Original Article

Effects of Different Light Environments with Varying Spectral Composition on the Axial Lengths and Scleral Specificity Protein 1 and Collagen Type I Expression in Juvenile Guinea Pigs

(guinea pigs / myopia / spectral composition / axial length / scleral specificity protein 1 / collagen type I)

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Abstract. The study aimed to investigate changes in the eye axial length in juvenile guinea pigs and the expression of scleral specificity protein 1 (Sp1) and collagen type I (Col-I) under different light environments with varying spectral composition. The animals were randomly divided into five groups: natural light (N), LED light with a low colour temperature (L), E light (E), Fulia light (F), and Gulia light (G). Axial lengths were measured every two weeks, and the expression of Sp1 and Col-I in the sclera was assessed by immunohistochemistry, Western blot and RT-qPCR. After 4, 6, 8, 10, and 12 weeks of light exposure, the L and G groups showed considerably longer axial lengths than the N group, with the L group exhibiting significantly longer axial lengths compared with the E and F groups. The protein and mRNA expression levels of Sp1 and Col-I, ranked from highest to lowest, were as follows: N, E, F, G, and L. The expression of Sp1 and Col-I was positively correlated, but both were negatively correlated with the length of the eye axis. The E group demonstrated higher Sp1 and Col-I expression than the other artificial light groups. Artificial light with a continuous, full spectrum lacking peaks and valleys can inhibit the elongation of the eye axis in juvenile guinea pigs and has a protective effect against myopia. There may be a certain relationship between Sp1 and Col-I, and the transforming growth factor-β1-Sp1-Col-I signalling pathway may play a crucial role in myopic scleral extracellular matrix remodelling.

Introduction

Myopia is highly prevalent among school-aged children and adolescents (Jiang et al., 2017), especially in Asia, where its prevalence reaches 70–80 % (Wen et al., 2013, Wu et al., 2016). In East Asia, the incidence of myopia among young people has risen to 80–90 % (Zhan et al., 2019). Studies have shown that China has the second-highest global incidence of myopia, following Japan accounting for 33 % of the world's myopia cases. This has imposed a significant burden on the social economy and adversely affects the quality of life (He et al., 2015). It is estimated that by 2050, the prevalence of myopia will reach 2.5 billion people, or 49.8 % of the world's population, with 9.8 % of the global population experiencing high myopia (≥ 6.00D). Myopia can cause serious and irreversible damage to the patients' vision (Wu et al., 2016; Hassen et al., 2017; Ikuno, 2017; Naidoo et al., 2019). In myopia, the human eye undergoes a series of changes such as excessive elongation of the ocular axis in some cases of high myopia, thinning of the sclera, and development of posterior staphyloma (McBrien and Gentle, 2001), which, in se-

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Abbreviations: Col-I – collagen type I, DA – dopamine, DAB – diaminobenzidine, ECM – extracellular matrix, HRP – horseradish peroxidase, IHC – immunohistochemistry, OD – optical densities, pRGCs – intrinsically photosensitive retinal ganglion cells, SD – standard deviation, Sp1 – scleral specificity protein 1, TGF-β1 – transforming growth factor β1, WB – Western blot.

vere cases, can lead to blindness (Bourne et al., 2013). It is evident that myopia has become a global public health concern (Marcus et al., 2011; Holden et al., 2016; Morgan et al., 2018; Flanagan et al., 2019). Therefore, understanding the mechanisms underlying myopia and developing preventive measures is an urgent social problem for ophthalmologists.

Myopia may be associated with scleral remodelling (Garcia et al., 2017). Specifically, in myopia, the sclera exhibits a decrease in collagen type I (Col-I) content, a decrease in proteoglycan content, abnormal morphology and structure, and thinning of collagen fibres. Collagen fibres are the most abundant component of the sclera, accounting for 90 % of its net weight, with Col-I fibres making up more than 75 % of the sclera. The expression of Col-I is significantly decreased during myopic scleral remodelling (Gentle et al., 2003; Pan et al., 2018). Remodelling of the scleral extracellular matrix (ECM) can alter the biomechanical properties of the sclera, leading to changes in the axial length (Wildsoet and Wallman, 1995; McBrien and Gentle, 2001; Rada et al., 2002; Zhu et al., 2003). Transforming growth factor β1 (TGF-β1) is involved in myopic scleral remodelling (Meng et al., 2015) and plays a critical role in the remodelling process of myopic sclera, regulating the synthesis and degradation of Col-I through downstream signal transcription genes (McBrien, 2013; Li et al., 2016b). During myopic scleral remodelling, the expression of both TGF-β1 and Col-I decreases, and there is a positive correlation between the two (Liu et al., 2007). Specificity protein 1 (Sp1), a transcription factor involved in many cellular processes such as cell differentiation and proliferation (Wang et al., 2018), is a downstream target of TGF-β1 and can regulate the synthesis and degradation of Col-I (Jobling et al., 2004; Tarzemany et al., 2015). This regulatory effect may result from the targeted binding of Sp1 to specific regions on the structure of Col-I precursors. Jiang et al. (2017) showed that Sp1 and Col-I are expressed in the scleral tissue of a myopic guinea pig model with form deprivation. As the duration of eye covering and the severity of myopia increased, the protein and mRNA expression levels of Sp1 and Col-I exhibited a downward trend, with Sp1 expression showing a positive correlation with Col-I expression (Jiang et al., 2017).

