noticeably shorter than those in the L group ($p<0.05$). After six, eight, 10, and 12 weeks of illumination, the axial lengths in the E, F, and G groups were considerably shorter than those in the L group and were noticeably longer in the L and G groups than those in the N group ($p<0.05$). The differences among the other groups in terms of their axial lengths were not statistically significant at any other time point.

Scleral histopathological morphology

Optical microscopy disclosed that scleral collagen fiber distribution was relatively uniform, orderly, and compact in both the N and E groups. In contrast, those of the other three artificial light groups had a disorganized distribution and wide gaps. The LED group displayed collagen fiber breakage (Figure 2).

Scleral tissue ultrastructure

At TEM, the scleral collagen fibers were closely and regularly arranged in the N and E groups, while being irregular and loose in the artificial light groups (Figure 3).

Expression of the MMP-2 and TIMP-2 protein in scleral tissue

MMP-2 and TIMP-2 in scleral tissue were detected by IHC and Western blot (WB). Both methods yielded the same results for the expression of MMP-2 and TIMP-2 protein. Compared to the N light group, the artificial light groups' scleral MMP-2 and TIMP-2 protein expression levels were considerably higher and lower, respectively ($p<0.01$). The L group had considerably higher levels of scleral MMP-2 protein expression and lower levels of scleral TIMP-2 protein expression than the E, F, and G groups ($p<0.01$). The F and G groups had considerably higher levels of scleral MMP-2 protein expression and lower levels of scleral TIMP-2 protein expression, respectively, compared to the E group ($p<0.01$).

Table 1. Comparison of the axial lengths of the eyes of guinea pigs exposed to various spectrum light at different periods (mm).

Time	N light group	LED light group	E light group	F light group	G light group
0 week	7.57±0.10	7.56±0.09	7.55±0.02	7.56±0.02	7.55±0.11
2 weeks	7.63±0.24	7.72±0.02	7.64±0.02	7.72±0.01	7.72±0.01
4 weeks	7.72±0.02	7.96±0.02#	7.74±0.03^	7.79±0.02°	7.94±0.01*
6 weeks	7.91±0.03	8.29±0.03#	7.89±0.03^	8.06±0.02°	8.11±0.02*°
8 weeks	7.97±0.01	8.40±0.02#	8.03±0.01^	8.11±0.02^	8.25±0.02#°
10 weeks	8.11±0.02	8.48±0.02#	8.15±0.02^	8.25±0.02^	8.36±0.02#°
12 weeks	8.19±0.02	8.61±0.02#*	8.25±0.02^	8.30±0.02^	8.43±0.02#°

*$p<0.05$, #$p<0.01$ compared with the natural light group at the same period; °p<0.05, ^p<0.01 compared with the LED light group at the same period.

