Intrascleral Fixation of Intraocular Lens Haptics: Histological Advantages in a Comparison with Scleral Suture Fixation in Rabbits

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ABSTRACT:

Purpose: To disclose histological advantages of intrascleral fixation of intraocular lens haptics, in comparison with scleral suture fixation, in a study with rabbits.

Methods: Ten white rabbits, 10 weeks of age, were used in this experimental histopathological study. After unilateral lensectomy and anterior vitrectomy, an intraocular lens haptic was inserted into one eye of each rabbit. Intrascleral fixation was performed in five rabbits, whereas scleral suture fixation was performed in the others. At postoperative 1 week, the globe was enucleated in two rabbits in each group; at postoperative 8 weeks, the globe was enucleated in the remaining three rabbits in each group. Sections of the sclera around haptics and sutures were evaluated with haematoxylin and eosin, and immunohistochemical staining methods. We assessed severity of inflammation on histopathological photos, taken near the haptic or suture in the sclera, by counting white blood cells. The Mann-Whitney U test was performed to analyse differences in the severity of inflammation between the groups.

Results: Samples in the intrascleral fixation group demonstrated reduced irregularity of collagen fibres; reduced infiltration of fibroblasts, giant cells, lymphocytes, neovascular cells, neutrophils, and eosinophils; and weaker staining for fibronectin (indicating tissue repair) and heat shock protein 70 (indicating cell damage). In addition, reduced white blood cell infiltration was observed in the intrascleral fixation samples at 8 weeks in both shallow sclera ($p=0.001$) and deep sclera ($p=0.002$).

Conclusions: Histological analysis showed that intrascleral fixation caused fewer inflammatory changes than scleral suture fixation, with reduced fibroblast migration and production of cytotoxic factors.

Keywords:

Intrascleral fixation; Scleral suture fixation; Histology; Inflammation

INTRODUCTION:

Intrascleral fixation and scleral suture fixation are currently the most popular techniques for posterior chamber intraocular lens (IOL) fixation. The intrascleral fixation technique is simple, but has achieved good results for correcting IOL tilt, decentration, and optical properties [1-4]. Subconjunctival haptic exposure is a major complication, although it is rare [3-5]. Scleral suture fixation was frequently performed before the popularization of the intrascleral fixation technique. The scleral suture technique also provides satisfactory postoperative outcomes. However, transscleral suturing is comparatively difficult and suture exposure is occasionally encountered in connection with this procedure [6,7].

Subconjunctival haptic and suture exposures are suggestive of tissue reaction to the haptic and suture, following inflammation [5,6]. Inflammation around the suture and haptic also carries a potential risk for cystoid macular oedema and suture erosion. However, no comparative histologic study of inflammation has been reported regarding intrascleral and scleral suture fixation techniques. The purpose of the present study was to characterise the histological advantages of the intrascleral fixation technique, in comparison with the scleral suture fixation technique, using rabbit eyes.

MATERIALS AND METHODS:

The Institutional Review Boards of Kozawa Eye Hospital (no. 011) and Dokkyo Medical School (no. 478) approved this study. This study was performed in accordance with the National Institutes of Health Guidelines on the Care and Use of Laboratory Animals in Research and the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

Ten female Japanese white rabbits, 10 weeks of age (body weight, 2.3–2.5 kg) were used in this experimental histopathological study. Only the right eye was used in each rabbit to avoid the issue of doubling number. All rabbits were maintained under identical conditions at Dokkyo Animal Center.

All surgeries were performed by a single surgeon (T.H.). The dorsotemporal area of the sclera was used. Intrascleral fixation and scleral suture fixation of IOL haptics were performed under general anaesthesia. IOL haptics were composed of polyvinylidene difluoride (PVDF) (NX-60; Santen Co., Ltd., Osaka, Japan). To avoid surgical complications, only haptics without IOLs were used for both surgeries.