Myopia results from both genetic and environmental factors (Chuang, 2017; Wang et al., 2017). Studies have shown that the light environment has a certain correlation with myopia (Morgan and Ashby, 2017; Torii et al., 2017), and the rising incidence of myopia rate may be associated with the introduction of artificial light sources (Prepas, 2008). Chickens, fish and guinea pigs are more prone to developing myopia under red light and hyperopia under blue light (Kröger and Fernald, 1994; Liu et al., 2011; Foulds et al., 2013; Jiang et al., 2014). Guinea pigs exhibit more severe axial elongation and myopia under 530 nm (green) light than under 480 nm (blue) illumination (Wang et al., 2011). These findings indicate that different monochromatic lights are closely associated with refractive development. Currently, artificial light primarily consists of multi-coloured light with various wavelengths, and there are various studies on the effects of artificial light with differing spectral composition on myopia. Outdoor natural light differs markedly from indoor artificial light in terms of illumination rhythm, stroboscopic effects and spectrum (Li et al., 2014). Outdoor activities have a good protective effect in preventing myopia (Sherwin et al., 2012; He et al., 2015; Wu, et al., 2018; Zadnik and Mutti, 2019), while the use of artificial light is a key factor in the formation of myopia, thereby affecting the development of the eyeball (Zhang et al., 2014). Our previous research has found that full-spectrum artificial light can increase serum and retinal dopamine (DA) levels and reduce retinal structural damage (Liu et al., 2015). Furthermore, we have found that continuous full-spectrum artificial light inhibits the elongation of the eye axis via the retinal dopaminergic and melanopsin systems (Xu et al., 2023). In addition, some models have explored the effects of spectral composition on the refractive development in animals (Hu et al., 2022; Muralidharan et al., 2022). These studies further confirm that light environments with varying spectral composition can induce differing refractive development and axial lengths in the eye, successfully establishing experimental myopia models.

At present, there are no reports on whether Sp1 is expressed in myopic sclera induced by light environments with different spectral composition, whether it is involved in the synthesis and degradation of Col-I, or the role of the TGF-β1 signalling pathway in myopic sclera remodelling. In response to this gap, this study used mixed light with varying spectral composition to irradiate guinea pigs, inducing formation of myopia models. Building on previous research, the study further explored the effects of artificial light with differing spectral composition on the growth of the guinea pigs' eye axes. Additionally, the expression of Sp1 and Col-I in the sclera and their relationship were observed, along with the potential role of the TGF-β1 signalling pathway in myopic sclera remodelling. Our findings provide new insights and directions for understanding the pathogenesis and prevention of myopia.

Material and Methods

Experimental animals and grouping

Thirty healthy three-week-old guinea pigs were purchased from Yizheng ANlimao Biotechnology Co. Ltd (Jiangsu, China). Excluding those with congenital myopia or other eye diseases, the guinea pigs were housed indoors under conditions of 22 °C, 55 % humidity and 12 h of light every day. The guinea pigs were provided with water, vitamin-enriched feed and fresh vegetables daily. Four types of artificial light sources, each composed of different spectra but with identical illuminance and irradiance, were used in this experiment to assess whether LED light based on the natural light (N light)

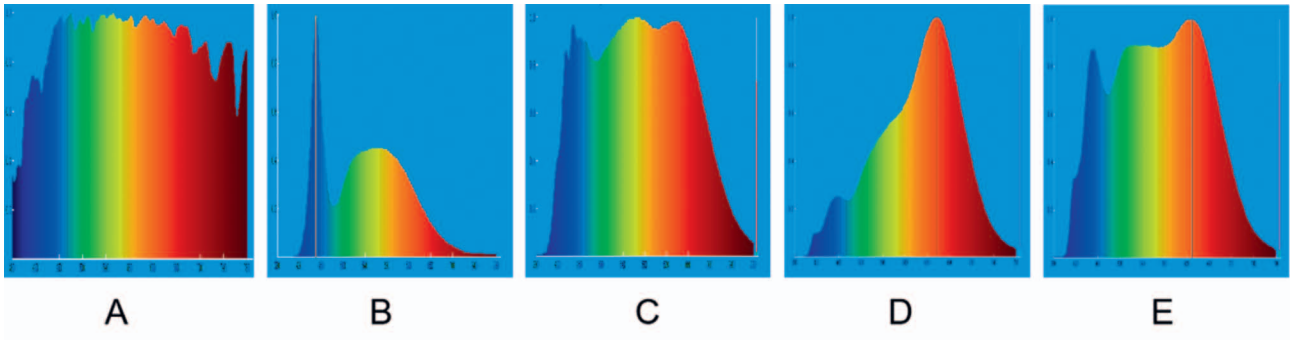

Fig. 1. The spectra of natural light and four artificial lights. (**A**) N light, (**B**) L light, (**C**) E light, (**D**) F light, (**E**) G light.