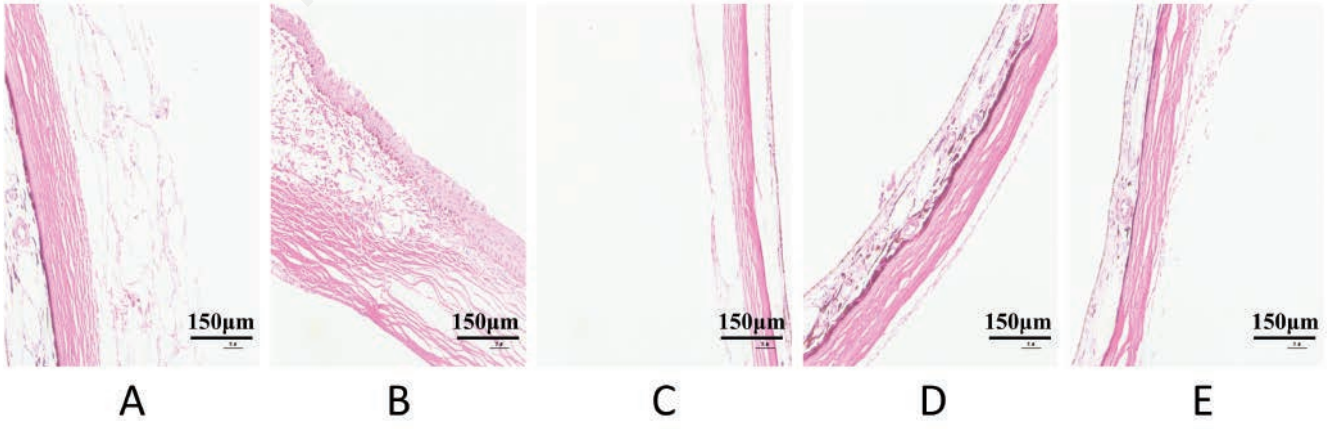

Figure 2. Different light-induced changes in scleral structure. **A**) N light group. **B**) L light group. **C**) E light group. **D**) F light group. **E**) G light group. Scleral collagen fiber distribution was more uniform, orderly, and compact in both the N and E groups than in the other three artificial light groups. Moreover, the L group displayed collagen fiber breakage. H&E staining.

The F group had considerably lower levels of scleral MMP-2 protein expression and higher levels of scleral TIMP-2 protein expression than the G group (p<0.01) (Figures 4 to 6).

Expression of the MMP-2 and TIMP-2 mRNA in scleral tissue

Compared to the N light group, the artificial light groups' scleral MMP-2 and TIMP-2 mRNA expression levels were considerably higher and lower, respectively (p<0.01). Compared to the L group, the mRNA expression levels of MMP-2 and TIMP-2 in the sclera of the other three artificial light groups were considerably lower and higher, respectively (p<0.01). compared to the E group, The F and G groups had considerably higher levels of scleral MMP-2 mRNA expression and lower levels of scleral TIMP-2 mRNA expression, respectively (p<0.01). The F group had considerably lower levels of scleral MMP-2 mRNA expression and higher levels of scleral TIMP-2 mRNA expression, respectively, compared to the G group (p<0.01) (Figure 7).

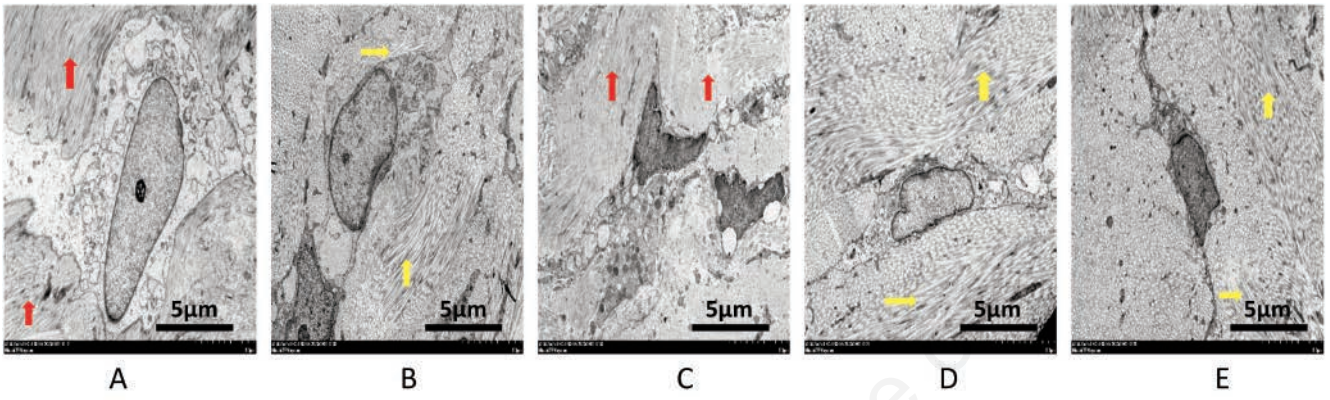

Figure 3. Ultrastructure of scleral tissue of guinea pig eyes exposed to different light sources and observed by transmission electron microscopy. **A**) N light group. **B**) L light group. **C**) E light group. **D**) F light group. **E**) G light group. In the N and E groups, collagen fibers were organized tightly and regularly (red arrows) whereas those in the other three artificial light groups were loose and irregular (yellow arrows).