In the intrascleral fixation group, each haptic was embedded in a scleral pocket made with a 25-gauge microvitreoretinal blade without a suture (Fig. 1a) [1,2]. In the scleral suture fixation group, each haptic was transsclerally fixed with a 9-0 polypropylene suture without a scleral flap. The suture was tied to the sclera under the conjunctiva (Fig. 1b) [7]. At the completion of each surgery, the corneal incision was sutured with 10-0 nylon.

Methylprednisolone acetate 0.1% (Neo-Medrol® EE ointment; Pfizer Japan Inc., Tokyo, Japan) was applied to the eye just after surgery. Betamethasone sodium phosphate 0.1% (Rinderon® ophthalmic solution; Shionogi Co., Ltd., Osaka, Japan), tropicamide (Mydrin®-P ophthalmic solution; Santen Co., Ltd., Osaka, Japan), levofloxacin hydrate 0.5% (Cravit® ophthalmic solution; Santen Co., Ltd., Osaka, Japan), and bromfenac sodium hydrate 0.1% (Bronuck® ophthalmic solution; Senju Pharmaceutical Co., Ltd., Osaka, Japan) were applied twice daily for 2 weeks after surgery. Two rabbit eyes in each group were enucleated at 1 week postoperatively; the remaining three rabbit eyes in each group were similarly enucleated at 8 weeks postoperatively. All rabbits were euthanised after enucleation.

Whole eyes were fixed for 24 hours in 10% formalin in 0.1 M phosphate buffer; gradually dehydrated in 70%, 80%, 90%, and 100% ethanol, with changes every 4 hours; then cleared with xylene until transparent, and embedded in paraffin (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Haptics with the corresponding sclera were excised and cut into 4-μm thick sections. Sections were stained with haematoxylin and eosin (HE). To evaluate inflammatory changes, immunohistochemical staining for fibronectin (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and heat shock protein 70 (HSP70; Enzo Life Science, Inc., Farmingdale, NY, USA) was performed.

We assessed the severity of inflammation in the histopathological samples by counting white blood cells (WBCs). We used 10 digital photographs of a 400 × 400-pixel square of the HE-stained specimens at 20× magnification, taken near the haptic or suture to the sclera. The sclera was divided into two areas for analysis: a shallow half and a deep half. ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA) was used to count WBCs.

Statistical analysis was performed with the Mann-Whitney U test or the Kruskal-Wallis one-way analysis of variance. P values of < 0.05 were considered to indicate statistical significance.

RESULTS:

1. Histopathological Findings of Enucleated Specimens 1 week after Surgery with HE Staining:

***a. Intrascleral fixation*:**

Slightly irregular arrangement of the collagen fibre layer was observed in the shallow sclera under low magnification (Fig. 2a). High magnification images revealed a few giant cells and fibroblasts around haptics in the shallow layer (Fig. 2b). In the deep scleral layer, slightly irregular arrangement of collagen fibres was observed at low magnification (Fig. 2c). On high magnification images around the haptics, a few giant cells, fibroblasts, and neovascular vessels were observed in the deep sclera (Fig. 2d).

b. Scleral suture fixation:

Markedly irregular arrangement of collagen fibres was observed around the sutured haptics under low magnification in the shallow sclera (Fig. 3a). Under high magnification, many fibroblasts, giant cells, neovascular vessels, and lymphocytes were observed around the suture near the ciliary sulcus, indicating a strong inflammatory reaction (Fig. 3b). In the deeper scleral layer, a slightly irregular collagen fibre arrangement was found around the sutured haptics in low-magnification images (Fig. 3c). A few fibroblasts and lymphocytes were observed around the suture under high magnification in this layer (Fig. 3d).

