One-Year Feasibility Study of Replenish MicroPump for Intravitreal Drug Delivery: A Pilot Study

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Purpose: To determine the feasibility of the surgical procedure and to collect some safety data regarding the bioelectronics of a novel micro drug pump for intravitreal drug delivery in a Beagle dog model for up to 1 year.

Methods: Thirteen Beagle dogs were assigned to two groups. The experimental group ($n = 11$) underwent pars plana implantation of MicroPump; the body of which was sutured episclerally, while its catheter was secured at a pars plana sclerotomy. The control group ($n = 2$) underwent sham surgeries in the form of a temporary suturing of the MicroPump, including placement of the pars plana tube. Baseline and follow-up exams included ophthalmic examination and imaging. The experimental animals were euthanized and explanted at predetermined time points after surgery (1, 3, and 12 months), while the control animals were euthanized at 3 months. All operated eyes were submitted for histopathology.

Results: Eyes were scored according to a modified McDonald-Shadduck system and ophthalmic imaging. Neither the implanted eyes nor the control eyes showed clinically significant pathological changes beyond the expected surgical changes. The operated eyes showed neither significant inflammatory reaction nor tissue ingrowth through the sclerotomy site compared with the fellow eyes.

Conclusion: This study shows that the Replenish Posterior MicroPump could be successfully implanted with good safety profile in this animal model.

Translational Relevance: The results of this study in a Beagle dog model are supportive of the biocompatibility of Replenish MicroPump and pave the way to the use of these devices for ocular automated drug delivery after further testing in larger animal models.

Introduction

Intravitreal (IVT) drug delivery of anti-angiogenic agents has revolutionized the care of patients with age-related macular degeneration (AMD),^{1–14} diabetic macular edema,^{15–21} and macular edema secondary to retinal vein occlusions.^{22–28} However, these patients require frequent office visits to receive these

injections, making it hard for many patients to comply with these therapies.^{16–21,29} In addition, serious complications such as cataract, vitreous hemorrhage, retinal detachment, and endophthalmitis may occur secondary to IVT.^{30–43}

Because of these limitations, various drug delivery systems have been proposed using a group of biodegradable and nonbiodegradable platforms to deliver anti-angiogenic drugs passively.^{44–54} However, one

limitation of these drug delivery platforms is that once implanted, they elute an unchangeable dosage of the drug and can be stopped only by explanting the device.⁴⁵

Moreover, some systems are confounded by the need to change the anti-angiogenic chemical properties (e.g., the change in anti-angiogenic properties required for them to bind to a polymer such as polyglycolic acid).^{55,56} There is neither a read out of the drug nor a way to regulate the dose short of device removal.^{57–60} Lasers have been used to release a drug from polymer to provide pulsed control with the same aforementioned shortcomings.^{61–63}

Electronic control of drug delivery for the eye has been pursued in the form of iontophoresis in which electrical current is used to drive transscleral diffusion of drug.^{58,64–72} However, this technique is potentially limited both by patient compliance and by the fact that only certain anterior parts of the eye are approachable.

Herein, we discuss the surgical procedure and biocompatibility of the first bioelectronic MicroPump (Replenish Inc., Pasadena, CA) developed for IVT drug delivery. This device is the first ocular implant that houses a rechargeable battery designed to be implanted in a manner similar to the current glaucoma drainage devices and to deliver a set volume of drug from its reservoir through a pars plana cannula into the vitreous cavity at preprogrammed periods of time.^{73–82} The MicroPump device is an example of the microelectromechanical systems (MEMS) devices being investigated for the treatment of chronic ocular diseases. The MicroPump is designed to last probably more than 5 years before needing replacement; and will deliver multiple micro-doses of a certain drug directly into the vitreous cavity such as anti-angiogenic therapy for AMD and steroid therapy for chronic uveitis. The device consists of a drug reservoir with a refill port, a battery, electronics, and an electrolysis chamber to precisely deliver the desired dosage volume when the formed gases (H₂ and O₂) generate pressure in the reservoir forcing the drug through the cannula then these gases recombine to form water when the pump is turned off. Battery and wireless inductive power transfer can be used to drive electrolysis.

Materials and Methods

Animal Preparation

Thirteen beagle dogs, 30 weeks of age or older, were selected for use as the preclinical canine eye model for the implant. All animal experiments

adhered to the regulations of the Institutional Animal Care and Use Committee of University of Southern California and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

For all animal procedures, dogs were anesthetized with intravenous propofol (4–6 mg/kg), then intubated and maintained under general anesthesia with isoflurane gas (3%) and oxygen. The pupils were dilated with topical application of phenylephrine hydrochloride 2.5% and tropicamide 0.5% eye drops.

Animal Subgroups

Animals were randomly divided into two groups: the experimental group ($n = 11$) and the control group ($n = 2$). The experimental group received the MicroPump implant with follow-up exams for up to 1 year after surgery, while the control group received the MicroPump implant followed by explantation in the same setting (i.e., sham surgery) with follow-up for 3 months after surgery (Table 1).

Ophthalmic Examination

One week before the implant surgery, all dogs had baseline ocular exams using slit-lamp biomicroscopy, tonometry, and indirect ophthalmoscopy. Clinical findings at each visit were graded according to a modified McDonald-Shadduck Ocular Scoring System.⁸³

All animals were scheduled for baseline optical coherence tomography (OCT) and fluorescein angiography (FA) imaging 1 week before surgery using a Cirrus HD-OCT Model 4000 system (Carl Zeiss Meditec, Inc., Dublin, CA). OCT acquisition protocols included retinal thickness and nerve fiber layer thickness change analysis at selected areas of the fundus.