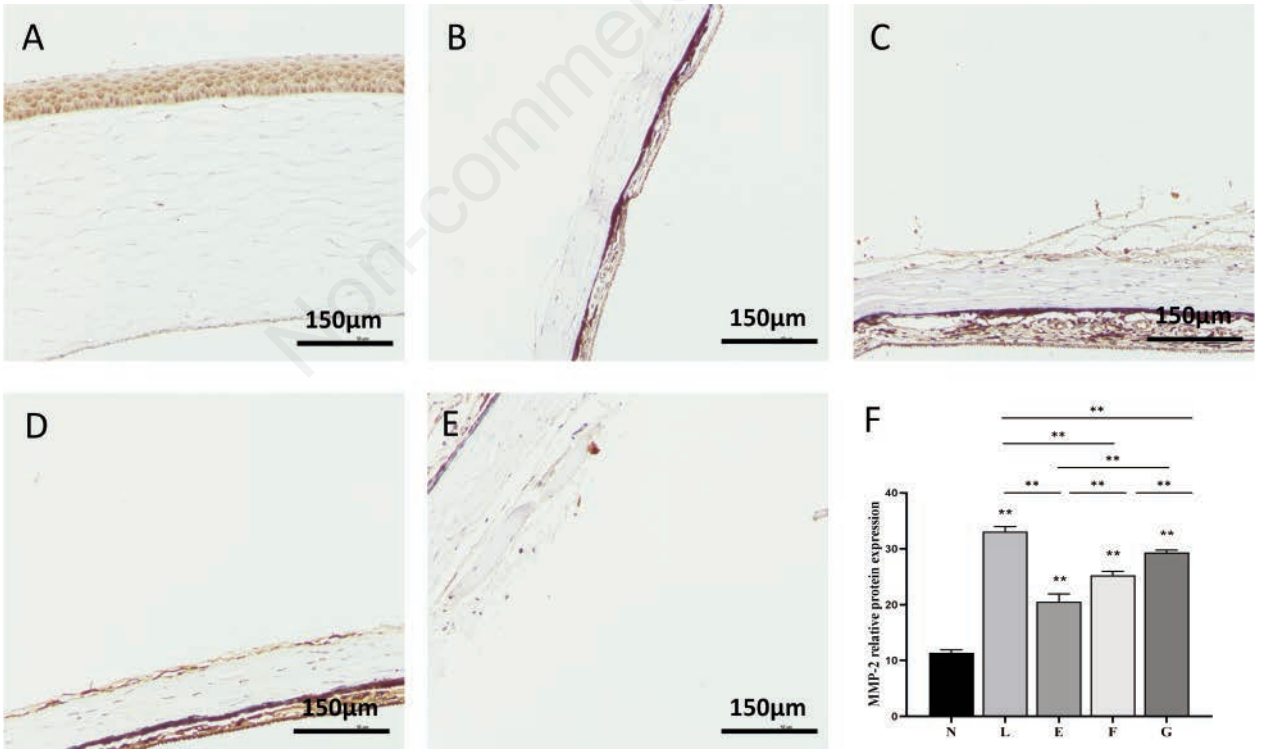

Figure 4. Photomicrographs of MMP-2 protein expression in guinea pig scleral tissue after a 12-week exposure to various light sources. MMP-2 protein is stained brown. The expression of scleral MMP-2 protein in the N group and four artificial light source groups from low to high were N group, E group, F group, G group, L group. The comparison between groups was statistically significant (p<0.01). **A**) N light group. **B**) L light group. **C**) E light group. **D**) F light group. **E**) G light group. **F**) Each group's relative MMP-2 protein expression levels are shown; *p<0.05, **p<0.01.

Discussion

Several animal models have been used to study the impact of light spectrum composition on refractive development and eye growth.[34-35] Wang *et al.* reported that guinea pigs presented more severe eye axis elongation and myopia under 530 nm (green) than 480 nm (blue) illumination.[7] Gawne *et al.* found that, compared to baby, older juvenile and adolescent tree shrews exposed to white fluorescent light, those exposed to red light showed more severe hyperopia.[11-12] In contrast, chickens are relatively more myopic and hyperopic in response to red and blue light, respectively.[36] Most earlier research focused on the impact of monochromatic light on scleral tissue. To the best of our knowledge, however, the effects of multi-spectral composition of mixed light sources on myopia have seldom been investigated. In a previous study, we found that continuous peaks-free or valleys-free artificial light with full-spectrum may prevent the elongation of the ocular axis by means of the

