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# A Novel Approach of Drug Delivery via Intrascleral Implantation of Latanoprost Imbedded Disk

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**Abstract :** This study evaluates the drug delivery and biocompatibility of latanoprost imbedded disk in rabbit eye by assessing pharmacokinetics (PK), clinical signs, and histopathologic findings. During 84 days of experimental period, 48 New Zealand Rabbit (NZW) eyes were divided into control group which received no treatment and test material group which latanoprost were implanted intrasclerally. Pharmacokinetic assessment was performed to evaluate the drug delivery for 3 months. For biocompatibility, clinical signs were observed and histopathological analysis was done at 3 months post-operatively. The concentration of latanoprost in the iris tissue was maintained during the experimental period and the highest level of latanoprost was found at 4 weeks. However, the latanoprost was not found in the aqueous humor. Macroscopically, there was no evidence of clinical signs except for temporary hyperemia, neovascularization and edema immediately after surgery. On histopathological examination, there were no abnormal findings such as hyperemia, neovascularization, and edema in the eye tissues. The latanoprost imbedded disks has effectively released the drug into the adjacent tissue with high compatibility. Therefore, this study suggests that the drug delivery system with intrascleral latanoprost imbedded implants might be a novel approach as a treatment option for glaucoma.

***Key words*** **:** drug delivery, intrascleral implantation, latanoprost, rabbit.

## INTRODUCTION

Glaucoma in terms of veterinary medicine can be defined as the elevation of intraocular pressure (IOP) and the consequences leading to loss of vision and health of the eye. IOP represents the net value of the balance between the production of aqueous humor and outflow (5). Any failure of these pathways may be highly responsible for the consequences of having glaucoma. For the diagnosis, the IOP measurement is an essential ophthalmic examination (26). Once glaucoma is diagnosed, a constant management of IOP is needed to avoid any further damage to the optic nerve or associated tissues (18). Two options can be offered to control glaucoma; medical or surgical therapy.

Latanoprost, a prostaglandin F2α (PGF2α) analog prodrug, is known to be an effective medical treatment for glaucoma (1,6). It increases uveoscleral outflow, and is thus commonly used as a treatment for glaucoma when applied topically, once daily, at a concentration of 0.005% (7). Since glaucoma requires constant medical treatment, such a process demands cooperative circumstances from both the animal owner and the patient in veterinary medicine. Despite education in drug application, there may be situations where owners cannot afford proper drug administration for their animals, which has led to the need for an alternative way to replace the current administration of latanoprost.

Drug delivery by bio-scaffold system is broadly used in human and veterinary medicine. These scaffold materials can provide fundamental structures for containing and constant releasing of the drugs with degradation. Because most scaffolds are implanted in the inner body, these scaffolds should consist of bio-compatible materials. Serum albumin (SA) is a protein which can be obtained from plasma, which is known to be a non-immunogenic, bio-compatible, and bio-degradable substance with no toxicity (3,9,15,23).

Previous studies emphasized nanoparticles to be used for targeting drug to organs and increasing drug bio-compatibility (4,19). Compared to other scaffold material, nanoparticles have its advantage of having higher storage along with in vivo stability, particles are a highly preferable technique to be used for drug delivery (10,11). Protein-based nanoparticles, which consists of serum albumin, are appropriate to be used as a carrier for drug delivery (3,8,23).

There are many different administrative ways when it comes to drug delivery system. Each and every drug delivery system suggests its own advantages and disadvantages. Commonly used topical administration limits absorption due to the extensive precorneal loss caused by the lacrimal system (4,19). Systemic penetration of drugs into the ocular system is restricted by the blood-ocular barrier, which requires large doses of drug, often resulting in general side effects (13). Periocular administration, drug delivered by passing through the sclera, in particular, can be considered as a loss of integrity (24). Intrascleral route of drug administration can be explained by diffusion. Diffusion occurs once the drug is absorbed through into the sclera. Into the anterior ciliary arteries, the drug is supplied to the uvea as well as other tissues in the anterior chamber. Due

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to the lack of lymphatics in the sclera, there may be less drug loss which makes it appropriate for managing a disease that requires constant drug administration (11).

