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## Research Article

# Comparison of Scleral Thickness and Fibroblast in Form Deprivation Animal Model (Experimental Study of Wistar Rat)

**Rini Kusumawar Dhany[1]**

**Gatut Suhendro[2]**

[1] Ophthalmology Department, Faculty of Medicine, Wijaya Kusuma University, Surabaya
[2]Vitreoretina Division, Ophthalmology Department, Faculty of Medicine, Airlangga University, Surabaya

**Abstract:**

**Background : Form deprivation (FD) is an effective approach to induce animal models of myopia. It disrupts the normal growth process, induces rapid axial elongation, and results in myopia in many species. In this investigation, three weeks old rat was deprived of form vision for 4 weeks and then compared to the fellow, non-deprived eye by histopathology of scleral thickness and fibroblast.**

**Methods : Ten of male three-weeks-old wistar rat weighing 200–300 g were obtained from the Airlangga University Laboratory Animal Center (Surabaya, Indonesia). All animals were examined clinically for confirming the corneal transparency of each eye and no injuries or infections of the eyes. Refractive errors were induced monocularly by covering right eye with adhesive tape in one group for 4 weeks which was named the FDM group. After enucleated under deep anesthesia, histopathology of scleral thickness and fibroblast was observed.**

**Results : There were no differences in baseline axial length variance among the groups (F = 1.006, *P* = .413). Statistical analysis using t-test, scleral thickness in the treatment group was reduced compared with the control group but the difference was not statistically significant (*p* =0.443; *p* > 0.05). Scleral fibroblast in the treatment group was reduced compared with the control group but the difference was not statistically significant (p= 0.990; *p* > 0.05). Logistic regression analysis showed that association of form deprivation myopia, scleral thickness and scleral fibroblast was not significant (p=0.997)**

**Conclusion : The scleral thickness and scleral fibroblast at conclusion of our study were reduced but not significant than in the control group. These results not validate the FDM model, further study with more samples and longer FDM periods is necessary.**

**Keywords: myopia, scleral thickness, fibroblast**

### Introduction

Myopia, the most prevalent refractive error, affects about 15–38.7 % of the population, and poses a significant public health burden and cost to society. The excessive axial eye size, especially the vitreous chamber elongation, is the most important determinant factor of this condition. The longer the axial length, the more severe the myopia. Form deprivation (FD) is an effective approach to induce animal models of myopia. It disrupts the normal growth process, induces rapid axial elongation, and results in myopia in many species.

A major advantage of the mouse is that it is a well-established animal model for a range of human diseases with a wealth of knowledge on its biochemistry and genetics. There is no other mammalian disease model that is available in so many transgenic versions, in which genes have been intentionally altered or inactivated. Further advantages are that the mouse can be easily obtained and bred. Disadvantages of the mouse model include small eye size (around 3.3 mm axial length), have poor optics, no accommodation and no fovea. Experimental manipulations of their visual experience with diffusers or spectacle lenses are demanding and the induced changes in refraction and ocular growth are slow to develop in comparison to chickens and guinea pigs. In contrast to guinea pigs, alert mice are not easy to handle and most researchers have experienced their sharp teeth. The importance of vision may have been underestimated in mice, since it was assumed that they are 'nocturnal'; however, direct observations of their diurnal activity, both of laboratory mice and mice in the wild, show that they are not truly nocturnal. Mouse spatial vision is relatively poor (0.5 to 0.6 cycles/degree, at least 60 times worse than that of humans) but this is not due to the size of the eye alone. Most recently, it was shown that mice lacking rods by functional knockout due to a mutation in the rod transducin alpha do not develop deprivation myopia. This could indicate that rods are implicated in the emmetropisation process, which may not be too surprising, given that emmetropisation in primates appears to be largely driven by the peripheral retina, where rods are predominant. The wide range of different findings on myopia in the mouse model were thoroughly

summarized by Pardue, Stone and Iuvone. A surprising issue in mice is that the effects on refraction are generally quite small. With a total power of the optics of the mouse eye above 500 D, the changes are typically only five dioptres or less, which is only one per cent of the total power, while in the chicken with a power of 140 D, 20 D of myopia can easily be induced, which is around 15 per cent of the power of the eye. It is not fully clear what this means. Similar neurochemical events appear to be involved in the development of myopia in chicks and mammals. In this investigation, three weeks old rat was deprived of form vision for 4 weeks and then compared to the fellow, non-deprived eye by histopathology of scleral thickness and fibroblast.

**Materials and methods**

**Laboratory animals and groupings**

Male three-weeks-old wistar rat weighing 200–300 g were obtained from the Airlangga University Laboratory Animal Center (Surabaya, Indonesia). All animals were examined clinically for confirming the corneal transparency of each eye and no injuries or infections of the eyes. A random number table was used to assign animals to the following 2 groups of 5 animals each: control, and treatment groups. The animals were housed at 22°C–28°C under natural light/dark cycle with consistent ventilation, and were provided with tap water, and standard food. All procedures were approved by the Animal Care and Ethics Committee at Airlangga University Laboratory Animal Center (Surabaya, Indonesia).