2. Histopathological Findings of Enucleated Specimens at 8 weeks after Surgery with HE Staining:

a. Intrascleral fixation:

A slightly irregular arrangement of the collagen fibre layer of the shallow sclera was observed under low magnification (Fig. 4a). A few fibroblasts and giant cells were present around the haptics in the shallow layer under high magnification (Fig. 4b). In the deep sclera, a slightly irregular arrangement of the collagen fibres was observed under low magnification (Fig. 4c). A few fibroblasts and lymphocytes were found in this layer under high magnification (Fig. 4d).

b. Scleral suture fixation:

Many neutrophils, lymphocytes, eosinophils, giant cells, fibroblasts, and neovascular vessels were present around the suture penetrating area in the shallow sclera, under low magnification (Fig. 5a). A few fibroblasts and lymphocytes were illustrated around the suture in the shallow sclera under high magnification (Fig. 5b). A slightly irregular arrangement of collagen fibres was observed around the suture in the deep sclera (Fig. 5c). A few fibroblasts and lymphocytes were detected around the suture under high magnification at this level (Fig. 5d).

3. Findings of Enucleated Specimens at 1 week after Surgery with Immunohistochemical Staining for Fibronectin:

a. Intrascleral fixation:

Slightly positive staining was observed around the haptics (Fig. 6, upper-left).

b. Scleral suture fixation:

Strongly positive staining (dark brown) was observed around the suture (Fig. 6, lower-left).

4. Findings of Enucleated Specimens at 8 weeks after Surgery with Immunohistochemical Staining for Fibronectin:

a. Intrascleral fixation:

Mildly positive staining was observed around the haptics in the shallow (Fig. 6, upper-middle) and deep layers (Fig. 6, upper-right) of the sclera.

b. Scleral suture fixation:

Mildly positive staining was observed around the suture in the shallow (Fig. 6, lower-middle) and deep layers (Fig. 6, lower-right) of the sclera.

5. Findings of Enucleated Specimens at 1 week after Surgery with Immunohistochemical Staining for HSP70:

a. Intrascleral fixation:

Slightly positive staining was observed around the haptics (Fig. 7, upper-left).

b. Scleral suture fixation:

Strongly positive HSP70 staining (light brown) was observed around the suture (Fig. 7, lower-left).

6. Findings of Enucleated Specimens at 8 weeks after Surgery with Immunohistochemical Staining for HSP70:

a. Intrascleral fixation:

Mildly positive staining was observed around the haptics in the shallow (Fig. 7, upper-middle) and deep layers (Fig. 7, upper-right) of the sclera.

b. Scleral suture fixation:

Mildly positive staining was observed around the suture in the shallow (Fig. 7, lower-middle) and deep layers (Fig. 7, lower-right) of the sclera.

7. Results of Analyses of Inflammation Grade:

The results of WBC counts in 10 square digital 400 × 400-pixel images at 40× magnification are shown in Table 1.

a. Differences at 1 week:

In the shallow portion of the sclera, WBC counts tended to be lower in the intrascleral fixation samples than in scleral suture fixation samples, although the statistical evidence was weak ($p = 0.06$). In the deep portion of the sclera, a statistically significant difference was observed between intrascleral fixation and scleral suture fixation ($p = 0.002$). The deep sclera had lower WBC counts than the shallow sclera after both intrascleral fixation ($p = 0.002$) and scleral suture fixation ($p = 0.001$).

b. Differences at 8 weeks:

The intrascleral fixation group showed lower WBC counts than the suture fixation group in both the shallow ($p = 0.001$) and deep ($p = 0.002$) layers of the sclera. The deep sclera demonstrated lower WBC counts than the shallow sclera after both scleral suture fixation ($p = 0.001$) and intrascleral fixation ($p = 0.05$).

DISCUSSION:

This is the first report of a histological comparison between the intrascleral fixation technique and the scleral suture fixation technique. The intrascleral IOL fixation technique showed a reduced inflammatory reaction, compared with the scleral suture fixation technique. These advantages were probably caused by a simple surgical manoeuvre. In addition, the haptic passed through the sclera without the use of a knot in the intrascleral fixation, while a suture knot in scleral suture fixation had small inside spaces, which may have permitted integration with exudate and unexpected tissue incarceration or growth. Because inflammation has a potential risk of suture erosion/exposure and cystoid macular oedema, reduced postoperative inflammation helps to achieve good vision and improves patient prognosis.