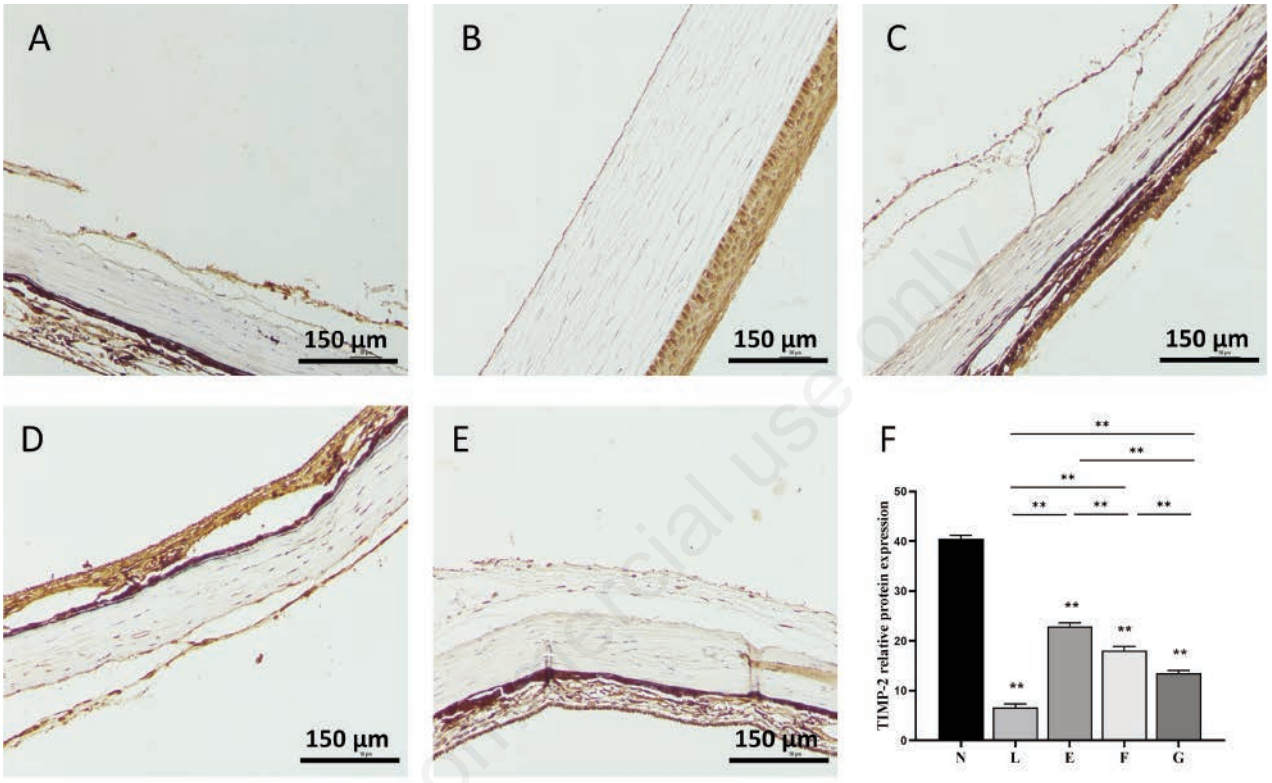

Figure 5. Photomicrographs of TIMP-2 protein expression in guinea pig scleral tissue after a 12-week exposure to various light sources. TIMP-2 protein is stained brown. The expression of scleral TIMP-2 protein in the N group and four artificial light source groups from high to low were N group, E group, F group, G group, L group. The comparison between groups was statistically significant ($p<0.01$). **A**) N light group. **B**) L light group. **C**) E light group. **D**) F light group. **E**) G light group. **F**) Each group's relative MMP-2 protein expression levels are shown; *$p<0.05$, **$p<0.01$.