**Experimental protocol and induction of form-deprivation myopia**

Refractive errors were induced monocularly by covering right eye with adhesive tape in one group of mice which was named the FDM group. (fig.1). The left eyes of mice untreated served as a contralateral eye control group. The covered eye were checked regularly to ensure they remained in proper position. Axial length measurements was recorded as the mean value of A-scan biomtery (BME-210 A/B Ophthalmic Ultrasound System, Tomey, Japan) on three repeated measurements.

**Scleral tissue morphologic observations**

Right eyes of all animals were enucleated under deep anesthesia (1000 mg/kg 20% urethane sodium injected intraperitoneally), and one right eye from each group was randomly selected. Sclera were removed on a cold clean working table by careful dissection. Right eyes of each treatment group were removed with 1–2-mm optic nerve tissue with a small limbus triangle incision and put in an ethanol–formaldehyde–acetic acid solution for 1 week of fixation. The ocular tissue on the coronal plane containing the optic nerves was removed with sections 3–5 μm in thickness. After gradient ethanol dehydration and paraffin embedding, three 4-μm sections were made, and one was used for HE staining.

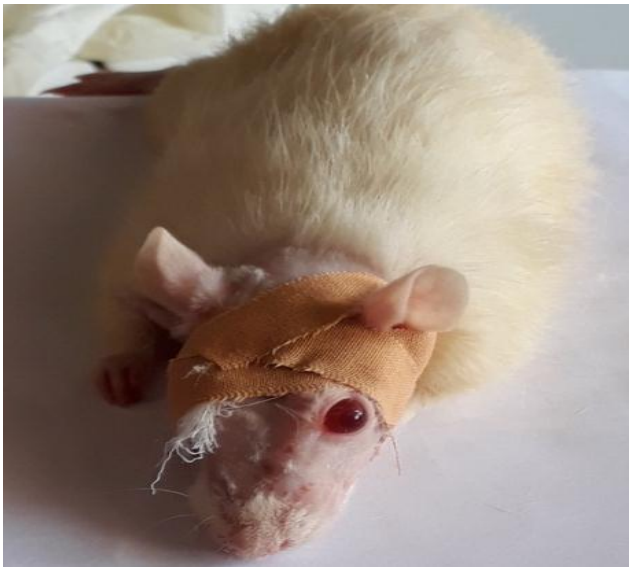

Fig. 1. Right eye occlusion of FDM group

Each layer of the ocular tissue structure was observed under a low power field (×100) light microscope, and the scleral thickness, the quantity of scleral fibroblasts were observed under a medium power field (×200). Ten visual fields of sclera at both ends of the optic disk were used for semi-quantitative measurements of scleral full-thickness in each group using an Nikon image analysis system (NIS), 10 times for each visual field. To exclude the impact of artificial fissures created during preparation of the tissue slices on scleral thickness measurements, the above-mentioned 10 visual fields of scleral tissue full-thickness areas were measured again. Taking the sum of 10 visual fields within the measured area expresses the thickness of sclera. The integral optical density (IOD) of fiber cell nuclei in these 5 visual fields inside the scleral tissue was also measured twice. The sum of the fiber cell nuclei IOD in the 5 visual fields showed the quantity of fibroblasts.