spectrum could protect axial development. An HR2000 fluorospectrophotometer (Oceatics Inc., Osaka, Japan) was utilized to measure the optical characteristics such as illuminance, irradiance and the spectral composition of the light. The 30 guinea pigs aged 21 days were randomly divided into five groups, with six guinea pigs in each group. Each group was exposed to a different light source, respectively: N light, LED light (L light) with a low colour temperature, E light, Fulia light (F light), and Gulia light (G light). The spectral characteristics of these five light sources have been described in our previous study (Yuan et al., 2024). The spectra of the five light groups are shown in Fig. 1, which references the figure published by Yuan et al. (2024). Except for group N, guinea pigs in all other groups were housed in cages with overhead lights and covered by black cloth. A device outside the cages automatically regulated the light illuminance and irradiance, synchronizing the lighting pattern with the natural light surroundings. The cage of the N light group was positioned near a south-facing window (quartz glass with sunlight), with only the top of the cage uncovered. In each group, the average light illuminance was set at 350 lux and the irradiance at 5W/m^2. In the N group, the distance between the window and the cage was adjusted based on weather conditions. These details are also described in our previous research (Xu et al., 2023; Yuan et al., 2024). The guidelines from the statement for the Use of Animals in Ophthalmic and Vision Research were followed in the handling and care of the animals, and the research procedure was approved by the Animal Protection and Ethics Committee of the Affiliated Hospital of Nanjing University of Chinese Medicine.

Analysis of ocular biology

A-scan ultrasonography (KN-1800; Kangning Medical Device Co. Ltd., Wuxi, China) was used to measure the guinea pigs' axial lengths before the start of the experiment and every two weeks thereafter. The detailed methods for measuring axial length have been described in detail in our previous research (Yuan et al., 2024) (Fig. 2).

Scleral histopathological observation

After 12 weeks of light exposure, the guinea pigs were euthanized via intraperitoneal injection of an overdose of sodium pentobarbital. All eyeballs were extracted, and the sclerae were separated and fixed in 4 % paraformaldehyde (PFA) for more than 24 h. After fixation, the treatment of the scleral tissue followed the procedures outlined in our previous studies (Yuan et al., 2024). Pathological changes in the sclerae were observed under an optical microscope (Axioplan 2 imaging; Carl Zeiss AG, Oberkochen, Germany).

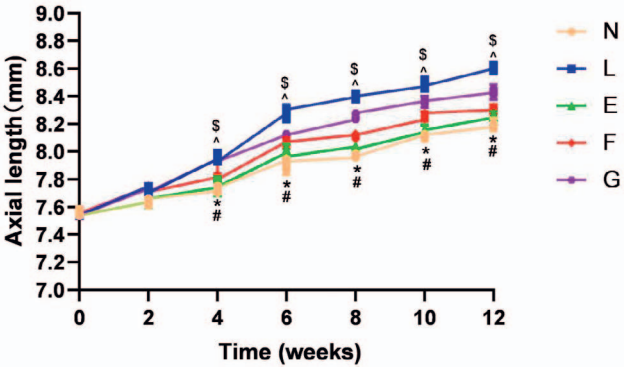

Fig. 2. Comparison of axial lengths at different time points under various spectrum light illumination (mm). After 4, 6, 8, 10, and 12 weeks of light exposure, the L and G groups showed considerably longer axial lengths than the N group ($^{*}P < 0.05$, $^{\#}P < 0.05$), with the L group exhibiting significantly longer axial lengths compared with the E and F groups ($^{\$}P < 0.05$, $^{\wedge}P < 0.05$).

Immunohistochemistry (IHC)

The experimental procedure was similar to that described in our previous study (Yuan et al., 2024). The sections were incubated overnight at 4 °C with anti-Sp1 (1 : 200; No. 21962-1-AP; Proteintech Group, Rosemont, IL) and anti-Col-I (1 : 200; No. 14695-1-AP; Proteintech Group) antibodies. They were then incubated for 2 h at room temperature with goat anti-rabbit immunoglobulin G that had been tagged with horseradish peroxidase (HRP) (IgG; 1 : 200; No. SA00001-2; Proteintech Group) for 2 h at room temperature. After colour development of the sections using diaminobenzidine (DAB), the sections

were counterstained with haematoxylin and rinsed with water. The slices were dehydrated with alcohol, anhydrous ethanol and xylene, and finally removed from xylene, dried slightly and sealed with neutral gum. The pre-test of positive and negative controls was performed before the experiment, and no false positives and false negatives were found. Image-Plus Pro software (Media Cybernetics Inc., Rockville, MD) was used to measure the average optical densities (OD) of five randomly selected visual fields taken from each image using a light microscope (Axioplan 2 imaging, Carl Zeiss AG; objective: 40×), and their means reflected the expression levels of scleral Sp1 and Col-I protein.

