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Controlled intraocular delivery of ganciclovir with use of biodegradable scleral implant in rabbits

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Abstract

We evaluated biodegradable scleral implants as a controlled intraocular delivery system of ganciclovir (GCV) for the treatment of cytomegalovirus retinitis in rabbits. The scleral implants (weight, 8.5 mg; length, 5 mm) were made of poly(DL-lactide) (PLA) or poly(DL-lactide-co-glycolide) (PLGA) and contained various amounts of GCV. The in vitro release studies demonstrated a triphasic release pattern. The 10% GCV-loaded scleral implant made from PLA with a molecular weight of 130 000 released GCV for 6 months. The in vivo release and biodegradation were studied using the 25% GCV-loaded implant made from PLGA(75/25) with a molecular weight of 121 000 in pigmented rabbits. The GCV concentration in the range of ED_{90} for human CMV was maintained in the vitreous for over 3 months and in the retina/choroid for over 5 months. The GCV concentration was greater in the retina/choroid than in the vitreous throughout the study. The scleral implants showed two phases of biodegradation: lagtime and erosion. In the erosion phase, the weight of PLGA dropped remarkably. All the scleral implants were separated into two pieces at the site of scleral penetration and displaced into the vitreous 10 weeks after implantation. The fragments disappeared from the vitreous and the subconjunctival space 5 months after implantation. Our findings suggest that the biodegradable scleral implant may be a promising device for the intraocular drug delivery of GCV.

Keywords: Biodegradable scleral implant; Intraocular controlled release; Poly(DL-lactide-co-glycolide); Ganciclovir; Cytomegalovirus retinitis

1. Introduction

Cytomegalovirus (CMV) retinitis is the most common opportunistic ocular infection to produce visual loss in patients with acquired immune deficiency syndrome (AIDS). CMV retinitis occurs in 20% to 25% of patients with AIDS during the course of their illness [1–6]. Systemic administration of ganciclovir (GCV), an acyclic nucleoside analogue, and foscarnet, a pyrophosphate analogue, is currently available for the treatment of CMV retinitis. Unfortunately, systemically administered drugs penetrate poorly into the eye due to the blood-retinal barrier [7]. This barrier consists of tight junctional complexes of the retinal pigment epithelium and the endothelium of retinal capillaries. Long-term treatment and high-dose intravenous injections of drugs are sometimes necessary to maintain the vitreous concentration of drugs in the therapeutic range. In the case of GCV, dose-related bone marrow suppression and neutropenia have been reported as systemic side effects [8–10]. GCV has also been success-

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fully injected intravitreally to control progressive CMV retinitis [11–14]. The injections have to be repeated at least every 1 or 2 weeks and may be associated with a risk of cataract, retinal detachment, and endophthalmitis.

To maintain the vitreous concentration of GCV in the therapeutic range for a certain period of time, liposomes containing the drug have been investigated as a vehicle [15–17]. Liposomes, however, have provided poor physicochemical stability and have entrapped hydrophilic drugs poorly. Moreover, small particles of liposomes suspended in the vitreous could impair the clarity of the ocular medium. Recently, an intraocular implant composed with ethylene-vinyl acetate copolymer and poly(vinyl alcohol) has been reported [18–21]. This device maintained the therapeutic range of GCV for more than 80 days in nearly zero-order release fashion. This device, however, required surgical implantation. Because it is not biodegradable, the implant also had to be removed when drug release was complete.

Previously, we reported on an implantable, biodegradable scleral implant containing sodium fluorescein as a model drug [22] and doxorubicin for the treatment of experimental proliferative vitreoretinopathy in rabbits [23]. These devices, which are implanted at the pars plana, release drugs directly into the vitreous without disturbing the transparency of the ocular medium. In the scleral implants with doxorubicin, the vitreous concentration of doxorubicin was maintained for 1 month in the therapeutic range, which is effective at reducing proliferation below 50% of control without evidence of in vitro cytotoxicity [24].

In this study, we have developed biodegradable scleral implants containing GCV to evaluate the feasibility as a long-term intraocular delivery system for the treatment of CMV retinitis in rabbits. We investigated the in vitro and in vivo release of GCV from the scleral implants and the degradation properties of the devices in pigmented rabbit eyes.