MicroPump

This MEMS drug pump is designed to be implanted episclerally in the superotemporal quadrant.^{72–81} The pump is custom contoured (13 × 16 × 5 mm) such that its front height is reduced (after implantation, this part of the pump is 9-mm posterior to the limbus). The MicroPump has two suture tabs for fixation on its anterior aspect. The materials of the pump that contact the ocular tissue include a titanium hermetic package on the bottom, a gold telemetry coil, a polycarbonate top surface to protect the pump, and a hybrid silicone-parylene cannula with suture tabs (Fig. 1). The MicroPump used in the study has

Table 1. Time Line of the Scheduled Procedures in Referral to the Implantation Day as Day: 0

	Experimental Group	Control Group
Baseline imaging (FA, OCT)	−7 d (<i>n</i> = 11)	−7 d (<i>n</i> = 2)
Surgery	D: 0 (<i>n</i> = 11)	D: 0 (<i>n</i> = 2)
Follow-up imaging (FA, OCT)	D: 7 (<i>n</i> = 11)	D: 7 (<i>n</i> = 2)
	Mo: 1 (<i>n</i> = 11)	Mo: 1 (<i>n</i> = 2)
	Mo: 2 (<i>n</i> = 9)	Mo: 2 (<i>n</i> = 2)
	Mo: 3 (<i>n</i> = 9)	Mo: 3 (<i>n</i> = 2)
	Mo: 6 (<i>n</i> = 2)	
	Mo: 9 (<i>n</i> = 2)	
	Mo: 12 (<i>n</i> = 2)	
Euthanasia	Mo: 1 (<i>n</i> = 2)	
	Mo: 3 (<i>n</i> = 7)	Mo: 3 (<i>n</i> = 2)
	Mo: 12 (<i>n</i> = 2)	

The number in brackets indicates the number of animals subject to the corresponding procedure at that time point.

the same components of the functioning device except that the reservoir is not filled with a drug.

Implantation Tool

A custom microsurgical loading and implanting tool was engineered to hold the pump firmly without damaging its cannula or the pump body.⁸² The reusable titanium tool can be locked once it engages the pump body. Once the pump is properly positioned, a button on the tool releases the pump and the tool can be removed. The smooth, cup-shaped docking platforms of the tool firmly hold the anterior aspect of the pump.

Surgical Procedure

The right eye was used for surgery in all animals in this study. Using sterile surgical instruments under the surgical microscope, a superotemporal conjunctival peritomy was performed followed by dissection of the underlying Tenon's capsule. A sizer tool with the

Figure 1. Schematic representation of the MicroPump implanted at the pars plana position. The green arrow points to the refill port, the red arrow points to a suture tab, while the blue arrow points to the pars plana cannula.

exact shape and size of the MicroPump was positioned into the superotemporal area under the surgically created Tenon's capsule and conjunctival pocket for the purpose of creation of enough space for device implantation.⁸⁴ The MicroPump was then loaded into the custom loading tool. Once over the intended implantation site, the tool was gently unlocked, deploying the pump onto the scleral surface. The two islet suture tabs on the anterior edge were secured to the sclera 9 mm from the limbus using 8/0 Ethilon black monofilament (Ethicon Inc., Somerville, NJ). With the pump body in position, a sclerotomy was made 4 mm from the limbus with a 19-G microvitrectomy blade (Alcon Laboratories Inc., Fort Worth, TX) and the cannula tip was introduced into the vitreous cavity. Once the cannula was visualized inside the vitreous cavity, the wings were sutured with 10/0 coated polyglactin Vicryl sutures through the islets in the wing structure. Once the device was properly secured, the conjunctiva and Tenon's capsule were closed in a single layer using 8/0 Vicryl sutures. All suture materials were purchased from Ethicon Inc.

For the control group, the MicroPump was introduced in the upper temporal quadrant of the eye, then sutured and the cannula was inserted into the eye at the pars plana site and sutured; all in a manner identical to the experimental group. The device was then removed, and the sclerotomy site and the overlying conjunctiva and Tenon's capsule were closed in a manner similar to the experimental eyes.

After surgery, 1 mL of dexamethasone sodium phosphate solution (2 mg/0.5 mL) and 1 mL cefazolin (100 mg/mL) were injected in the subconjunctival space. Postoperative treatment included topical neomycin/polymyxin/bacitracin ophthalmic ointment and prednisolone acetate suspension eyedrops (Pred Forte; Allergan Inc., Irvine, CA) four times daily for 5 days to the operated eyes in both the experimental and the control groups.

Follow-Up Exams

After surgery, ophthalmic examinations and imaging were conducted at the scheduled time points (1 week, and 1, 2, 3, 6, 9, and 12 months after implant). For the experimental group, the follow-up period was extended to 1 ($n = 2$) and 3 months ($n = 7$), and 1 year ($n = 2$). For the control group, the follow-up period was extended to 3 months after surgery.

Euthanasia and Explantation

Euthanasia was performed by intravenous injection of pentobarbital (Beuthanasia-D; Schering Plough Animal Health, Omaha, NE). After euthanasia, the MicroPump devices were explanted from the experimental animals under microscopic visualization.

Light Microscopic Examination

Enucleated eyes were immersed in Davidson's fixative solution (two eyes from each group) overnight and then dehydrated in a series of graded alcohol solutions over the next 24 to 48 hours before paraffin embedding. Blocks were obtained from cuts through the whole-globe oriented perpendicularly to the optic disc. Sections 5- μ m thick were obtained by microtome, stained with hematoxylin and eosin (H&E), and examined by light microscopy. Retinal regions four disc diameters below the optic disc were photographed at the same magnification and the retinal thickness in the right eye was compared with that of the left eye. The sclerotomy site in the operated eyes was evaluated for tissue ingrowth or cellular infiltration.