The deep portion of the sclera showed less inflammation than the shallow portion, in both intrascleral fixation and scleral suture fixation. The shallow sclera is closer to the episclera with rich vessels, resulting in vulnerability to a greater inflammatory response in the deeper sclera [6]. This result suggests that deep scleral fixation under a thick scleral flap prevents suture erosion in suture IOL fixation.

Fibronectin is a glycoprotein that is secreted from migrated fibroblasts during wound healing. Fibronectin promotes the migration of extracellular macrophages, fibroblasts, vessel endothelial cells, and epithelial cells [8]. Slightly positive immunohistochemical staining for fibronectin at 8 weeks, therefore, indicated chronic inflammation with a wound healing process. The intrascleral fixation samples demonstrated weaker staining for fibronectin, compared with scleral suture fixation, at 1 week and 8 weeks, suggesting reduced migration of fibroblasts occurred when using this technique.

HSP70 is a heat shock protein produced during tissue regeneration by cytotoxic factors; it is related to cell proliferation and differentiation [9]. The intrascleral fixation samples demonstrated weaker staining for HSP70, compared with scleral suture fixation, at 1 week and 8 weeks; this indicated a reduction in the production of cytotoxic factors, with corresponding reductions in cell proliferation and differentiation, when using this technique.

A PVDF haptic was chosen in our study based on a previous article by Yamane et al. [10]. An IOL with a haptic made from PVDF, polymethylmethacrylate (PMMA), polyamide, or polypropylene is commonly used for posterior chamber IOL fixation. PVDF, PMMA, and polyamide have similar polymer characteristics, including

high strength and minimal tendency to cause a kink in the haptics. However, a PMMA haptic is easily scratched and requires coating with protectant. Polyamide can eexperience degeneration due to oxidative stress. Polypropylene also has high strength, but exhibits a lower capacity for shape memory, causing postoperative IOL decentration [11]. Yamane et al. recommended the use of an IOL with a PVDF haptic, based on its stability against intraoperative stretching force.

For suture scleral fixation, we used polypropylene sutures, which have sufficient strength, heat tolerance, and biochemical stability. Polyamide (nylon) sutures are also used; however, as mentioned above, polyamide can be degenerated by oxidative stress [12].

The use of 9-0 polypropylene in this study was planned based on the report by Price et al. [13], which indicated that 10-0 polypropylene suture-fixated PC-IOLs could be dislocated over time due to degradation of the suture material. Instead, the use of a larger diameter polypropylene suture, such as a 9-0 suture, was recommended because it had a lower risk of degeneration with respect to the long-term biochemical stability of scleral-fixated posterior chamber IOLs [13].

We did not perform topical anti-inflammatory treatment after the postoperative third week. This was based on the results of our previous study, which showed that inflammation in the anterior segment disappeared within 2 weeks after cataract surgery with topical corticosteroid administration; moreover, no further inflammation was observed in the anterior chamber, 2 weeks after stopping topical corticosteroid administration in rabbit eyes [14].

The purpose of the present study was simply to compare postoperative inflammation between the two groups. However, the use of markers for B and T cells, endothelial cells, and macrophages, and Western blot and real-time polymerase chain reaction analyses would provide additional information regarding the immune reaction and underlying biological phenomena.

This study had several limitations. We only used a limited number of eyes in an animal model. Only haptics were placed in the eye, rather than whole IOLs. Other factors contributing to inflammation, such as the type of haptic, type of suture, size of suture knot, and amount of tissue incarceration, were not investigated.

In conclusion, in our histological analysis, the intrascleral fixation technique was shown to cause reduced inflammation, compared with the scleral suture fixation technique, with concomitant reductions in fibroblast migration and production of cytotoxic factors.