2. Materials and methods

2.1. Materials

Poly(DL-lactide) with a weight-average molecular weight of 20 000 (abbreviated hereafter as PLA-20 000); poly(DL-lactide-co-glycolide), with a weight-average molecular weight of 20 000, whose copolymer ratio of DL-lactic acid to glycolic acid is 75:25 (abbreviated as PLGA(75/25)-20 000); and PLGA(50/50)-20 000 were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). PLA-130 000, PLGA(75/25)-121 000, and PLGA(50/50)-95 000 were supplied by Du Pont (Medisorb®, Wilmington, DE, USA). The weight-average molecular weight was determined by gel permeation chromatography (GPC) by the suppliers. Ganciclovir (Denosine®) was purchased from Nippon Syntex K.K. (Tokyo, Japan). Other chemicals were of reagent grade.

2.2. Preparation of the scleral implants

The scleral implants were prepared by dissolving the polymer and GCV in acetic acid which is a good solvent for the polymer and the drug. The resultant solution was lyophilized (FDU-830, Tokyo Rikakikai, Tokyo, Japan) to obtain a homogeneous cake. The cake then was compressed into a scleral implant on a hot plate (Model HM-19, Koike Precision Instruments, Osaka, Japan) at a temperature ranging from 80 to 100°C. It was confirmed that GCV degradation did not occur during preparation process of the implants. The implants had loadings of 10, 25 and 40 wt%. The scleral implant weighed 8.5 mg and was 5 mm in length and 1 mm in diameter. The implant was shaped similarly to metallic scleral plugs, which are used temporarily during vitrectomy (Fig. 1).

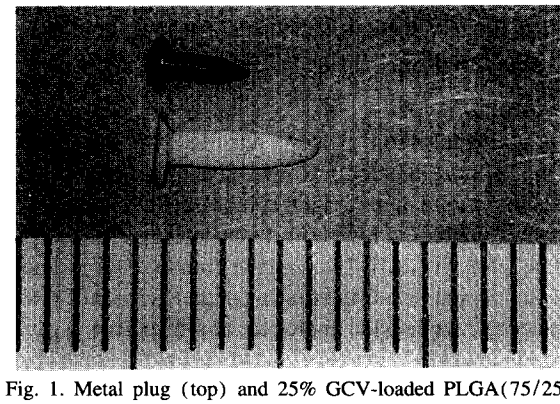

Fig. 1. Metal plug (top) and 25% GCV-loaded PLGA(75/25)-121 000 scleral implant (bottom).

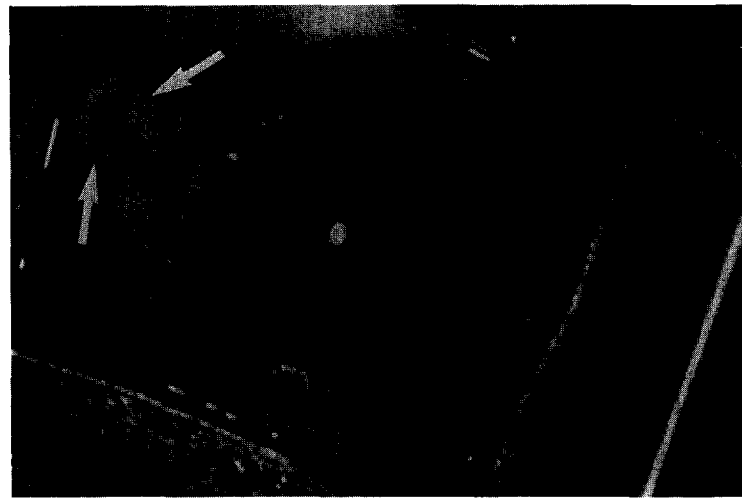

Fig. 2. Implantation site in pigmented rabbit eye.

2.3. In vitro release study

The scleral implant was incubated in 2 ml of phosphate-buffered solution (0.1 M, pH 7.4) in a shaking water bath (BT-25, Yamato Scientific Co., Ltd., Tokyo, Japan) at 37°C. At predetermined intervals, the entire buffer volume was sampled and 2 ml of fresh medium was added to the sample vial to approximate perfect sink conditions. It was confirmed that GCV degradation did not occur during release studies. The amount of GCV released into the medium was measured by spectrophotometry (Model DU-64, Beckman Instruments Inc., USA) at a wavelength of 254 nm.