Immunohistochemistry

Enucleated eyes (two eyes from each group) were fixed in 4% paraformaldehyde for 2 hours. Following the removal of the anterior segment of the globe, eyecups were fixed further in paraformaldehyde overnight and then transferred to 25% sucrose solution for an additional 24 hours. Blocks were cut from the posterior eyecup four disc diameters below the optic nerve head and embedded in optimal cutting temperature compound with 2-methylbutane and dry ice. Cryosections (5- μ m thick) were prepared at -20°C , fixed in cold methanol for 15 minutes, rinsed in 1X phosphate-buffered saline (PBS) for 5 minutes, and incubated with 10% goat serum for 45 minutes at room temperature.

All antibodies were purchased from Abcam (Cambridge, MA). The primary antibodies were anti-caspase 9 antibody (rabbit polyclonal, 1:50) and anti-acrolein antibody (mouse monoclonal immunoglobulin G [IgG], 1:100) as markers for apoptosis. Incubation with the primary antibody was performed for 1 hour at 37°C . Sections were then washed three times with 1X PBS and incubated with either Alexa Fluor 555 (anti-rabbit FITC, 1:200) or Texas red (antimouse IgG, 1:200) conjugated secondary antibody at room temperature for 1 hour. Retinal sections

Figure 2. Color photos of the right eyes of one dog from the experimental group and one from the control group 3 months after surgery, showing comparable conjunctival injection in both dogs.

were washed for 30 minutes with 0.1 M PBS and cover-slipped with mounting medium (Vectashield, Vector Laboratories Inc., Burlingame, CA) containing 4,6-diamidino-2 phenylindole (DAPI). Sections were then analyzed using a confocal microscope (LSM 510; Carl Zeiss, Inc., Thornwood, NY). Fluorescein isothiocyanate (FITC) staining was captured using the 488-nm argon laser. Texas red staining was captured using a 543-nm He-Ne laser. DAPI staining was captured using an 800-nm titanium sapphire laser. Immunofluorescence images were processed using Zeiss software (LSM-PC; Carl Zeiss Inc., Thornwood, NY). Negative controls were prepared by substituting the primary antibody with 10% normal goat serum in PBS.

Statistical Analysis

Data are shown as mean $\pm$ SD. Comparison between means was conducted by running the paired *t*-test. Statistical significance was defined as *P* less than 0.05. All analyses were conducted using the SPSS 17.0 statistical package (SPSS Inc., Chicago, IL).

Results

Ophthalmic Examination and Imaging

The intraocular portion of the cannula was not in contact with the crystalline lens. Apart from grade-I conjunctival edema and hemorrhage in three eyes (two experimental and one control) and grade-I hemorrhage localized to the vicinity of the sclerotomy site (in all eyes), which resolved 2 weeks after the implantation surgery, no other adverse event was noted (Fig. 2).

FA imaging showed no significant differences

between the right eyes (experimental and control) and left eyes at any time during the follow-up visits, namely no retinal, optic disc, or choroidal structural changes. OCT measurements of the average retinal thickness, optic disc measurements, and nerve fiber layer thickness revealed no statistically significant changes at any time point during the follow-up period when the experimental eyes were compared with both the control eyes and the left eyes (Fig. 3).

Histological Evaluation

Eyes submitted for H&E stain at different time points after surgery showed no major histopathological changes affecting the different ocular structures apart from a mild chronic inflammatory cell infiltration around the sclerotomy site with a thin band of fibrous tissue on the scleral surface (Fig. 4). The retina did not show any toxic changes such as cell necrosis, vacuolation, nerve fiber layer changes, or retinal pigment epithelium degenerative changes that can be attributed to cell damage.

Eyes submitted for immunohistochemistry showed no significant staining differences between the right and left eyes (experimental and control; Figs. 5, 6).

Discussion

To our knowledge, other than the retinal prostheses, no bioelectronic devices have been implanted chronically in and around the eye. Unlike the retinal prostheses, which require a lot more power and do so more continuously,^{85–87} the MicroPump turns on and off intermittently at the time of dosage. Also unlike the retinal prostheses, which have electrode arrays, the MicroPump has a cannula, a drug reservoir, and a pumping mechanism. Hence, although we can have

Figure 3. OCT scan taken for the right eye from of a dog from the experimental group and the control group after 3 months after surgery showing normal retinal thickness and optic nerve structure.

Figure 4. H&E-stained slide from the right eye of a dog in the experimental group 12 months after surgery, showing the posterior 2/3 of the globe (magnification is ×10). The inset (magnification is ×40) refers to the sclerotomy site, confirming the absence of any fibrous or epithelial ingrowth.

Figure 5. Photomicrographs of vertical retinal sections from the midperipheral retina (taken from the right eye of a dog from the experimental group, the right eye of a dog from the control group, and the left eye of a dog from the control group 3 months after surgery) processed for anti-acrolein immunofluorescence (A, D, G) with DAPI nuclear counterstaining shown in images (B, E, H), and merged images (C, F, I). The secondary antibody was Texas red-conjugated anti-mouse IgG. Images show comparable expression of acrolein in the three eyes. Scale bar, 50 μ m. GCL, ganglion cell layer; IPL, inner plexiform layer; OPL, outer plexiform layer; IS, inner segments of the photoreceptor layer.

some level of comfort that retinal prostheses are tolerated in and around the eye, it is certainly not evident that a bioelectronic MicroPump will also be well tolerated. Demonstration of the compatibility of an implanted ocular medical device is a difficult task, especially when many of the materials have never been used in and around the ocular tissue.