Western blot

The experimental procedure prior to the incubation of PVDF membrane was detailed in our previous study (Yuan et al., 2024). After washing the membranes, the PVDF membranes were incubated overnight at 4 °C with anti-Sp1 (1 : 5,000; No. ab308364; Abcam, Cambridge, UK), anti-Col-I (1 : 1,000; No. ab260043; Abcam) and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1 : 5,000; No. 10494-1-AP; Proteintech Group). Following washing with Tris-buffered saline with Tween 20 (TBST), the samples were incubated with HRP-labelled goat anti-rabbit IgG (1 : 5,000; No. SA00001-2; Proteintech Group) at room temperature for 1.5 h, followed by additional washing with TBST. Then, the membranes were exposed to HRP colour-developing substrate, and an automatic chemiluminescence analyser was used to detect and image the signals. The signals were semi-quantitatively analysed with Image-Plus Pro software (Media Cybernetics Inc., Rockville, MD). GAPDH was used as the internal reference protein.

RT-qPCR detection

The process of RT-qPCR detection was described in detail in our previous research (Yuan et al., 2024). Three replicates were prepared for each sample, and GAPDH was used as the internal control. The relative expression levels of the target gene mRNA were calculated using the $2^{-\Delta\Delta CT}$ method. The sequences (5'-3') of the primers (Shenggong Bioengineering Co. Ltd., Shanghai, China) were as follows: *Sp1*, CTCAAAGGAACAGAGTGGCA and GAGCTGGGAGTCAAGGTAGC; *ColI*, ACAAGCGATTACACACCCAA and TTAGTTTCCTGCCTCTGCCT; *GAPDH*, TCGCTCCTGGAAGATGGTG and TCATTGACCTCCAGTACATGG.

Statistical analysis

Normality and mean square error tests were performed before selecting appropriate statistical tests. The eye axes of guinea pigs were represented as mean ± standard deviation (SD). An independent samples *t*-test and one-way analysis of variance (ANOVA) were used to compare the mean values between two samples and the differences across groups, respectively. All statistical analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL). A P value of $P < 0.05$ was considered statistically significant, while a P value of < 0.01 was considered highly significant.

Results

Impact of varying light sources on axial lengths

Before the experiment, there was no considerable difference in the axial lengths among the groups ($P > 0.05$). The axial lengths of guinea pigs in each group increased with irradiation time. After 4 weeks of irradiation, guinea pigs in the L and G groups had axial lengths that were significantly longer than those in the N group, while guinea pigs in the E and F groups had axial lengths that were considerably shorter than those in the L group ($P < 0.05$). After 6, 8, 10, and 12 weeks of irradiation, the axial lengths of guinea pigs in the E and F groups were significantly shorter than those in the L group and were considerably longer in the L and G groups than those in the N group ($P < 0.05$). More detailed information on axial lengths can be found in our previous study (Yuan et al., 2024).

Histopathological morphology of the sclera

The distribution of scleral collagen fibres in groups N and E appeared relatively uniform, orderly and dense under the optical microscope. However, the collagen fibres in the other three artificial light groups (groups L, G, and F) were arranged in a disorganized manner with large gaps. The L group showed collagen fibre breakage (Fig. 3).

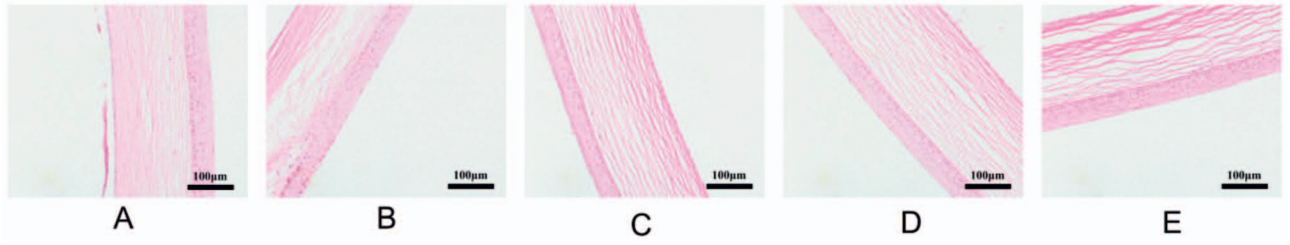

Fig. 3. Scleral structural changes caused by different lights. (**A**) N group, (**B**) L group, (**C**) E group, (**D**) F group, (**E**) G group. The distribution of scleral collagen fibres in N and E groups was more uniform, orderly and dense than in L, G, and F groups. Moreover, collagen fibre fracture was seen in the L group. Haematoxylin and eosin (HE) staining; Magnification 400×.

Expression of Sp1 and Col-I protein detected by IHC

Compared with the N group, the protein expression levels of Sp1 and Col-I in the scleral tissue of the four artificial light groups were considerably decreased ($P < 0.05$). The E, F, and G groups exhibited considerably higher levels of scleral Sp1 and Col-I protein expression compared with the L group ($P < 0.05$). Both the F and G groups showed considerably lower levels of scleral Sp1 and Col-I protein expression compared with the E group ($P < 0.05$). Additionally, the F group showed significantly higher levels of scleral Sp1 and Col-I protein expression than the G group ($P < 0.05$) (Figs. 4–5).