2.4. In vivo release study

The implantation method used in this study was the same as described previously [23]. In brief, 30 eyes of 30 pigmented rabbits, weighing 1.9–2.5 kg each, were used for this study. All animals were handled according to the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research. The rabbits were anesthetized with a mixture (1:1) of xylazine hydrochloride (2 mg/kg) and ketamine hydrochloride (5 mg/kg). Then the ocular surface was anesthetized with a topical instillation of 0.4% oxybupurocaine hydrochloride. After the sclera was exposed, a 1 mm sclerotomy was made with an 18 gauge needle 3.5 mm from the limbus. The 25% GCV-loaded PLGA(75/25)-121 000 implant was inserted at the sclerotomy site. The conjunctival wound was sutured with 8-0 virgin silk (Fig. 2). Animals were killed with an overdose of intravenous sodium pentobarbital at weeks 1, 2, 4, 8, 12 and 20 after implantation, and the eyes were enucleated. The enucleated eyes were stored at −85°C until the GCV concentrations were determined. The scleral implants and samples of ocular tissue (aqueous humor, vitreous and retina/choroid) were retrieved from the eyes.

2.5. Weight change of PLGA scleral implant in vivo

The retrieved implants were washed three times with distilled water, weighed and dried under reduced pressure at room temperature for 1 week. Biodegradation of PLGA scleral implant in the vitreous was estimated as weight change represented by Eq. 1.

$$\text{Weight change } (\%) = 100(W_0 - W_t)/W_0, \qquad (1)$$

where W_0 and W_t are the initial weight of polymer and the residual weight of polymer after drying, respectively. W_0 and W_t are calculated as follows:

W_0 = (initial weight of implant)

− (weight of initial GCV)

W_t = (weight of implant)

− (weight of GCV remaining in implant)

2.6. Determination of GCV in implants and ocular tissues

The amount of GCV remaining in the scleral implants and samples of ocular tissue was determined by HPLC using a C-18 reverse-phase column (250 mm × 4.6 mm I.D., YMC-Pack ODS-A303, YMC Co., Ltd., Kyoto, Japan) at 45°C (oven; CTO-10A, Shimadzu, Kyoto, Japan) at a flow rate of 1.0 ml/min (pump; LC-10AS, Shimadzu, Kyoto, Japan, degasser; KT-16, Showa Denko Co., Ltd., Tokyo, Japan) and was detected with a UV spectrophotometer at 254 nm (detector; SPD-6A, Shimadzu, Kyoto, Japan). The mobile phase was 2% of acetonitrile in 30 mM phosphate buffer containing 5 mM 1-heptanesulfonate sodium salts (pH 2.0). Acyclovir (Sigma Chemical Co., MO, USA) was used as the internal standard. GCV was extracted from the tissues by the following procedures. 0.5 ml of the internal standard solution (1.5 μg/ml) and 3.0 ml of acetonitrile were added in each tissue sample. The mixture was homogenized and centrifuged at 3000 rpm for 15 min. The supernatant was collected and dried under reduced pressure using a centrifugal concentrator (VC-960, Taitec Co., Saitama, Japan). The residue was dissolved with 0.5 ml of mobile phase. 50 μl was injected into HPLC as described above. The recovery of GCV spiked into placebo vitreous, retina/choroid and aqueous humor during the above extraction procedure were 105.7, 105.2 and 103.0%, respectively, indicating the valid evaluation of the drug concentrations in the tissues. Under these conditions, the detection limit for GCV was 30 ng/ml. The GCV concentrations in ocular tissues were represented as GCV weight per weight of wet tissue.

3. Results and discussion

3.1. In vitro release of GCV

The cumulative release of GCV from the scleral implants at a constant GCV loading of 10% has been plotted in Fig. 3. These data are consistent with triphasic profiles. The three phases are (1) an initial burst, (2) a second stage that is derived from diffusional release before erosion and swelling of the polymer begins, and (3) a sudden burst due to swelling and