Before proceeding to the animal studies, the MicroPump materials underwent extensive biocompatibility testing in accord with International Organization for Standardization (ISO) 10,993 using in vitro and in vivo tests. The particular design and engineering of the MicroPump took into consideration the special curved geometry of the eyeball and the relatively small subconjunctival space for implantation. Microelectronics are not made on curved platforms, and this was a special challenge to make the base of the pump curved to match the contour of the eye wall. In addition, certain microelectronic and mechanical surfaces are rough and toxic and can

mechanically or chemically degrade the eye tissue; hence, the MicroPump also had to take this constraint into its design.

The surgical implantation procedure and tool had to be designed to safely implant the MicroPump without damaging it. Unlike glaucoma setons, which are purely mechanical and can sustain a fair amount of pulling on the cannula and body, our MicroPump with its electronics and electronic sensors cannot be handled with as much force. The tool design had to take into consideration the handling requirements of the soft tissue surrounding the globe as well. The tool needed to have smooth edges at a certain angle so that it could easily slide back out of the pocket after the pump was placed in the implant area without being trapped or stuck in the surrounding tissue.

Moreover, our MicroPump is taller than the glaucoma setons^{88–91}; and therefore, we had to contour the top surface to dramatically reduce the height of the front of the device, thereby reducing the

Figure 6. Photomicrographs of vertical retinal sections from midperipheral retina (taken from the right eye of a dog from the experimental group, the right eye of a dog from the control group, and the left eye of a dog from the control group 3 months after surgery) processed for anticaspase immunofluorescence (A, D, G) with DAPI nuclear counterstaining shown in images (B, E, H) and merged images (C, F, I). The secondary antibody was FITC-conjugated anti-rabbit IgG. Images show comparable expression of caspase-9 in the three eyes. Scale bar, 50 μ m.

risk of extrusion. This study further confirmed our engineering design as neither any of the MicroPumps partially or completely extruded nor there was any conjunctival thinning. As the human conjunctiva, especially in the elderly, is thinner than that of young dogs, we will have to finally verify this aspect of the MicroPump in human patients. Moreover unlike most glaucoma setons,^{90,91} our cannula is closed and only opens intermittently to deliver drug into the eye. This was confirmed by the fact that no fluid egress from the eye was seen and no blebs were observed in any of the implanted eyes. This bodes well as large filtering blebs, reported with glaucoma setons, have been associated with a greater incidence of endophthalmitis.^{90,91}

It is important to note that the beagle eye is approximately 15% smaller in diameter than the human eye with a lens that is 50% thicker in size.^{92,93} However, these differences were acceptable, because

we wanted to make a device with no likelihood of touching the crystalline lens.

Histology findings of minimal chronic inflammation and fibrous capsule around the MicroPump were expected. Such fibrous capsules are found around glaucoma setons and scleral buckles. Also, there was no evidence of fibrous ingrowth at the cannula insertion site. Fibrous ingrowth is an uncommon complication of glaucoma setons.^{94–97} We anticipated even less incidence if at all with the MicroPump, not only because of the meticulous surgical dissection of conjunctiva and Tenon's away from the sclerotomy site, but also because the cannula does not drain fluid, and therefore does not lead to hypotony, which has been associated with increased risk of epithelial ingrowth.

Our study has some drawbacks. First, the size of the study makes it too small to give a strong conclusion regarding the safety of the MicroPump in this animal model. However, this was a pilot study

and further testing of the MicroPump is required in a larger study to support these data.

Second, the device was never activated during the study is one of the study limitations. Although our device met the acceptance criteria of biological risk assessment in accordance with ISO 10,993-1:2009 (cytotoxicity, sterilization, ocular irritation, sub-chronic toxicity IP 30 day toxicity, and implantation tests; data not shown), it is not known whether activation of a fibroblastic response around the device can incur from the electromagnetic field when the device is turned on, and hence the importance of further studies with the device on to evaluate this possibility.

Third, the cost of such technology to deliver drugs into the vitreous cavity may be a limitation when compared with the cost of IVT when using the regular syringe and needle. However, when dealing with patients with wet AMD as an example of the device application, monthly IVT needle injection presents a risk as well as a burden to patients. Potential serious risk associated with each IVT procedure includes traumatic cataract, retinal detachment, and endophthalmitis.^{30–43} Moreover, monthly treatment or monitoring, which may continue for a patient's lifetime, is a major burden to patients, their caregivers, and the healthcare system. On the other hand, our study of the efficacy of microdosing of Lucentis has shown comparable results to the full dose (unpublished data). Besides, novel emerging anti-vascular endothelial growth factor therapy that can be administered in micro-doses are on their way to the market. Microdosage is expected to increase the safety profile of the administered drug, increases the interval between our pump refills, and hence improves the patient compliance with the refill procedure.

Fourth, it has been a concern that the pars plana clip may create a path for fluid drainage or fibrous/epithelial ingrowth. However, our cannula design is essentially the same as the one used with Ahmed valve (New World Medical, Rancho Cucamonga, CA) designed for pars plana implantation. The latter did not show any of these adverse effects since its introduction in clinical use.^{98–102} Moreover, our study did not show any of these complications either.