For an antiglaucoma agent, it can be said that the penetration of the active site is completed once the drug reaches the ciliary body through the ciliary arteries. Therefore, the study of latanoprost delivery can easily be explained by the measurement of the drug level at the targeted site by pharmacokinetic assessment (24).

This study suggests a new way of controlling glaucoma by using a latanoprost imbedded disk material to be implanted intrasclerally. In order to prove the drug delivery system along with bio-compatibility, assessments of ocular pharmacokinetics (PK), clinical signs, and histopathological evaluation were carried out.

## Materials and methods

### Animals

Twenty-four healthy male New Zealand White Rabbits (NZW) were used. Prior to the experiment, they were assessed by ophthalmic examination to see any abnormalities using a slitlamp direct ophthalmoscope (Takagi SM-70N, Nagano-Ken, Japan), and TonoVet (Icare®, Jorgensen Laboratories, USA) rebound tonometer which has been widely adopted in veterinary medicine for ophthalmic examinations.

NZW were randomly divided into 2 groups; 3 in a control group and 21 in latanoprost group. In the latanoprost group, 3 were used for histopathological assessment, the rest (18 rabbits) were used for pharmacokinetics and clinical signs including intraocular pressure and conjunctival hyperemia.

At each time point, the rabbits were put down using intravenous injection of 20 ml mEq potassium chloride (KCl inj®, Je-il Pham., Korea), followed by enucleation.

The procedures in this study were approved by Institutional Animal Care and Use Committees of Kyungpook National University (No. KNU 2018-59).

### Implant material

Rabbit serum albumin (RSA) was dissolved to reach a concentration of 40 mg/mL and 25 mL of RSA was attained.

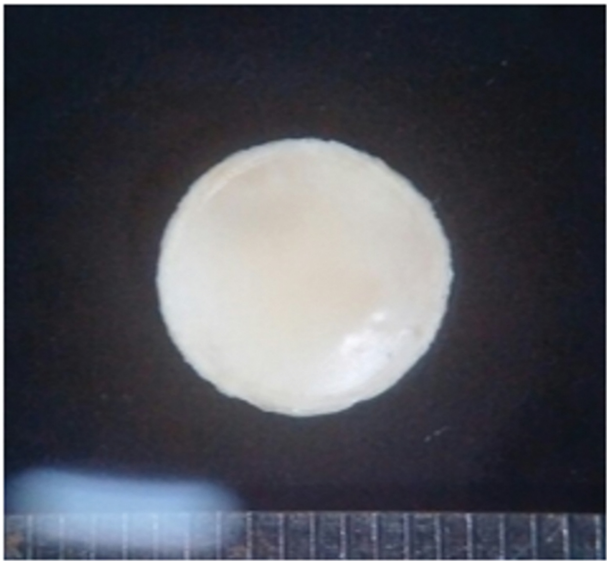

**Fig 1.** Latanoprost imbedded RSA nanoparticles disk material. Diameter 4.5 mm × height 0.5 mm disk material.

The pH of the RSA was set to be 8-8.5. With the same method, 2-iminothiolane hydrochloride (2-It.HCL) was dissolved in distilled water (DW) to reach an amount of 25 mL with a concentration of 2 mg/mL. The same proportions of RSA and 2-It solution were combined. In order to remove 2-It, the solution was centrifuged for 10 minutes at 400 rpm. Thiolated RSA was then collected, followed by mixing of two solutions; (1) 4 mL DW + 40 mg RSA, (2) 200 μL dimethyl sulfoxide (DMSO) + 50 μL ethanol + 24 mg latanoprost.

The two solutions were mixed by stirring with a mild magnetic bar. Nanoparticles were then collected and lyophilized in a 0.5% glycol chitosan solution. Lyophilized powders were pressed to make a convex-shaped, disk-type implant material which had a diameter of 4.5 mm and a height of 0.5 mm. The disk material was coated with a 5% polycaprolactone (PCL) solution (Fig 1).