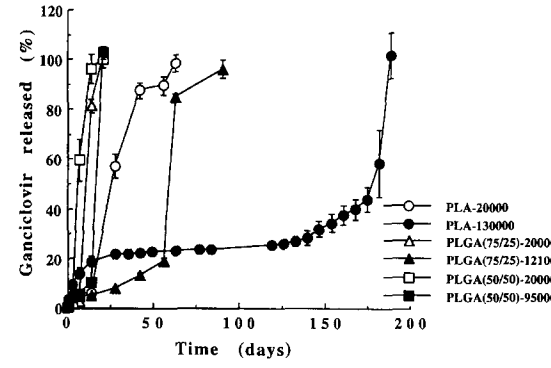

Fig. 3. Effects of the molecular weight and LA/GA ratio of PLA(PLGA) on in vitro release from scleral implants at a constant GCV loading of 10%. The values are shown as mean ± SD of $n = 5$.

disintegration of the polymeric matrix. This result was similar to previous reports about the release of various drugs from biodegradable polymer matrices [25–27]. The initial burst may have been due to the rapid release of the drugs deposited on the surface and in the water channels in the matrix. During the second stage, the drug was released slowly, possibly controlled by the degradation speed of the polymer. The release rate increased with the decrease of the molecular weight and lactide contents in PLGA. This rate was probably due to the difference in the degradation rate of the polymer.

Degradation of PLGA could be influenced by the composition of LA and GA: the higher the glycolide content, the faster the degradation rate [28]. In the higher molecular weight and higher lactide content polymer, the diffusional phase (the second stage), which is governed mainly by the degradation of polymer, was more prolonged. Although the scleral implants composed of PLA-130 000 released GCV for 180 days, they were not suitable for therapeutic application because of their long lagtimes. Therefore, we selected PLGA(75/25)-121 000 as the matrix material for the following experiments.

An increase in GCV loading of the scleral implant made of PLGA(75/25)-121 000 resulted in a higher rate of GCV release, as seen in Fig. 4. At lower GCV loading (10%, 25%), a triphasic release pattern from the PLGA(75/25)-121 000 scleral implant was clearly observed. The initial burst occurred during the first 3 days. At the second stage, during days 3–42, GCV was released slowly, the rate of which was regulated by the diffusion of the drug in the rigid matrix. The third phase

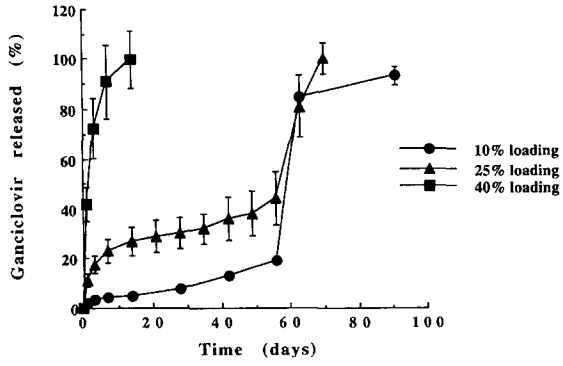

Fig. 4. Effect of the loading of GCV on in vitro release from the PLGA(75/25)-121 000 scleral implant. The values are shown as mean ± SD of $n = 5$.

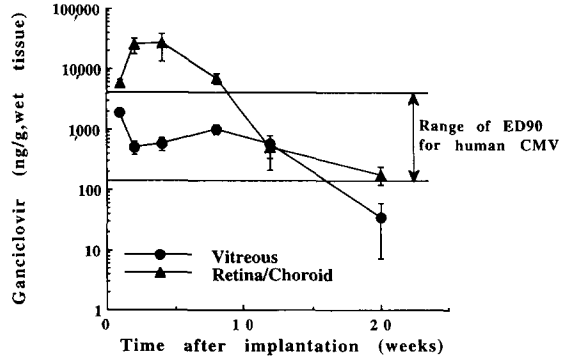

Fig. 5. Concentrations of GCV in the vitreous and the retina/choroid after implantation of the 25% GCV-loaded PLGA(75/25)-121 000 scleral implant. The values are shown as mean ± SD of $n = 5$.