Fifth, one of the most common problems with implanted devices such as this would be conjunctival erosion over the device or the tube and we do not exactly know the actual incidence of such complications with our device as the number of animals that had the tube implanted for more than 3 months was

only two so that the safety of the device related to such complications from this study is limited.

Last, the duration of our study may not be enough to test the long-term safety profile of a device that may be left in place for 5 years or so. Hence, further studies are required to assess the possibility of development of any complication after years of implantation of the MicroPump.

Finally, two questions may arise. First, whether a patch graft (e.g., preserved sclera) is needed to protect the sclerotomy site. However, we did not feel there was a need for that, as we did not see any conjunctival erosion in our study. Furthermore, the sclerotomy site is away from the limbus and not exposed to much eyelid rubbing as seen with setons.

Second, it is still an unproven assumption that drug delivery to the posterior segment of the eye is better with needle injection compared with drug infusion through a pars plana device based on drug delivery deep into the vitreous gel. However, the MicroPump can assure a steady state concentration of the drug in the vitreous cavity (data not shown), as well as allowing repeated microdosing of the drug while avoiding the aforementioned complications associated with repeated needle injections.

Conclusion

The implantation of a novel bioelectronics MicroPump for IVT drug delivery has been shown to be feasible and the safety data collected so far both clinically and pathologically in this pilot animal model are sound. Larger studies are required to test the safety of this pump over longer follow-up periods before testing both the safety and efficacy of this pump in patients.

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References

- Melamud A, Stinnett S, Fekrat S. Treatment of neovascular age-related macular degeneration with intravitreal bevacizumab: efficacy of three consecutive monthly injections. *Am J Ophthalmol*. 2008;146:91–95.
- Heier JS, Boyer D, Nguyen QD, et al. The 1-year results of CLEAR-IT 2, a phase 2 study of vascular endothelial growth factor trap-eye dosed as-needed after 12-week fixed dosing. *Ophthalmology*. 2011;118:1098–1106.
- Keane PA, Sadda SR. Development of anti-VEGF therapies for intraocular use: a guide for clinicians. *J Ophthalmol*. 2012;2012:483034.
- Lally DR, Gerstenblith AT, Regillo CD. Preferred therapies for neovascular age-related macular degeneration. *Curr Opin Ophthalmol*. 2012;23:182–188.
- Kovach JL, Schwartz SG, Flynn HW Jr, Scott IU. Anti-VEGF treatment strategies for wet AMD. *J Ophthalmol*. 2012;2012:786870.
- Verner-Cole EA, Davis SJ, Lauer AK. Aflibercept for the treatment of neovascular age-related macular degeneration. *Drugs Today*. 2012;48:317–329.
- Kaiser PK. Emerging therapies for neovascular age-related macular degeneration: drugs in the pipeline. *Ophthalmology*. 2013;120:S11–S15.
- Tozer K, Roller AB, Chong LP, et al. Combination therapy for neovascular age-related macular degeneration refractory to anti-vascular endothelial growth factor agents. *Ophthalmology*. 2013;120:2029–2034.
- Fong AH, Lai TY. Long-term effectiveness of ranibizumab for age-related macular degeneration and diabetic macular edema. *Clin Interv Aging*. 2013;8:467–483.
- Michels S, Rosenfeld PJ. Treatment of neovascular age-related macular degeneration with ranibizumab/Lucentis [article in German]. *Klin Monbl Augenheilkd*. 2005;222:480–484.
- Michels S, Schmidt-Erfurth U, Rosenfeld PJ. Promising new treatments for neovascular age-related macular degeneration. *Expert Opin Investig Drugs*. 2006;15:779–793.
- Holz FG, Helb HM, Bindewald-Wittich A, Scholl HP. Modern pharmacotherapy of age-related macular degeneration. *Internist (Berl)*. 2006;47:192–198.
- Zhou B, Wang B. Pegaptanib for the treatment of age-related macular degeneration. *Exp Eye Res*. 2006;83:615–619.
- Eter N, Krohne TU, Holz FG. New pharmacologic approaches to therapy for age-related macular degeneration. *BioDrugs*. 2006;20:167–179.
- Pearson PA, Comstock TL, Ip M, et al. Fluocinolone acetonide intravitreal implant for diabetic macular edema: a 3-year multicenter, randomized, controlled clinical trial. *Ophthalmology*. 2011;118:1580–1587.
- Bandello F, Berchicci L, La Spina C, Battaglia Parodi M, Iacono P. Evidence for anti-VEGF treatment of diabetic macular edema. *Ophthalmic Res*. 2012;48(suppl 1):16–20.
- Al Shamsi H, Ghazi NG. Diabetic macular edema: new trends in management. *Expert Rev Clin Pharmacol*. 2012;5:55–68.
- Azad R, Sain S, Sharma YR, Mahajan D. Comparison of intravitreal bevacizumab, intravitreal triamcinolone acetonide, and macular grid augmentation in refractory diffuse diabetic macular edema: a prospective, randomized study. *Oman J Ophthalmol*. 2012;5:166–170.
- Ho AC, Scott IU, Kim SJ, et al. Anti-vascular endothelial growth factor pharmacotherapy for diabetic macular edema: a report by the American Academy of Ophthalmology. *Ophthalmology*. 2012;119:2179–2188.
- Zechmeister-Koss I, Huic M. Vascular endothelial growth factor inhibitors (anti-VEGF) in the management of diabetic macular oedema: a systematic review. *Br J Ophthalmol*. 2012;96:167–178.
- Thomas BJ, Shienbaum G, Boyer DS, Flynn HW Jr. Evolving strategies in the management of diabetic macular edema: clinical trials and current management. *Can J Ophthalmol*. 2013;48:22–30.
- Ramchandran RS, Fekrat S, Stinnett SS, Jaffe GJ. Fluocinolone acetonide sustained drug delivery device for chronic central retinal vein occlusion: 12-month results. *Am J Ophthalmol*. 2008;146:285–291.
- Jonas J, Paques M, Monés J, Glacet-Bernard A. Retinal vein occlusions. *Dev Ophthalmol*. 2010;47:111–135.
- Nghiem-Buffet S, Cohen SY. Retinal vein occlusion: anti-VEGF treatments [article in French]. *J Fr Ophtalmol*. 2009;32:679–686.
- Höh AE, Schaal KB, Dithmar S. Central and branch retinal vein occlusion. Current strategies for treatment in Germany, Austria and Switzerland [article in German]. *Ophthalmologe*. 2007;104:290–294.