Expression of Sp1 and Col-I protein detected by WB

The protein expression levels of Sp1 and Col-I in the four artificial light groups were considerably lower than those in the N group ($P < 0.05$). Compared with the L group, the E, F, and G groups showed significantly higher levels of scleral Sp1 and Col-I protein expression ($P < 0.05$). Both the F and G groups had considerably lower levels of scleral Sp1 and Col-I protein expression than the E group ($P < 0.05$). Additionally, the F group exhibited significantly higher levels of scleral Sp1 and Col-I protein expression compared with the G group ($P < 0.05$) (Fig. 6).

Expression of Sp1 and Col-I mRNA detected by RT-qPCR

Compared with the N light group, the mRNA expression levels of scleral Sp1 and Col-I in the four artificial light groups were considerably lower ($P < 0.05$). The levels of scleral Sp1 and Col-I mRNA expression in the E and F groups were significantly higher than in the L group, and the levels of Col-I mRNA expression in the G group were also considerably higher than in the L group ($P < 0.05$). However, the difference in Sp1 mRNA expression between the L and G groups was not statistically significant ($P > 0.05$). Compared with the E group, the F and G groups exhibited considerably lower levels of scleral Sp1 and Col-I mRNA expression ($P < 0.05$). Additionally, the F group exhibited significantly higher levels of scleral Sp1 and Col-I mRNA expression than the G group ($P < 0.05$) (Fig. 7).

Discussion

Myopia, particularly common in Asia, is a global health issue (Wen et al., 2013; Wu et al., 2016). The light environment has a certain correlation with myopia (Morgan and Ashby, 2017; Torii et al., 2017), and the rising prevalence of myopia rate may be associated with the advent of artificial light sources (Prepas, 2008). Many previous studies have confirmed that different monochromatic lights are closely linked to refractive

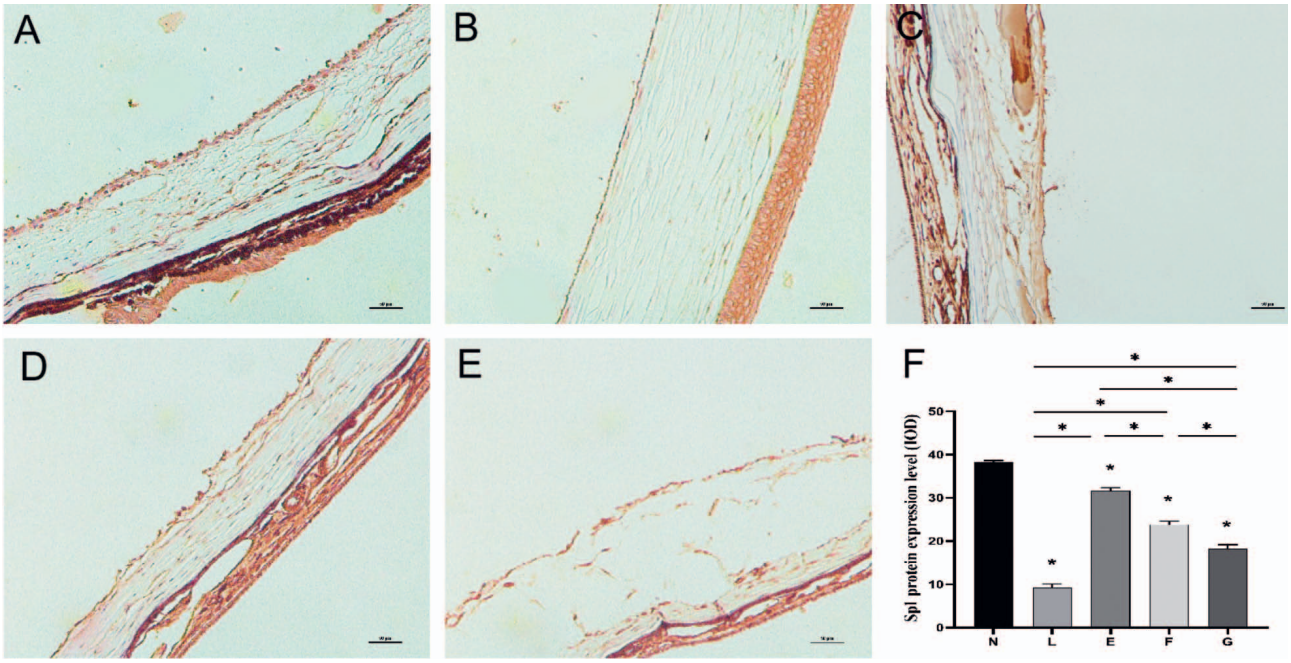

Fig. 4. Microphotographs of Sp1 protein expression in guinea pig scleral tissue after a 12-week exposure to different light sources. The Sp1 protein is stained brown. The expression of Sp1 protein in the five light source groups from high to low were N, E, F, G, and L. Pairwise comparison was statistically significant ($P < 0.05$). (**A**) N group, (**B**) L group, (**C**) E group, (**D**) F group, (**E**) G group, (**F**) the relative Sp1 protein expression levels in each group. $^{*}P < 0.05$.