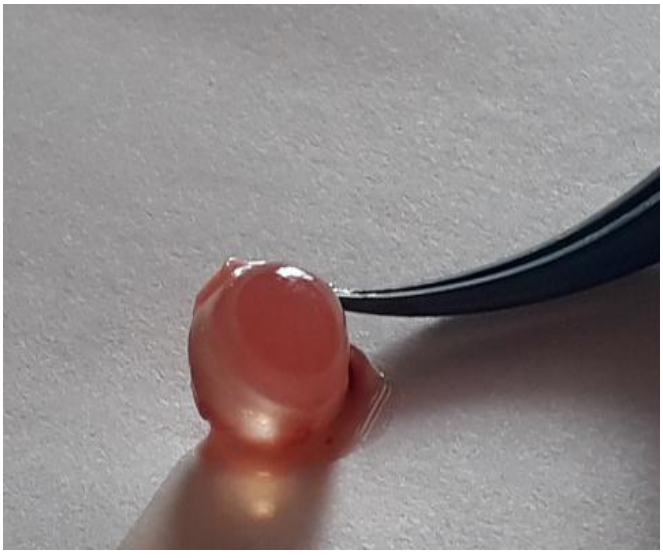

Fig. 2. Enucleated eye

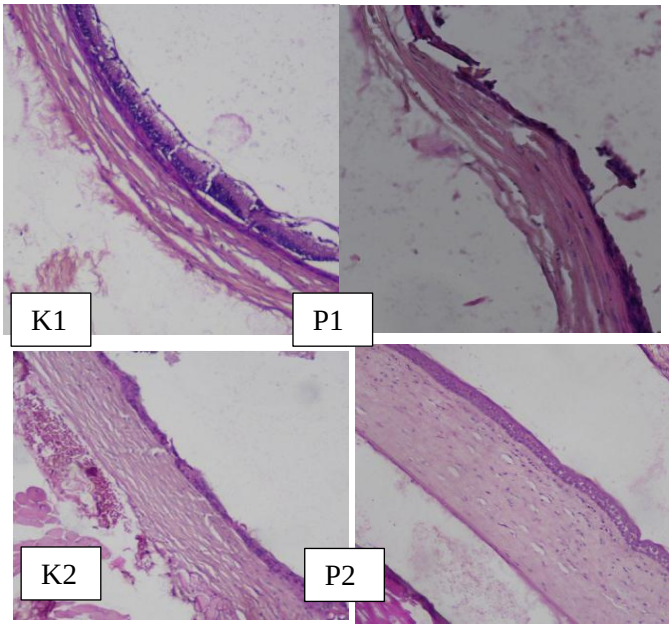


Fig. 3 Scleral histomorphology among groups. K1K2: In the control group, the scleral collagen fibers were aligned in an orderly manner and fibroblast arrangement was uniform. P1P2: In the treatment group, the scleral collagen fibers were disordered and fibroblasts were decreased and disordered.

**Statistical analysis**

Data were processed using SPSS17.0 statistical software (IBM, Armonk, NY, USA) and are expressed as mean (SD). Normal distribution and Levene's homogeneity test of variances were also performed. Scleral thickness and fibroblast was compared among groups before and after treatment using the paired *t*-test, and changes in dioptre and the axial length among groups were analysed by one-way ANOVA. The homogeneity of variances was measured with the least significant difference test, and the heterogeneity of variance was measured by Dunnett's test; $P < .05$ indicated differences between samples were statistically significantly

**Results**

From total 10 wistar rats sample, 1two rats were died. So there was 3 rat or six eyes samples left.

Table 1. Scleral Thickness and Fibroblast Count

| | Scleral thickness (µm) | fibroblast (per 5 visual field) |
|---|---|---|
| control 1 (K1) | 42.07 | 302 |
| control 2 (K2) | 46.01 | 363 |
| control 3 (K3) | 59.20 | 215 |
| treatment 1 (P1) | 41.21 | 293 |
| treatment 2 (P2) | 35.25 | 360 |
| treatment 3 (P3) | 47.89 | 208 |

There were no differences in baseline axial length variance among the groups ($F = 1.006$, $P = .413$). Hematoxylin and eosin stained slices of rat right sclera tissue were observed under a microscope. In the control group, scleral thickness was moderate, fibroblasts were apparent and had an even distribution, and the scleral fibroblasts and fiber alignment were arranged in an orderly manner (Fig. 1A). Scleral thickness in the treatment group was reduced compared with the control group but the difference was not statistically significant ($P > .05$). Scleral fibroblast in the treatment group was reduced compared with the control group but the difference was not statistically significant ($P > .05$)

Table 2. Statistic Analysis

| Variable | T-Test sig | Logistic regression sig |
|---|---|---|
| Scleral thinning | 0.443 | 0.997 |
| fibroblast | 0.990 | 0.997 |
| constant | | 0.997 |

**Discussion**

Reduced scleral collagen content is associated with the formation of myopia. This study used 3-week-old wistar rat in which FDM was induced in the right eye. The sclera plays an important role in maintaining the axial length and the depth of the vitreous chamber during the formation of myopia. Previous studies have shown that in human myopia, the sclera thins, the collagen content decreases significantly, the diameter and tissue of collagen fibbers alter, and the dry weight of the sclera decreases. In mammals, the sclera is mainly composed of a small number of fibroblasts and a large amount of ECM. Collagen is the main component of the ECM and accounts for 90% of the dry weight of the sclera and is produced by fibroblasts. Scleral fibber is mainly made up of collagen I and small amounts of collagens III and IV. It is widely recognized that genetic and environmental factors cause abnormal production and degradation of scleral collagen, causing scleral thinning. When the sclera becomes too weak for normal intraocular pressure and expansion it leads to excessive axial extension. In the induced myopia model, collagen synthesis is reduced when mitotic activity of fibroblasts is diminished. During scleral remodelling collagen I expression decreases significantly, but the degradation of collagen fibbers and ECM increases. ECM is mainly synthesized by scleral fibroblasts.

**Conclusion**

The scleral thickness and scleral fibroblast at conclusion of our study were reduced but not significant than in the control group. These results not validate the FDM model, further study with more samples and longer FDM periods is necessary.

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