26. Schaal KB, Höh AE, Scheuerle A, Schutt F, Dithmar S. Bevacizumab for the treatment of macular edema secondary to retinal vein occlusion [article in German]. *Ophthalmologe*. 2007;104:285–289.
27. Stahl A, Agostini H, Hansen LL, Feltgen N. Bevacizumab in retinal vein occlusion-results of a prospective case series. *Graefes Arch Clin Exp Ophthalmol*. 2007;245:1429–1436.
28. Brand CS. Management of retinal vascular diseases: a patient-centric approach. *Eye*. 2012;26(suppl 2):S1–S16.
29. Ip MS, Domalpally A, Hopkins JJ, Wong P, Ehrlich JS. Long-term effects of ranibizumab on diabetic retinopathy severity and progression. *Arch Ophthalmol*. 2012;130:1145–1152.
30. Jeganathan VS, Verma N. Safety and efficacy of intravitreal anti-VEGF injections for age-related macular degeneration. *Curr Opin Ophthalmol*. 2009;20:223–225.
31. Sampat KM, Garg SJ. Complications of intravitreal injections. *Curr Opin Ophthalmol*. 2010;21:178–183.
32. Roth DB, Realini T, Feuer WJ, et al. Short-term complications of intravitreal injection of triamcinolone acetonide. *Retina*. 2008;28:66–70.
33. Gelissen F, Ziemssen F, Voelker M, Bartz-Schmidt KU, Inhoffen W. Retinal pigment epithelial tears after single administration of intravitreal bevacizumab for neovascular age-related macular degeneration. *Eye*. 2009;23:694–702.
34. Kook D, Wolf A, Neubauer AS, et al. Retinal pigment epithelial tears after intravitreal injection of bevacizumab for AMD. Frequency and progress [article in German]. *Ophthalmologe*. 2008;105:158–164.
35. Aiello LP, Brucker AJ, Chang S, et al. Evolving guidelines for intravitreal injections. *Retina*. 2004;24:S3–S19.
36. Weber M, Cohen SY, Tadayoni R, et al. Evolving intravitreal injection technique [article in French]. *J Fr Ophthalmol*. 2008;31:625–629.
37. Konstantopoulos A, Williams CP, Newsom RS, Luff AJ. Ocular morbidity associated with intravitreal triamcinolone acetonide. *Eye*. 2007;21:317–320.
38. Jager RD, Aiello LP, Patel SC, Cunningham ET Jr. Risks of intravitreal injection: a comprehensive review. *Retina*. 2004;24:676–698.
39. El-Ashry MF, Dhillon B. The article by Fintak et al on the incidence of endophthalmitis related to intravitreal injection of bevacizumab and ranibizumab. *Retina*. 2009;29:720–721.
40. Fintak DR, Shah GK, Blinder KJ, et al. Incidence of endophthalmitis related to intravitreal injection of bevacizumab and ranibizumab. *Retina*. 2008;28:1395–1399.
41. Mason JO III, White MF, Feist RM, et al. Incidence of acute onset endophthalmitis following intravitreal bevacizumab (Avastin) injection. *Retina*. 2008;28:564–567.
42. Ranchod TM, Leverenz VR, Chintala SK, Cheng M, Trese MT, Giblin FJ. Re: Infectious endophthalmitis after intravitreal injection of antiangiogenic agents. *Retina*. 2010;30:1752–1754.
43. Diago T, McCannel CA, Bakri SJ, Pulido JS, Edwards AO, Pach JM. Infectious endophthalmitis after intravitreal injection of antiangiogenic agents. *Retina*. 2009;29:601–605.
44. Hsu J. Drug delivery methods for posterior segment disease. *Curr Opin Ophthalmol*. 2007;18:235–239.
45. Zhang K, Zhang L, Weinreb RN. Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. *Nat Rev Drug Discov*. 2012;11:541–559.
46. Kyyrönen K, Hume L, Benedetti L, Urtti A, Topp E, Stella V. Methylprednisolone esters of hyaluronic acid in ophthalmic drug delivery: in vitro and in vivo release studies. *Int J Pharm*. 1992;80:161–169.
47. Okabe K, Kimura H, Okabe J, Kato A, Kunou N, Ogura Y. Intraocular tissue distribution of betamethasone after intrascleral administration using a non-biodegradable sustained drug delivery device. *Invest Ophthalmol Vis Sci*. 2003;44:2702–2707.
48. Yasukawa T, Ogura Y, Sakurai E, Tabata Y, Kimura H. Intraocular sustained drug delivery using implantable polymeric devices. *Adv Drug Deliv Rev*. 2005;57:2033–2046.
49. Daull P, Paterson CA, Kuppermann BD, Garrigue JS. A preliminary evaluation of dexamethasone palmitate emulsion: a novel intravitreal sustained delivery of corticosteroid for treatment of macular edema. *J Ocul Pharmacol Ther*. 2013;29:258–269.
50. Haghjou N, Soheilian M, Abdekhodaie MJ. Sustained release intraocular drug delivery devices for treatment of uveitis. *J Ophthalmic Vis Res*. 2011;6:317–329.
51. Hunter RS, Lobo AM. Dexamethasone intravitreal implant for the treatment of noninfectious uveitis. *Clin Ophthalmol*. 2011;5:1613–1621.