### Surgical implantation

21 NZW were given surgical implantation on both sides.

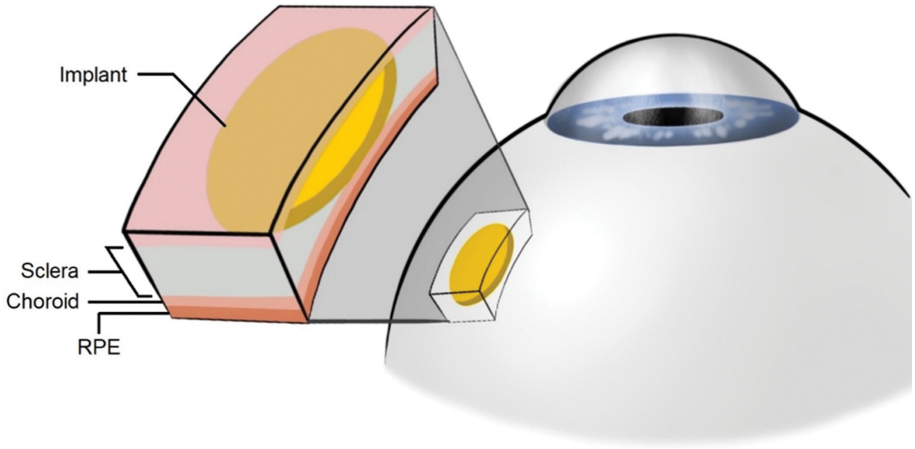


**Fig 2.** Location of implant material. Latanoprost-imbedded disk implant material located in the suprascleral plane. The scleral pocket is formed. Implant material sits securely in the sclera by surgical suture. RPE, Retina pigment epithelium.

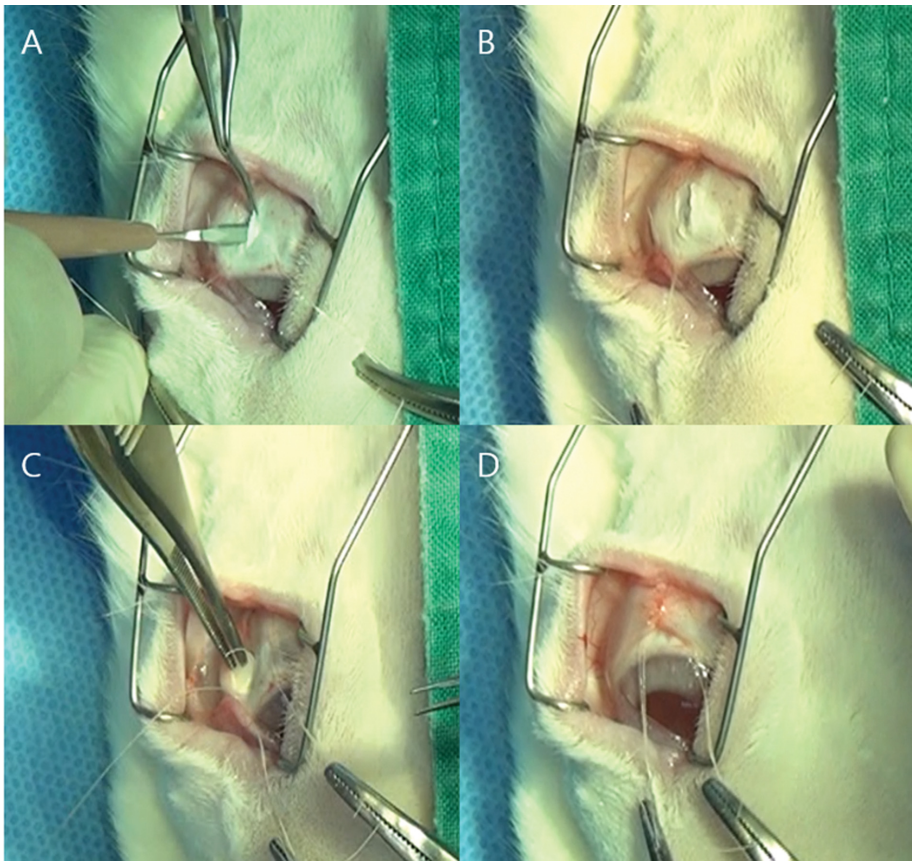


**Fig 3.** Surgical implantation of disk material. (A) Incision with beaver blade for the exposure of site, (B) Pocket for disk to be implanted, (C) Suture sclera after placing disk material, (D) Suture conjunctiva.