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COMPLIANCE WITH ETHICAL STANDARDS

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Ethical Approval: All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

Other Contributors: None.

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FIGURE LEGENDS:

Figure 1: Schematic diagrams of surgery.

(a) Intrascleral fixation technique. Insertion of the end of the haptic into the scleral tunnel and fixation to the sclera without sutures.

(b) Scleral suture fixation. Suturing of the haptic to the sclera without scleral flaps.

Figure 2: An enucleated sample at 1 week after intrascleral fixation (haematoxylin and eosin staining).

(a) Slightly irregular arrangement of collagen fibres in the shallow sclera (4× magnification; scale bar = 500 μm).

(b) Enlargement of the rectangle in Figure 2a. A few foreign body giant cells and fibroblasts are present around the haptic in the shallow sclera (20× magnification; scale bar = 50 μm).

(c) Slightly irregular arrangement of collagen fibres in the deep sclera (4× magnification; scale bar = 500 μm).

(d) Enlargement of the rectangle in Figure 2c. A few foreign body giant cells, fibroblasts, and neovascular vessels are present around the haptic in the deep sclera (20× magnification; scale bar = 50 μm).

Figure 3: An enucleated sample at 1 week after scleral suture fixation (haematoxylin and eosin staining).

(a) Irregular collagen fibre arrangement in the shallow sclera (4× magnification; scale bar = 500 μm).

(b) Enlargement of the rectangle in Figure 3a. Many foreign body giant cells and fibroblasts are present around the suture knot in the shallow sclera (20× magnification; scale bar = 50 μm).

(c) Slightly irregular arrangement of collagen fibres in the deep sclera (4× magnification; scale bar = 500 μm).

(d) Enlargement of the rectangle in Figure 3c. A few fibroblasts and lymphocytes are present around the suture in the deep sclera (20× magnification; scale bar = 50 μm).

Figure 4: An enucleated sample at 1 week after intrascleral fixation (haematoxylin and eosin staining).

(a) Slightly irregular arrangement of collagen fibres around the haptic in the shallow sclera (4× magnification; scale bar = 500 μm).

(b) Enlargement of the rectangle in Figure 4a. A few fibroblasts and foreign body giant cells are present around the haptic of the shallow sclera (20× magnification; scale bar = 50 μm).

(c) Slightly irregular arrangement of collagen fibres around the haptic in the deep sclera (4× magnification; scale

bar = 500 μm).

(d) Enlargement of the rectangle in Figure 4c. A few fibroblasts and lymphocytes are present around the haptic in the deep sclera (20× magnification; scale bar = 50 μm).

Figure 5: An enucleated sample at 1 week after scleral suture fixation (haematoxylin and eosin staining).
(a) Many neutrophils, lymphocytes, eosinophils, foreign body giant cells, fibroblasts, and neovascular vessels are present in the shallow sclera (4× magnification; scale bar = 500 μm).
(b) Enlargement of the rectangle in Figure 5a. A few fibroblasts and lymphocytes are present around the suture in the shallow sclera (20× magnification; scale bar = 50 μm).
(c) Slightly irregular arrangement of collagen fibres around the suture in the deep sclera (4× magnification; scale bar = 500 μm).
(d) Enlargement of the rectangle in Figure 5c. A few fibroblasts and lymphocytes are present around the suture in the deep sclera (20× magnification; scale bar = 50 μm).

Figure 6: Immunohistochemical staining for fibronectin (20× magnification; scale bar = 50 μm).
The intrascleral fixation technique caused reduced fibronectin staining, compared with the scleral suture fixation technique, at 1 week and 8 weeks postoperatively (arrows).

Figure 7: Immunohistochemical staining for heat shock protein 70 (20× magnification; scale bar = 50 μm).
The intrascleral fixation technique caused reduced heat shock protein 70 staining, compared with the scleral suture fixation technique, at 1 week and 8 weeks postoperatively (arrows).