52. London NJ, Chiang A, Haller JA. The dexamethasone drug delivery system: indications and evidence. *Adv Ther.* 2011;28:351–366.
53. Schwartz SG, Flynn HW Jr. Fluocinolone acetonide implantable device for diabetic retinopathy. *Curr Pharm Biotechnol.* 2011;12:347–351.
54. Campochiaro PA, Hafiz G, Shah SM, et al. Sustained ocular delivery of fluocinolone acetonide by an intravitreal insert. *Ophthalmology.* 2010;117:1393–1399, e1393.
55. Murata N, Takashima Y, Toyoshima K, Yamamoto M, Okada H. Anti-tumor effects of anti-VEGF siRNA encapsulated with PLGA microspheres in mice. *J Control Release.* 2008;126:246–254.
56. Carrasquillo KG, Ricker JA, Rigas IK, Miller JW, Gragoudas ES, Adamis AP. Controlled delivery of the anti-VEGF aptamer EYE001 with poly(lactic-co-glycolic)acid microspheres. *Invest Ophthalmol Vis Sci.* 2003;44:290–299.
57. Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverge R. Ophthalmic drug delivery systems—recent advances. *Prog Retin Eye Res.* 1998;17:33–58.
58. Behar-Cohen F. Drug delivery to target the posterior segment of the eye [article in French]. *Med Sci (Paris).* 2004;20:701–706.
59. Bourges JL, Bloquel C, Thomas A, et al. Intraocular implants for extended drug delivery: therapeutic applications. *Adv Drug Deliv Rev.* 2006;58:1182–1202.
60. Mundada AS, Shrikhande BK. Design and evaluation of soluble ocular drug insert for controlled release of ciprofloxacin hydrochloride. *Drug Dev Ind Pharm.* 2006;32:443–448.
61. Kapoor KG, Shultz RW, Iezzi R. Vitreous pillow for sustained-release intravitreal drug delivery implants. *Ophthalmic Surg Lasers Imaging Retina.* 2013;44:181–182.
62. Liu S, Jones L, Gu FX. Nanomaterials for ocular drug delivery. *Macromol Biosci.* 2012;12:608–620.
63. Mishra GP, Bagui M, Tamboli V, Mitra AK. Recent applications of liposomes in ophthalmic drug delivery. *J Drug Deliv.* 2011;2011:863734.
64. Cohen AE, Assang C, Patane MA, From S, Korenfeld M; for the Avion Study Investigators. Evaluation of dexamethasone phosphate delivered by ocular iontophoresis for treating noninfectious anterior uveitis. *Ophthalmology.* 2012;119:66–73.
65. Eljarrat-Binstock E, Orucov F, Aldouby Y, Frucht-Pery J, Domb AJ. Charged nanoparticles delivery to the eye using hydrogel iontophoresis. *J Control Release.* 2008;126:156–161.
66. Güngör S, Delgado-Charro MB, Ruiz-Perez B, et al. Trans-scleral iontophoretic delivery of low molecular weight therapeutics. *J Control Release.* 2010;147:225–231.
67. Kompella UB, Kadam RS, Lee VH. Recent advances in ophthalmic drug delivery. *Ther Deliv.* 2010;1:435–456.
68. Li SK, Liddell MR, Wen H. Effective electrophoretic mobilities and charges of anti-VEGF proteins determined by capillary zone electrophoresis. *J Pharm Biomed Anal.* 2011;55:603–607.
69. Molokhia SA, Jeong EK, Higuchi WI, Li SK. Transscleral iontophoretic and intravitreal delivery of a macromolecule: study of ocular distribution in vivo and postmortem with MRI. *Exp Eye Res.* 2009;88:418–425.
70. Patane MA, Cohen A, From S, Torkildsen G, Welch D, Ousler GW III. Ocular iontophoresis of EGP-437 (dexamethasone phosphate) in dry eye patients: results of a randomized clinical trial. *Clin Ophthalmol.* 2011;5:633–643.
71. Rawas-Qalaji M, Williams CA. Advances in ocular drug delivery. *Curr Eye Res.* 2012;37:345–356.
72. Singh RP, Mathews ME, Kaufman M, Riga A. Transcleral delivery of triamcinolone acetonide and ranibizumab to retinal tissues using macrophages. *Br J Ophthalmol.* 2010;94:170–173.
73. Pang C, Jiang F, Shih J, Caffey S, Humayun M, Tai YC, inventors; Manufacturer, assignee. *MiniPumps.* US Patent 08,529,538. September 10, 2013.
74. Pang C, Jiang F, Shih J, Caffey S, Humayun M, Tai YC, inventors; Manufacturer, assignee. *MiniPumps.* US Patent 08,486,278. July 16, 2013.
75. Pang C, Jiang F, Shih J, Caffey S, Humayun M, Tai YC, inventors; Manufacturer, assignee. *MiniPumps.* US Patent 20,120,323,218. December 20, 2012.
76. Pang C, Jiang F, Shih J, Caffey S, Humayun M, Tai YC, inventors; Manufacturer, assignee. *MiniPumps.* US Patent 08,231,608. July 31, 2012.
77. Pang C, Jiang F, Shih J, Caffey S, Humayun M, Tai YC, inventors; Manufacturer, assignee. *MiniPumps.* US Patent 08,231,609. July 31, 2012.
78. Pang C, Jiang F, Shih J, et al., inventors; Manufacturer, assignee. *MiniPumps.* US Patent 20,090,306,594-A1. December 10, 2009.