For the anaesthesia, ketamine (35 mg/kg, Yuhan Ketamine® 50 inj., Yuhan Yanghaeng, Korea) and xylazine (5 mg/kg, Rompun® 2%, Bayer, Korea) were combined and injected intramuscularly. The surgical site was prepared by shaving the periocular surface and sterilizing the globe by flushing it with a 0.2% povidone iodine solution. By placing NZW in lateral recumbency, the surgical procedure started with the placement of eye speculum, followed by two stay sutures for the convenience of exposing the site. The disk material was placed at the dorsal part of the suprascleral plane by surgical procedure (Fig 2). With a short incision on the conjunctiva and tenon's capsule, the scleral surface was exposed. By using a microsurgical crescent knife (Stylus®, Angiotech, USA), the pocket is achieved for implanting the material securely. Then, Polyglactin 910 6-0 (Vicryl®, Ethicon, USA) was used in order to suture the incision line (Fig 3). The whole surgical implantation procedure was completed within 30 minutes from the initial move. The NZW were given enrofloxacin (50 mg/ml, Baytril 50 inj.®, Bayer, Korea) 0.2 ml/kg/IM postoperatively to prevent postoperative inflammation.

### Ocular PK

PK assessment was measured in the iris at six time points over a 3 - month period (1, 7, 14, 28, 56, and 84 days postoperatively). Following careful enucleation with the preservation of the implanted area, the iris was frozen and kept at −70°C storage, diluted with PBS (Phosphate Buffer Saline) which was three times the weight of the tissue. The tissue was then ground with a homogenizer. Centrifuged at 10,000 rpm for 3 minutes, the upper layer was used as a sample for the measurement (Table 1). The assessment of PK for the iris was carried out by a liquid chromatography technique coupled with tandem mass spectrometry (LC-MS/MS); 20 μL of the sample specimen was added to acetonitrile 180 μL (internal standard included), followed by centrifuge at 4°C 15,000 rpm for 5 minutes. The collected top coat was evaluated by LC-MS/MS (Table 2). The procedure provided latanoprost concentrations attained from the iris before and after the implantation.

**Table 1.** Conditions of HPLC system

| | |
|---|---|
| HPLC system | Nexera XR system (Shimadzu, Japan) |
| Column | Kinetex XB-C18 column (2.1 × 100 mm, 2.6 μm, particle size; Phenomenex, USA) |
| Injection volume | 2 μl |
| Mobile phase | (A) 0.1% formic acid in water<br>(B) 0.1% formic acid in acetonitrile |
| Sample analysis time | 3.5 min |
| Retention time | 2.31 min |

### Clinical signs

Starting from day 1 of the procedure when 6 rabbits were

**Table 2.** Conditions of mass spectrometry

| | |
|---|---|
| System of analysis | TSQ vantage triple quadruple (Thermo, USA) |
| Molecular weight | 432.59 g/mol |
| Ion source type & Ionization mode | Turbo Spray Ionization, Positive mode<br>- MRM transition (*m/z*): Q1 433.19, Q3 91.06<br>- CE (V): 56, S-lens: 65 |
| Lower Limit of Quantification (LLOQ) | Iris: 10 ng/ml |
| Standard Curve Range | Iris: 10-1000 ng/ml |

treated with the surgical procedure intrasclerally, all observations of clinically significant effects of the surgical implantations were analyzed. The analysis was divided into three parts; any change in the conjunctiva, iris, and IOPs. The observations for IOPs were made at day 0, 1, 7, 14, 28, 42, 56, and 84 postoperatively.