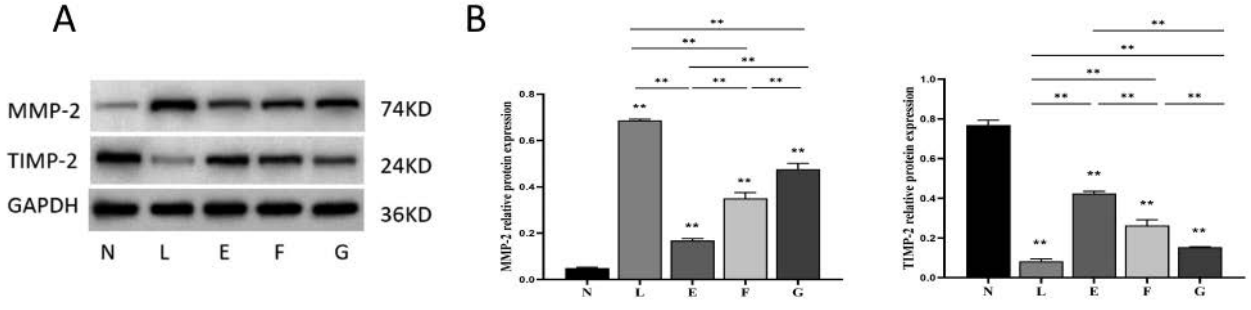

Figure 6. A) Levels of MMP-2 and TIMP-2 protein expression by Western blot. **B**) Each group's relative expression levels of MMP-2 and TIMP-2 protein were adjusted to GAPDH, as demonstrated. The expression of scleral MMP-2 protein from low to high were N group, E group, F group, G group, L group. However, the expression of scleral TIMP-2 protein from high to low were N group, E group, F group, G group, L group. The comparison between groups was statistically significant ($p<0.01$); *$p<0.05$, **$p<0.01$.

melanopsin and dopaminergic systems in the retina,[22] but we did not really assess whether blocking that systems would also block the response. This is important for further verification of the melanopsin pathway, therefore, intrinsically photosensitive retinal ganglion cell (ipRGC) ablation or melanopsin knockout experiments should be performed to evaluate the response of blocking this pathway later. Here, we used scleral tissue to determine the impacts on axial growth induced by artificial light with various spectral components and whether full-spectrum artificial light protects refractive development in guinea pigs.

The mechanism by which melanopsin affects refractive development remains unknown.[22] Melanopsin plays a critical role in the non-imaging visual form system, with a peak absorption of 479 nm.[37] Melanopsin responds more effectively to blue wavelength. Methodological differences may lead to inconsistent results of melanopsin studies. Liu *et al.* found that when mice were fed in an environment where 480 nm wavelength light (the maximum excitation wavelength of melanopsin) was absent, the activation degree of melanopsin was reduced, and the effect of form deprivation on myopia was significantly reduced.[38] Chakraborty *et al.* found that opn4$^{-/-}$ mice raised in a normal environment were more myopic after 4 weeks, and opn4 $^{-/-}$ mice were more myopic than opn4$^{+/+}$ mice after 3 weeks of form deprivation.[39] Our previous research demonstrated that melanopsin may play a role in refractive development.[22] The blue spectral region (310-450 nm) can cause retinal damage, specifically directly affecting the ability of retinal ganglion cell mitochondria.[40] In the present study, after 4 weeks of light exposure, the guinea pigs in the L and G groups had axial lengths that were considerably (p<0.05) longer than the animals in the N group. while the guinea pigs in the E and F groups had considerably shorter ones (p<0.05) than the ones in the L group. However, axial length did not significantly differ among the E, F, and N groups. The differences among treatments in terms of their spectral characteristics might explain the foregoing observations. Low color temperature LED light exhibits a blue peak and a blue valley at 430-460 nm and 480 nm, respectively. E light is a continuous spectrum in the range of ~390-780 nm, and its spectral profile resembles those of F and G light. However, G light has a blue peak and a small valley at 450 nm and 480 nm, respectively, while F light is filtered at wavelengths <400 nm but its spectrum is continuous and lacks blue peaks and valleys. Hence, the peaks and valleys in the mixed light spectrum play vital roles in eyeball growth and development. Our results can be explained by strong light suppression, namely the peak light of the light source will produce strong light suppression on the visual cells, so that the relative low light segment will not be perceived, forming an incoherent multifocal plane phenomenon. The persistence of this phenomenon will produce defocusing signals different from natural light, which will cause the elongation of the axial length and drift towards myopia.[12,22] The blue light peak of about 450 nm in group L and Group G inhibited the blue light valley at 480 nm, resulting in the formation of incoherent multifocal planes and lengthening of guinea pig axial length. Our research showed the artificial E light source most strongly inhibited eye axis elongation. But the mechanism of the influence of light on refractive development is complex, and more research will be needed.