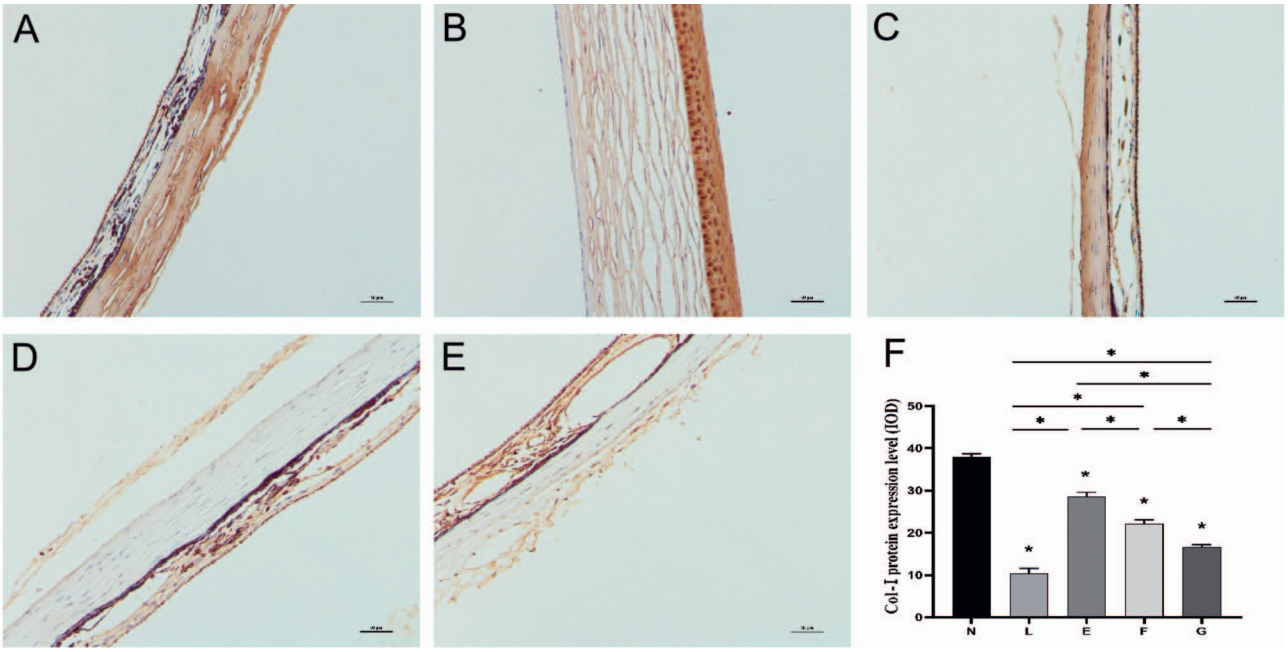

Fig. 5. Microphotographs of Col-I protein expression in guinea pig scleral tissue after a 12-week exposure to different light sources. The Col-I protein is stained brown. The expression of Col-I protein in the five light source groups from low to high were L, G, F, E, and N. Pairwise comparison was statistically significant ($P < 0.05$). (**A**) N group, (**B**) L group, (**C**) E group, (**D**) F group, (**E**) G group, (**F**) the relative Col-I protein expression levels in each group. $^{*}P < 0.05$.

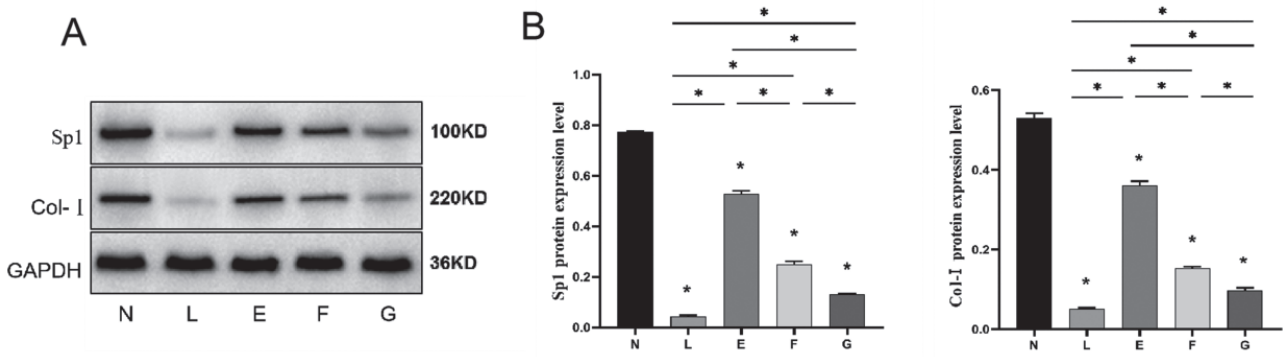

Fig. 6. (**A**) Levels of Sp1 and Col-I protein expression detected by Western blot (WB), (**B**) the relative expression levels of Sp1 and Col-I proteins in each group were adjusted to GAPDH, as shown in the figure. The expression levels of scleral Sp1 and Col-I proteins in the five light source groups from high to low were N, E, F, G, and L. $^{*}P < 0.05$.

development (Kröger and Fernald, 1994; Seidemann and Schaeffel, 2002; Liu et al., 2011; Wang et al., 2011; Foulds et al., 2013; Jiang et al., 2014). Guinea pigs are ideal animals for experimental myopia models owing to their docile temperament, easy coordination, rapid visual development, large eyeballs, low cost and high fecundity. Our previous studies have found that full-spectrum artificial light may prevent elongation of the ocular axis through the melanopsin and dopaminergic systems in the retina, providing good protection against myopia (Xu et al., 2023). However, we did not assess whether blocking the melanopsin system would inhibit this protective response. Therefore, we plan to conduct future studies involving ablation of intrinsically photosensitive retinal ganglion cells (ipRGCs) or melanopsin knockout models. Scleral remodelling plays a crucial role in the development of myopia, which is characterized by changes in the refractive state and ocular morphology, especially excessive elongation of the ocular axis (Liu et al., 2017; Hu et al., 2018).