Clinical findings were evaluated from the conjunctiva and iris at day 0, 1, 3, 5, 7, 10, 14, 21, 28, 42, 56, and 84 postoperatively.

The assessment was appraised through a camera for the conjunctiva. Gathered data were put into a conjunctival hyperemia scoring system (17). The criteria for assessing clinical signs regarding any abnormal changes in the conjunctiva were as follows: The grades of redness and hyperemia were divided into 5 scalar units; Grade 0 for normal, Grade 1 for mild, Grade 2 for moderate, Grade 3 for severe, Grade 4 for extremely severe.

The parameters for the system regarded the vessel covering the surface area, reduction of the white surface areas due to the emergence of episcleral vasculature, the dilation of conjunctival vessels, and the proximal invasiveness to the limbus.

In order to assess any abnormal changes in the iris, a slit lamp microscope (Takagi SM-70N) at magnification of 10x was used. Any changes in the surface neovascularization of the pupillary zone, ciliary zone, color of iris, and collarette were observed and evaluated by a grading system for iris neovascularization (25). Depending on the surface area of neovascularization, the grading system is classified from 0 to 4.

IOPs were measured by TonoVet rebound tonometry (mmHg). NZW were placed on the table where the surface of the cornea could be measured at a 90° on the tonometer. The TonoVet was held to aim at the center of the cornea (22). IOPs measurement was performed on both eyes, repeated three times while NZW held gently to avoid any rise in IOPs. The observation was carried out prior to conjunctival observation. The observations for IOPs were made at day 0, 1, 7, 14, 28, 42, 56, and 84 postoperatively.

### Histopathologic assessment

In order to assess the tissue toxicity of the implanted disk, any histopathological changes have been evaluated. Tissues were collected from the cornea, iris, sclera, and retina at day 84. Any abnormal changes were assessed by microscopical observation.

Collected tissues were fixed with 10% neutral formalin. The fixed tissues were trimmed to be made into paraffin blocks of a sufficient size. The paraffin blocks were dissected by 4 μm using a micro-slicer and made into slides with Hematoxylin and Eosin staining (H&E). The criteria of evaluation included any microscopic changes involving edema, neovascularization, fat infiltration, necrosis, fibrosis, and inflammation.

### Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). Multiple data tests were put into a paired student *t*-test which assumes distributive normality. A Mann-Whitney U test was adopted for a non-parametric comparison test. Statistical analyses were obtained by using SigmaPlot (Version 12.3, Systat Software, Inc, UK). p values less than 0.05 were considered statistically significant.

## Results

### Ocular PK

Evaluation was taken at six time points; 1, 7, 14, 28, 56, and 84 days postoperatively. Mean value and standard error of the mean are plotted in Fig 4. The PK evaluation showed an initial detection of latanoprost 1 day after the surgical implantation, after which it increased and reached the maximum level at day 56 (50.4 ng/mL), and started to decrease after (Fig 4).

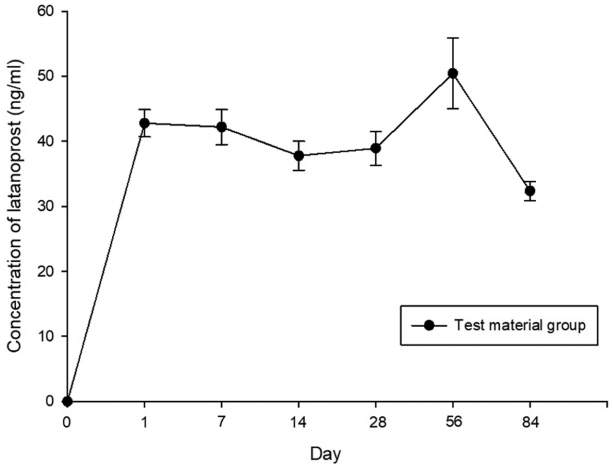


**Fig 4.** Ocular PK for latanoprost in the iris. The PK value was to be seen in the iris the day after surgical implantation. It remained at a concentration within 42 and 48 ng/ml for 84 days. After reaching maximum value at day 56, it starts to decrease.