Guinea pigs are docile and highly coordinated, and even neonates have well-developed visual systems that can readily distinguish various objects and linear directions. Their large eyeballs are useful in research as they facilitate the measurement of various parameters. Moreover, these animals are cost-effective, reproduce reliably, and are genetically distinct.[41,42] For these reasons, guinea

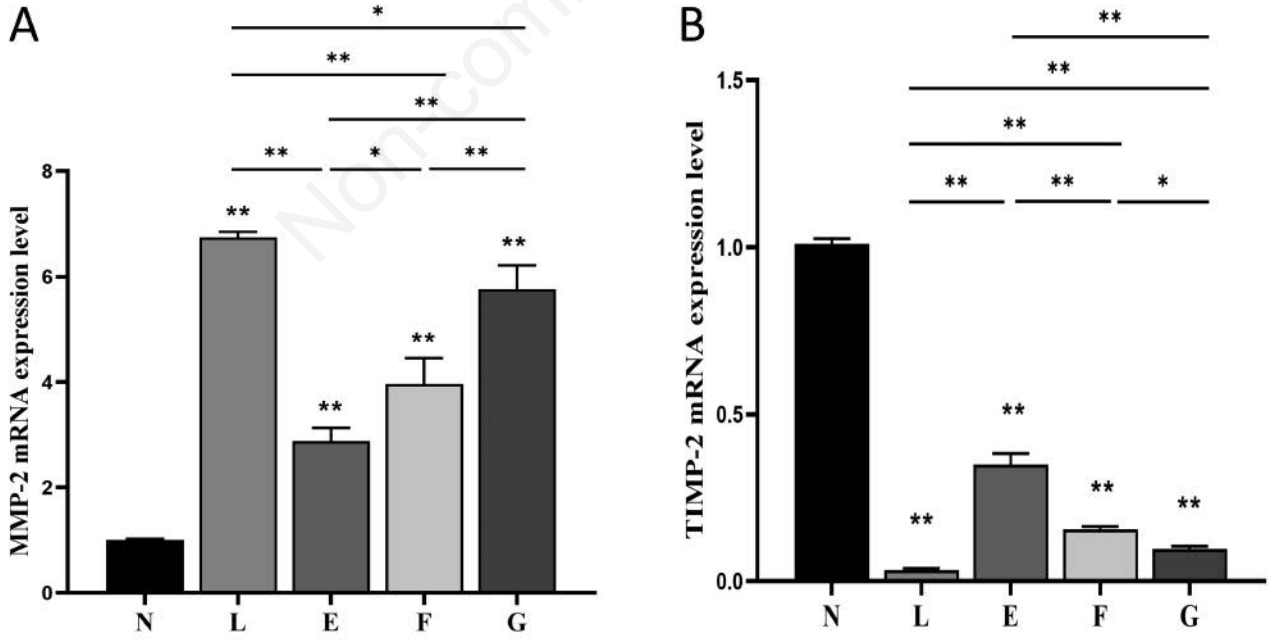

Figure 7. Levels of scleral MMP-2 and TIMP-2 mRNA expression by RT-qPCR. **A**) MMP-2 mRNA expression level. **B**) TIMP-2 mRNA expression level. The expression of scleral MMP-2 mRNA from low to high were N group, E group, F group, G group, L group. However, the expression of scleral TIMP-2 mRNA from high to low were N group, E group, F group, G group, L group. The comparison between groups was statistically significant (p<0.01); *p<0.05, **p<0.01.

pigs are ideal as experimental myopia models. The sclera is a dense connective tissue composed of cells (mainly fibroblasts) and ECM (mostly collagen fibers). In normal persons, collagen fibers are interwoven or lamellar. Gottlieb *et al.* stated that in human myopia, abnormal interwoven collagen replaces the normal lamellar form and partially attenuates the capacity of the sclera to resist expansion.[43] Here, optical microscopy revealed that the scleral collagen fiber distribution was relatively uniform, orderly, and compact in both the N and E groups but disorganized and widely separated particularly in the other three artificial light groups. Moreover, the L group displayed collagen fiber breakage. Ultrastructural observation of the sclerae disclosed that the N and E groups' collagen fibers were organized tightly and regularly whereas those in the other three artificial light groups were loose and irregular. An earlier study indicated that collagen fiber interlacing and diameter decreased, and the number of serrated and star-shaped fibers increased with the severity of experimental myopia.[44] Our results suggest that the multi-spectral light sources induced pathological scleral collagen fiber remodeling during the onset of myopia and that the E group presented the least amount of scleral collagen fiber remodeling of all treatments.