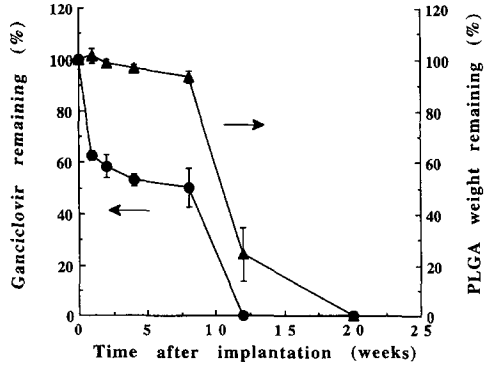

Fig. 6. Release of GCV from the 25% GCV-loaded PLGA(75/25)-121 000 scleral implant and PLGA weight change in vivo. The values are shown as mean ± SD of $n = 5$.

of GCV release occurred when the scleral implant was swollen into an ovoid and was about to disintegrate (42 days). At the highest GCV loading (40%), a high initial burst was observed in the first 7 days. A large amount of GCV was released rapidly before swelling and disintegration of the implant occurred. Elution of this GCV would leave an extensive pore structure to facilitate the more rapid release of GCV from the implant before the onset of erosion and swelling. Water channels, which facilitate drug release, might be produced by the osmotic effect [29].

3.2. Kinetics of GCV in ocular tissues

The concentrations of GCV in the vitreous and in the retina/choroid after implantation of the 25% GCV-loaded PLGA(75/25)-121 000 implant have been plotted in Fig. 5. In general, the level of GCV in the retina/choroid was significantly higher than in the vitreous at all times. In the vitreous, the maximum concentration was obtained 1 week after implantation (1.92 ± 2.12 μg/g). On the other hand, in the retina/choroid, the maximum concentration was 26.16 ± 12.70 μg/g 2 months after implantation. In the aqueous humor, GCV was below the detection limit during the study period.

The in vitro 90% effective dose (ED_{90}) of GCV for human CMV has been determined to be about 0.15–4.00 μg/ml [30]. In the vitreous, GCV concentration was maintained in the ED_{90} range for 3 months. Fortunately, in the retina/choroid, which is the target site of GCV, the concentration was greater than or within the ED_{90} range for 5 months.

It has been reported that the partition coefficient (log P) of GCV is −2.07 or −2.10 in an octanol/pH 7.4 buffered solution system at 37°C [31,32]. Conse-

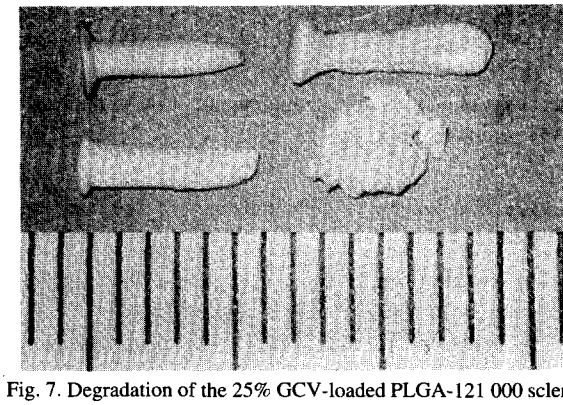

Fig. 7. Degradation of the 25% GCV-loaded PLGA-121 000 scleral implant. Upper left: before implantation; lower left: 1 month after implantation; upper right: 2 months after implantation; lower right: 3 months after implantation.

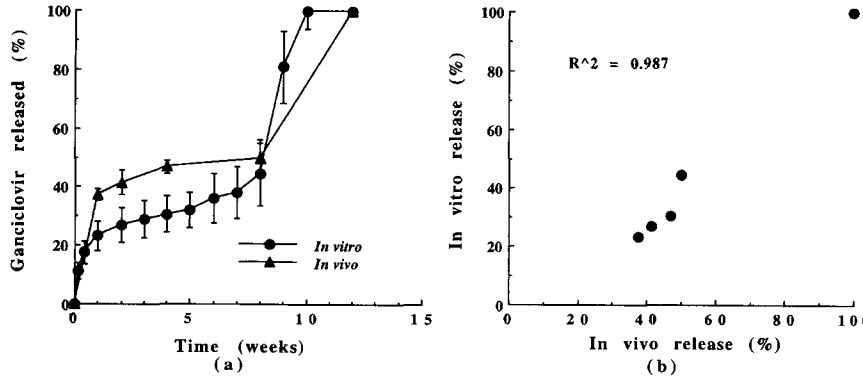

Fig. 8. Time course of the in vivo and in vitro release of GCV (a) and correlation of the in vivo and in vitro release (b).

quently, we will not discuss the good permeability of GCV into the retina/choroid by means of the hydrophilicity/lipophilicity balance.