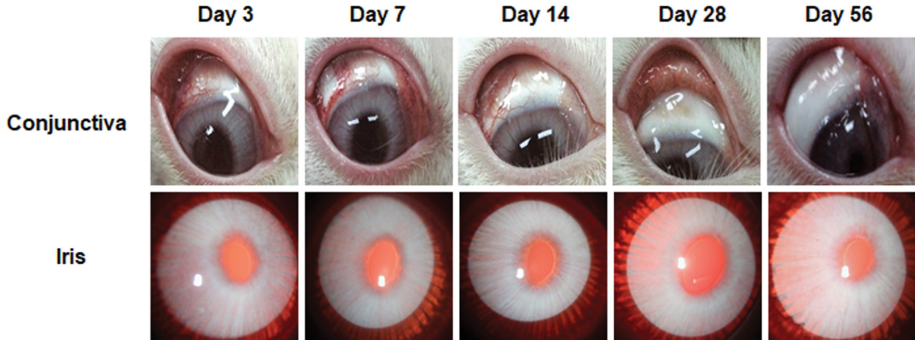


**Fig 5.** Clinical signs showing conjunctival hyperemia. Conjunctival hyperemia after surgical implantation. Note that at day 3, severe hyperemia was observed with massive dilated vessels covering most of the white part of the eye. At day 7, hyperemia was observed with dilated vessels covering white part of the eye. At day 14, hyperemia was observed with some vessels covering white part of the eye. After day 28, hyperemia was not seen. Iris observation taken with slit-lamp microscopy. No evidence of adverse effects throughout the experimental period, including any changes of the marginal area of pupillary zone, ciliary zone, color of iris, and collarette.

Thus, based on the data achieved, the drug release for the disk material through intrascleral method has been proven to be at least 84 days.

### Clinical signs

According to the conjunctival grading system (17), the results showed a diminishing pattern of hyperemia after severe conjunctival hyperemia starting from the day after surgery (Fig 5).

Data showed a spiking peak the day after implantation, with severe hyperemia covering the white surface areas along with the dilation of vessels. Such conditions can be explained as a normal variation of the site following surgical manipulation. It constantly decreased and vanished as it reached day 42 (Fig 6). Gradual decrease in conjunctival hyperemia was observed, with reduction of vessels covering the surface area, dilation of conjunctival vessels, and proximal invasiveness to the limbus.

The observation taken by slit lamp microscopy showed no changes to the iris (Fig 5). According to the grading system for iris neovascularization, the grade of the iris remained the same before and after the implantation. The marginal area of pupillary zone, ciliary zone, color of iris, and collarette seemed to remain identical throughout the experimental period.

After the measurements for IOP were collected, the mean values for the data were analyzed. IOPs remained within normal range for the rabbit during the experimental period (Fig 7). $P < 0.05$ is considered significantly different from each baseline.

### Histopathological assessment

The histopathologic assessment, which has been made to examine the microscopical changes of tissues from the eyes, showed no evidence of abnormal changes in edema, neovascularization, fat infiltration, necrosis, fibrosis, and inflammation (Fig 8). Such findings indicate that neither the tissue adjacent to the surgically manipulated area, nor the major compartments of the eyes were affected by any means of tissue toxicity.