Active remodeling of the scleral ECM has a significant impact on human eye development, as well as other animals.[45] The onset and progression of myopia are associated with scleral remodeling.[24] Degradation and biosynthesis of the scleral ECM increase and decrease, respectively, the scleral tissue thins, and the ocular axis lengthens with myopia progression.[46] Abnormal scleral ECM metabolism contributes to the development of axial myopia during scleral remodeling. MMP-2 and TIMP-2 regulate scleral ECM metabolism.[47-49]

The gelatinase MMP-2 is regulated by multiple signaling pathways. It is upregulated during the onset of myopia, hydrolyzes scleral ECM, and induces scleral remodeling.[29] Therefore, it may be directly implicated in scleral collagen degradation during myopic eye elongation. In myopia sufferers, the MMP-2 is strongly expressed in their aqueous humor.[50] MMP-2 was upregulated in the scleral tissues of certain animals subjected to myopia induction.[31] Guggenheim *et al*. performed a shape perception experiment on masked and unmasked tree mice and found that most of the MMP-2 was inactive in the sclerae of the unmasked animals.[51] In contrast, MMP-2 activity was considerably higher in the posterior polar sclerae of the masked mice presenting form deprivation myopia than in those of the unmasked control mice. After the masks were removed, however, the MMP-2 activity in the former declined to a lower level than that in the latter. Rada *et al.* discovered that chicks masked for 11 days had considerably greater amounts of MMP-2 protein and mRNA in their posterior pole sclerae than those unmasked controls.[52] Here, IHC, qRT-PCR, and WB revealed that the expression levels of MMP-2 mRNA and protein were the lowest and there was minimal scleral remodeling in the guinea pigs exposed to natural light. Furthermore, the expression levels of MMP-2 mRNA and protein were lower and there was less scleral remodeling in the E group than in the other three artificial light groups.

TIMPs are natural MMP inhibitors secreted by scleral fibroblasts. Both TIMPs and MMPs affect scleral remodeling, and their activation and balance affect normal eyeball development as well as the onset and progression of myopia.[53] In studies on experimentally induced myopia, imbalances in the expression of MMP-2 and TIMP-2 decreased and increased scleral ECM biosynthesis and degradation, respectively. Other studies showed that tumor invasion, metastasis, and prognosis are strongly associated with MMP-2 and TIMP-2 imbalances.[54-56] TIMP-2 forms a complex with MMP-2 to inhibit it.[55] Rada *et al.* found that the TIMP-2 mRNA level was 53% lower in the posterior pole sclerae of chickens masked for 11 d than in those of the unmasked control chickens.[53] In the former case, the TIMP-2 level increased one day after unmasking. In the present study, IHC, RT-qPCR, and WB disclosed that the expression levels of TIMP-2 mRNA and protein were the highest, and scleral remodeling was the weakest in the guinea pigs exposed to natural light. The expression levels of TIMP-2 mRNA and protein were higher and scleral remodeling was weaker in the E group than in the three other artificial light groups. The preceding results suggest that the E treatment was the best simulated natural light source and most effectively protected against myopia.[22]

The findings of the present work suggested that artificial light sources with full spectrum simulating the natural light spectrum and lacking any peaks or valleys might confer protection against myopia in guinea pigs by downregulating MMP-2 and upregulating TIMP-2 in the sclerae. Future investigations should apply scleral fibroblast culture and *in vivo* detection to evaluate the changes that occur in scleral MMP-2 and TIMP-2 content and activity. In this manner, we may be able to elucidate the mechanisms by which full-spectrum artificial light sources resembling natural light protect against the onset and progression of myopia. This information could lay the foundation for research and development into innovative prophylaxes against myopia.

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