79. Pang C, Jiang F, Shih J, Caffey S, Humayun M, Tai YC, inventors; Manufacturer, assignee. *MiniPumps*. US Patent 20,090,311,133. December 17, 2009.
80. Pang C, Jiang F, Shih J, Caffey S, Humayun M, Tai YC, inventors; Manufacturer, assignee. *MiniPumps*. US Patent 20,090,312,742. December 17, 2009.
81. Pang C, Shih J, Jiang F, Caffey S, Humayun M, Tai YC, inventors; Manufacturer, assignee. *MiniPumps*. US Patent 20,090,306,585. December 10, 2009.
82. Journey M, Caffey S, Humayun M, Huculak J, inventors; Manufacturer, assignee. *MiniPumps*. US Patent 20,130,018,412. January 17, 2013.
83. Altmann S, Emanuel A, Toomey M, et al. A quantitative rabbit model of vaccinia keratitis. *Invest Ophthalmol Vis Sci*. 2010;51:4531–4540.
84. Journey ML, Shih J, Caffey S, Harbers G, inventors; Manufacturer, assignee. *Replenish*. US Patent 20,130,090,676. April 11, 2013.
85. Lazzi G. Thermal effects of bioimplants. *IEEE Eng Med Biol Mag*. 2005;24:75–81.
86. Opie NL, Burkitt AN, Meffin H, Grayden DB. Thermal heating of a retinal prosthesis: thermal model and in-vitro study. *Conf Proc Eng Med Biol Soc*. 2010;2010:1597–1600.
87. Opie NL, Greferath U, Vessey KA, et al. Retinal prosthesis safety: alterations in microglia morphology due to thermal damage and retinal implant contact. *Invest Ophthalmol Vis Sci*. 2012;53:7802–7812.
88. Aref AA, Gedde SJ, Budenz DL. Glaucoma drainage implant surgery. *Dev Ophthalmol*. 2012;50:37–47.
89. Aminlari AE, Scott IU, Aref AA. Glaucoma drainage implant surgery - an evidence-based update with relevance to sub-Saharan Africa. *Middle East Afr J Ophthalmol*. 2013;20:126–130.
90. Patel S, Pasquale LR. Glaucoma drainage devices: a review of the past, present, and future. *Semin Ophthalmol*. 2010;25:265–270.
91. Reinthal EK, Rohrbach JM, Grisanti S. Glaucoma drainage implants [article in German]. *Klin Monbl Augenheilkd*. 2010;227:49–55.
92. Tuntivanich N, Petersen-Jones SM, Steibel JP, Johnson C, Forcier JQ. Postnatal development of canine axial globe length measured by B-scan ultrasonography. *Vet Ophthalmol*. 2007;10:2–5.
93. Williams DL. Lens morphometry determined by B-mode ultrasonography of the normal and cataractous canine lens. *Vet Ophthalmol*. 2004;7:91–95.
94. Ho JC, Yuen NS, Chan CW. Capsule membrane growth complicating glaucoma and corneal decompensation after neodymium: YAG capsulotomy. *J Cataract Refract Surg*. 2012;38:1492–1494.
95. Jacob JT, Burgoyne CF, McKinnon SJ, Tanji TM, LaFleur PK, Duzman E. Biocompatibility response to modified Baerveldt glaucoma drains. *J Biomed Mater Res*. 1998;43:99–107.
96. Sidoti PA, Minckler DS, Baerveldt G, Lee PP, Heuer DK. Epithelial ingrowth and glaucoma drainage implants. *Ophthalmology*. 1994;101:872–875.
97. Bayer A, Wilson RR, Eagle RC Jr. Tube occlusion from the external ostium after implantation of an aqueous shunt. *Ophthalmic Surg Lasers Imaging*. 2002;33:493–496.
98. Wallsh JO, Gallemore RP, Taban M, Hu C, Sharareh B. Pars plana Ahmed valve and vitrectomy in patients with glaucoma associated with posterior segment disease. *Retina*. 2013;33:2059–2068.
99. García-Delpech S, Sanz-Marco E, Martínez-Castillo S, et al. Ahmed valve, suture-less implantation: a new approach to an easier technique. *J Glaucoma*. 2013;22:750–756.
100. Diaz-Llopis M, Salom D, García-Delpech S, Udaondo P, Millan JM, Arevalo JF. Efficacy and safety of the pars plana clip in the Ahmed valve device inserted via the pars plana in patients with refractory glaucoma. *Clin Ophthalmol*. 2010;4:411–416.
101. Faghihi H, Hajizadeh F, Mohammadi SF, Kadkhoda A, Peyman GA, Riazi-Esfahani M. Pars plana Ahmed valve implant and vitrectomy in the management of neovascular glaucoma. *Ophthalmic Surg Lasers Imaging*. 2007;38:292–300.
102. Schlote T, Ziemssen F, Bartz-Schmidt KU. Pars plana-modified Ahmed Glaucoma Valve for treatment of refractory glaucoma: a pilot study. *Graefes Arch Clin Exp Ophthalmol*. 2006;244:336–341.