The pharmacokinetics of drugs after intravitreal injection in normal eyes has been reviewed by Maurice [33]. Drugs are eliminated from the vitreous via two major routes: the anterior route via aqueous outflow and the retinal route. In particular, organic anions such as iodopyracet and penicillins are thought to be eliminated via the retinal route by active transport [34]. Moreover, Mahony et al. [35] reported that GCV permeates the human erythrocyte membrane primarily by the purine nucleobase carrier and secondarily by the nucleoside transporter. Since no GCV was detected in the aqueous humor in the present study, we speculated that hydrophilic GCV permeation into the retina/choroid probably occurred by these mechanisms. Also, the clearance pathway for GCV from the vitreous was mainly via the retinal route; therefore, GCV concentration was higher in the retina/choroid than in the vitreous at all times. Furthermore, the difference in times for the maximum concentration in the vitreous and in the retina/choroid after implantation can be explained by the above hypotheses.

3.3. *In vivo release of GCV and biodegradation*

The profile of in vivo GCV release at the implantation site from the 25% GCV-loaded PLGA(75/25)-121 000 scleral implant, which was estimated as the initial percentage of GCV remaining in the implant, is shown in Fig. 6. These data indicate that an initial burst occurred at the first week, followed by a diffusional phase for 2 months. Three months after implantation, GCV release was completed. These findings were similar to the in vitro release behaviors.

Fig. 6 also plots typical weight changes in PLGA after implantation of the PLGA(75/25)-121 000 scleral implant in rabbit vitreous. The weight loss had two phases: in the first phase the weight did not change (lagtime). In the second phase, the weight changed remarkably (erosion phase). The drug release from the scleral implant was much more rapid than the polymer weight change. This finding suggests that the drug can diffuse through the water channels in the polymer matrix during biodegradation, but the weight of the polymer did not change until the production of oligomers, which are small enough to be soluble in water by a random hydrolytic chain scission mechanism. The third phase of release occurred when the polymer weight loss began.

Fig. 7 demonstrates typical changes in the form of implanted PLGA scleral implant during biodegradation in the eye. During 2 months after implantation only modest deformation was observed, although the size of the implant was unchanged. All the scleral implants were separated into two pieces at the site of scleral penetration and displaced into the vitreous 10 weeks after implantation. The separated fragments stayed near the vitreous base. Biocompatibility of PLGA in the intraocular site has been reported [22,23,36–38]. In this study, we observed that the fibroblasts, neutrophils and multinucleated giant cells were infiltrating into the matrix pores in the course of degradation, but no significant inflammatory reaction was observed in the vitreous. No retinal detachment occurred around the scleral implant. At 3 months after implantation, the broken implant was swollen and had begun to disinte-

grate in the vitreous. Finally, the fragments completely disappeared from the vitreous and the subconjunctival space 5 months after implantation. We also observed the sclerotomy site had been closed with fibrous connective tissues and the infiltration of inflammatory cells had been further decreased around the sclerotomy site.

Because advanced safety of the scleral implant must be ensured in clinical application, further studies are required.

3.4. Comparison of in vitro and in vivo release

Fig. 8 compares the in vitro and in vivo release from the scleral implant. A good linear fit was obtained. The correlation coefficient (r^2) was 0.987. This finding was in agreement with a previous report on PLGA microspheres subcutaneously injected [39]. Our results indicated that PLGA was hydrolyzed by a non-enzymatic process in the vitreous and in the subconjunctiva.

4. Conclusion

We have shown that a biodegradable scleral implant provides an effective method for long-term intraocular GCV delivery in the treatment of CMV retinitis. GCV release in the vitreous from the 25% GCV-loaded PLGA(75/25)-121 000 scleral implant approximately correlated with the in vitro release. GCV concentration in the vitreous was maintained within the range of ED_{90}. In the retina/choroid, GCV concentration was greater than or within the reported range of ED_{90} for human CMV for more than 5 months after implantation in the pigmented rabbit eye.

Our study has shown that modulated release can be obtained with the scleral implant by varying the molecular weight, LA/GA ratio of PLGA and drug loading. The biodegradable scleral implant could be used as an effective intraocular drug delivery system for several months for the treatment of various vitreoretinal disorders.

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