Melanopsin plays a key role in the non-image-forming visual system, circadian rhythm and pupillary reflex,

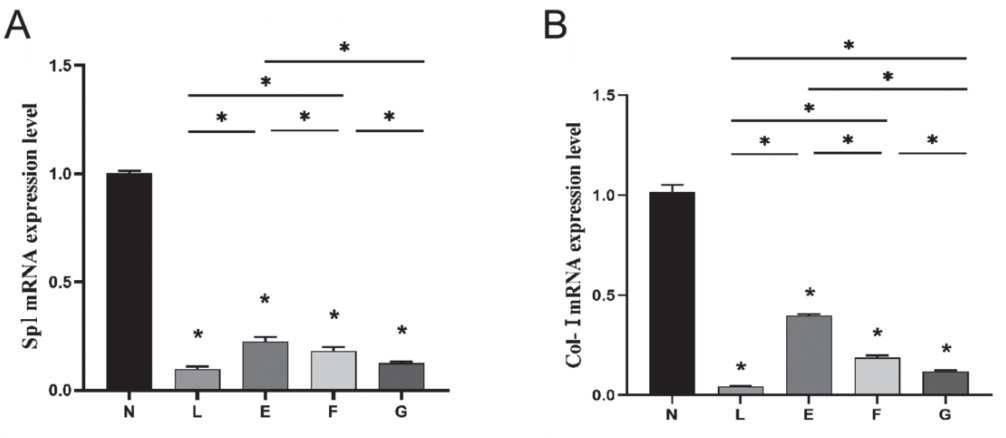

Fig. 7. Levels of scleral Sp1 and Col-I mRNA expression were detected by RT-qPCR. (**A**) Sp1 mRNA expression level, (**B**) Col-I mRNA expression level. The expression levels of scleral Sp1 and Col-I mRNA in the five light source groups from high to low were N, E, F, G, and L. $^{*}P < 0.05$.

with a peak absorption at 479 nm (Hannibal et al., 2013). Melanopsin drives the day-night cycle of the retina and pineal organs and affects the retinal DA and melatonin cycles (Xu et al., 2023). However, the specific mechanism by which melanopsin influences refractive development under varying light exposure remains unclear (Yuan et al., 2024). Our previous studies have found that the expression of retinal melanopsin varies under different lighting conditions, and the group comparisons showed consistency with the axial length, suggesting that melanopsin may play a role in refractive development (Xu et al., 2023). Melanopsin is more responsive to blue wavelengths. However, differences in experimental methods used to study melanopsin can lead to inconsistent results (Yuan et al., 2024). Liu et al. (2022) found that in the absence of light at 480 nm, melanopsin activation in mice was reduced, considerably decreasing the impact of form deprivation on myopia. Chakraborty et al. (2022) found that after three weeks of form deprivation, opn4$^{-/-}$ mice developed more severe myopia than opn4$^{+/+}$ mice. Retinal damage can be caused by the blue spectral region, which also directly affects the capacity of RGC mitochondria (Osborne et al., 2017). In this study, after 6, 8, 10, and 12 weeks of light exposure, guinea pigs in the L and G groups had considerably longer axial lengths ($P < 0.05$) than those in the N group, whereas the guinea pigs in the E, F, and G groups had considerably shorter axial lengths ($P < 0.05$) than those in the L group. Notably, the E group had the shortest axial length among the four artificial light groups. These results may be attributed to the differences in spectral characteristics. The natural light spectrum is continuous, without peaks or valleys. LED light with a low colour temperature shows a blue peak at 430–460 nm and a blue valley at 480 nm. In the range of ~390–780 nm, E light has a continuous spectrum with a profile similar to F and G lights. G light exhibits a blue peak at 450 nm and a valley at 480 nm.

Although F light is filtered below 400 nm, its spectrum remains continuous, without blue peaks or valleys. Therefore, the peaks and valleys in the mixed light spectra have a significant impact on ocular axial growth. Continuous E light, without peaks or valleys, exerts a significant inhibitory effect on axial elongation, consistent with our previous research results (Xu et al., 2024; Yuan et al., 2024). However, the total duration of our study was only 12 weeks. In future studies, we will conduct more extensive studies on myopia progression and scleral remodelling in guinea pigs beyond the 12-week period.