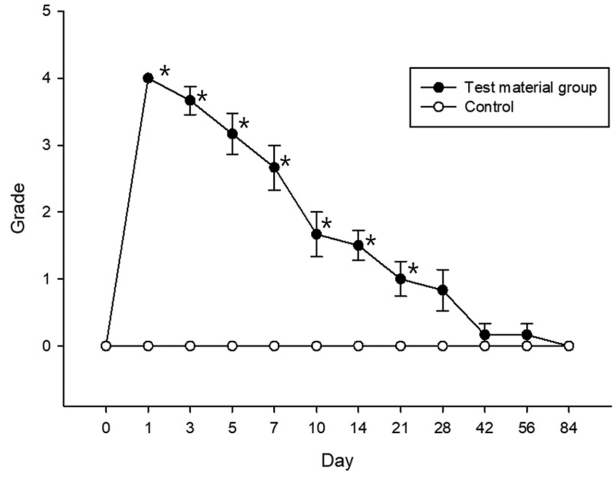


**Fig 6.** Conjunctival hyperemia grading system. Conjunctival grading system ranging from 0 to 4; 0 for normal, 1 for mild, 2 for moderate, 3 for severe, and 4 for extremely severe. Data evaluated at day 1, 3, 5, 7, 10, 14, and 21 shows significantly different result from the baseline. *$p < 0.05$; significantly different with each baseline.

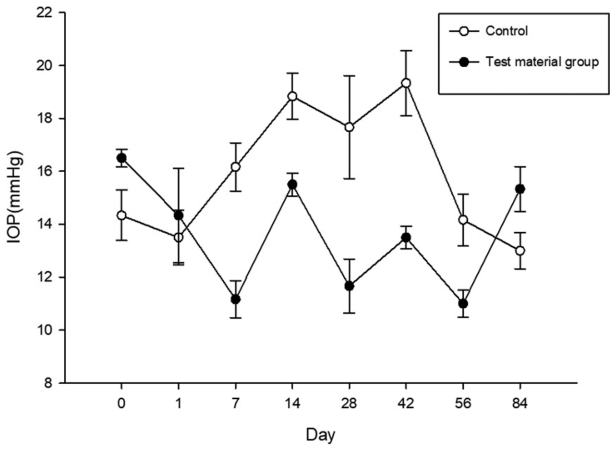


**Fig 7.** Changes of IOPs during the experiment. Note that data from days 7, 28, 42, and 56 showed significantly low IOPs in the test material group. *$p < 0.05$; significantly different with each baseline.

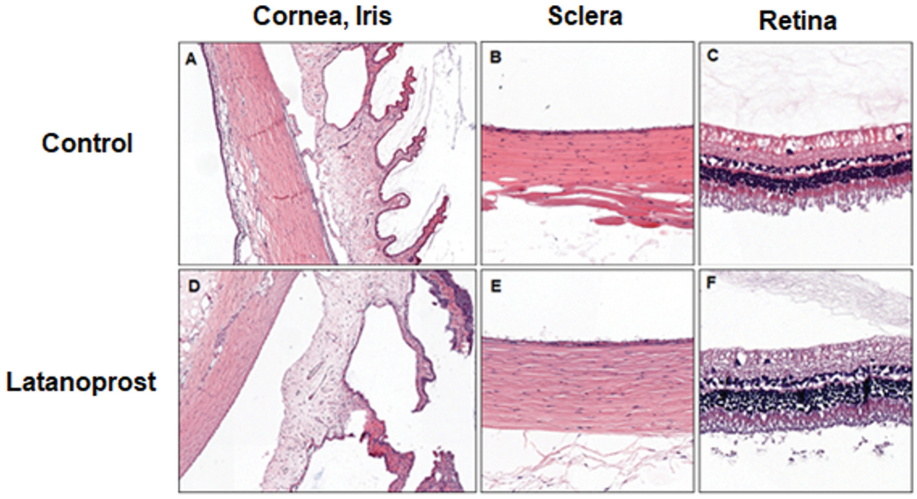


**Fig 8.** Histopathologic assessment (H&E staining). Histopathologic assessment taken at day 84. The microscopic findings of histopathologic assessment showed no evidence of abnormal changes from cornea, iris, sclera and retina in both control and test groups; edema, neovascularization, fat infiltration, necrosis, fibrosis, and inflammation. H&E, Hematoxylin and Eosin.

## Discussion

In this study, the feasibility of applying a bio-degradable RSA into sclera has been demonstrated by evaluating ocular pharmacokinetics, clinical signs, and histopathological assessments. RSA is the most abundant plasma protein in rabbits (15). It is known to be a bio-compatible material with no toxicity, also shown to have constant drug releasing properties (8).