Strong light suppression may account for our findings. The peak light of the light source exerts notable light suppression on the visual cells, preventing us from perceiving lower light levels. This results in the formation of incoherent multi-focal planes. This phenomenon continuously generates defocusing signals that differ from those produced by natural light, contributing to the elongation of the eye axis and a shift towards myopia (Gawne et al., 2017; Xu et al., 2023). The blue peak at 450 nm in the L and G groups suppressed the blue valley at 480 nm, further contributing to the formation of incoherent multi-focal planes and axial lengthening. However, the mechanisms by which light influences refractive development are complex, and further research is needed.

During myopic scleral remodelling, the diameter of collagen fibre bundles decreases, and the fibre gaps increase (Wang et al., 2021). In this study, the distribution of scleral collagen fibres in the groups N and E was relatively uniform, orderly and dense under the optical microscope. However, in the other three artificial light groups (groups L, G, and F), the collagen fibres were distributed in a disorderly manner with large gaps. The L group showed collagen fibre breakage. Our study showed that different spectral artificial light sources can induce pathological remodelling of scleral collagen fi-

bres during myopia, with the changes in scleral collagen remodelling induced by E light being the least pronounced among all treatments. TGF-β plays a key role in the synthesis and degradation of ECM components (Frangogiannis, 2020), and the expression of all three subtypes decreases during scleral remodelling (Li et al., 2016b; Yuan et al., 2018). *In vitro* studies have confirmed that TGF-β1 is the growth factor that most effectively enhances the proliferative capacity of scleral fibroblasts among the three subtypes, and it regulates the synthesis and degradation of Col-I through downstream signalling transcription genes (McBrien, 2013; Li et al., 2016b). Studies have shown that as myopia progresses, the expression levels of TGF-β1 and Col-I are gradually down-regulated during the synthesis and degradation of myopic scleral collagen, and there is a certain positive correlation between them (Li et al., 2016b).

Sp1 is expressed in all mammalian cells and regulates a variety of cellular processes, including the cell cycle processes, proliferation, growth, metabolism and apoptosis (Beishline et al., 2015; Hellweg et al., 2016). Sp1 functions as a downstream factor of TGF-β1 (Li et al., 2016b). During gum wound healing, TGF-β1 regulates the expression of connexin 43 in fibroblasts through transcription factors such as Sp1 (Tarzemany et al., 2015). Studies by Martin-Gallausiaux et al. (2018) have shown that TGF-β1 can bind to transcription factor Sp1 and activate it. Li et al. (2016a) found that the TGF-β1 signalling pathway plays a role in regulating fibrosis. Sp1 is activated by TGF-β1 and promotes the expression of Col-I (Jiang et al., 2017).

In the field of ophthalmology, there are limited reports on the expression and role of Sp1 and Col-I in myopic scleral remodelling. Yu et al. (2015) showed that miR-29b inhibited proliferation of follicular fibrocytes through the Sp1-collagen I signalling pathway following glaucoma surgery, leading to good filtration function and stable intraocular pressure. Jiang et al. (2017) found that Sp1 may regulate Col-I in the process of myopic scleral remodelling. At present, no studies have reported the expression and effects of Sp1 and Col-I in the sclera of experimental myopic models induced by artificial light of different spectra. Therefore, we conducted this study. Guinea pigs were euthanized after 12 weeks of experimentation, and IHC, WB and RT-qPCR were used to detect the protein and mRNA expression levels of Sp1 and Col-I in the sclera of the guinea pigs. The results showed that the expression of scleral Sp1 and Col-I in the N group and the four artificial light source groups, from highest to lowest, was as follows: N group, E group, F group, G group, and L group. The expressions of Sp1 and Col-I were positively correlated with each other but negatively correlated with the axial length. The findings suggest that continuous full-spectrum E light, without peaks and valleys, results in the shortest eye axis and the lowest degree of myopia among the four artificial light groups. Sp1 and Col-I may be involved in the process of myopic scleral remodelling. Sp1 may bind to specific regions on the chain of Col-I precursor component Col1α1, thereby influencing the synthesis and degradation of Col-I and regulating scleral remodelling.

In summary, artificial light with varying spectral composition affects the axial eye length, inducing formation of myopia models. Artificial light sources with a full spectrum that simulates natural light can inhibit the elongation of the eye axis in young guinea pigs, demonstrating a protective effect against myopia. Both Sp1 and Col-I were expressed in varying amounts during myopic scleral remodelling. The expressions of Sp1 and Col-I were positively correlated with each other but negatively correlated with the axial length. The protein and mRNA expression levels of Sp1 and Col-I in the E light group, characterized by a continuous spectrum without peaks and valleys, were higher than in the other artificial light groups. There may be a possible relationship between Sp1 and Col-I with the TGF-β1-Sp1-collagen I signalling pathway, potentially playing a key role in myopic scleral remodelling. In the future, we aim to further investigate the progression of myopia and scleral remodelling in guinea pigs after 12 weeks of exposure to different light sources. We will also study the dynamic expression changes of Sp1 and Col-I and their relationship over different time points, providing new insights into the pathogenesis, prevention and treatment of myopia.

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Conflicts of interest

All authors swear to the accuracy of the work reported here and state that they have no competing interests.

Ethics approval

The Animal Care and Ethics Committee of the Affiliated Hospital of Nanjing University of Chinese Medicine gave its approval for this study (2022DW-49-01).

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