The large surface area along with high degree of hydration of the sclera allows a wide range of molecular weights to be permeable (2,10,16,19). Past studies suggested that proteins with large molecular weight could diffuse rapidly in the sclera (2). Another explanation for the rapid release can be due to the surgical thinning of the sclera (19). Assuming the scleral thickness of rabbits to be less than 100 μm, the depth of pocket was half the thickness of the sclera (19). Thus, surgical thinning attained by scleral pocket formation may have contributed to significantly enhanced permeation of drugs (19,20).

However, the ocular PK detected in the aqueous humor was below the lower marginal limit. This may be due to the insufficiency of drug dose loaded at the disk to reach the aqueous humor. SA nanoparticles have high storage capacity for drugs, which enables the implant material to load drugs with broad range (8). The latanoprost loaded on each disk was approximately 462 μg. Throughout the experimental period, the concentration of latanoprost maintained a constant level at iris, proving the ability of RSA as a nanoparticle to have constant drug releasing properties. This can imply the possibility of disk material to replace topical application of latanoprost.

Conjunctival hyperemia was observed instantly after surgical implantation. However, symptoms were resolved as it reached complete wound healing phase. The iris is a main component of the uvea, located adjacent to the sclera, and is an eye compartment with high vasculature (9). Such a structure can present inflammatory or toxic change after surgical implant with slit lamp examination. The observation of the iris showed no adverse effects to the marginal area of the pupillary zone, ciliary zone, color of iris, or collarette.

IOP changes, can also be taken into consideration when discussing clinical side effects of ocular surgeries. It is well known from previous studies that IOP-lowering response of latanoprost lacks in rabbits (12). Since the purpose of IOP measurement in this study was to evaluate the safety of implant material, it is limited in its ability to see any clinical proof including therapeutic value through drug delivery system (12). The normotensive IOPs of NZW throughout the experiment implies that there were no significant adverse effects involving clinical change of IOPs.

Histopathological findings showed no evidence of edema, neovascularization, fat infiltration, necrosis, fibrosis, and inflammation in cornea, iris, sclera, and retina.

Results of clinical signs showing the condition of conjunctiva, iris, IOPs and histopathological findings highly support the feasibility of disk materials to be applied clinically since there were no significant clinical signs to be regarded as adverse effects.

There are fewer lymphatics in the sclera, which makes it suitable for the placement of such disk materials since there will be less loss of drugs (11). Nonetheless, the thin layer of sclera is hardly the optimum place for surgical treatment to be performed. The surgical approach itself requires a skilled surgeon. It can easily involve tearing of sclera, choroid, or even more, perforation of the globe, which relates to the leakage of vitreous (21). Considering the complicity of the surgical approach, more simplified ways can be studied.

The convex-shaped disk material tended to irritate the surface of the sclera by introducing pressure onto it. The thin, highly muscular rigid layer of the outer sclera was sutured with a tension to hold the disk material in its pocket (14). Considering the size of a rabbit eye, the volume of disk material was not appropriate to be fitted intrasclerally. Further studies should consider more compact-sized disks to be made in order to be used in veterinary medicine.

Limitations to this study are also secondary to the small population of animals tested. The small number limits any definitive conclusion and further research with a larger number would be needed to investigate this study. This may firmly support the suggested clinical application for managing glaucoma. Also, considering clinical use in veterinary medicine, the need for clinical trials with dogs can be discussed further.

Even though there have been numerous studies suggesting a substitutional method of applying eyedrops by using scaffold systems, no one has studied the intrascleral route of latanoprost application by disk implantation. In this regard, this study is very meaningful in using the new technique to be applied in clinical veterinary medicine.

The latanoprost imbedded disk material via intrascleral implantation effectively released the drug into the adjacent organ, throughout the assessment period. The evaluation of PK, clinical signs, and histopathological findings have revealed that implant material has high biocompatibility with no adverse effects including tissue toxicity and clinical signs. This study suggests that the drug delivery system by intrascleral latanoprost imbedded disk might be a novel approach as a treatment option for glaucoma.

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