Development of an Experimental Drug Eluting Suprachoroidal Microstent as Glaucoma Drainage Device

Marina Hovakimyan1*, Stefan Siewert1*, Wolfram Schmidt1, Katrin Sternberg1, Thomas Reske1, Oliver Stachs2, Rudolf Guthoff1, Andreas Wree3, Martin Witt3, Klaus-Peter Schmitz1, and Reto Allemann2

1 Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany
2 Department of Ophthalmology, Rostock University Medical Center, Rostock, Germany
3 Department of Anatomy, Rostock University Medical Center, Rostock, Germany

Correspondence: Marina Hovakimyan, Institute for Biomedical Engineering, Rostock University Medical Centre, Friedrich-Barnewitz Strasse 4, D-18119 Rostock, Germany. e-mail: marina.hovakimyan@med.uni-rostock.de

Received: 26 February 2015
Accepted: 28 April 2015
Published: 30 June 2015

Keywords: glaucoma; experimental models; drainage devices


Purpose: A novel glaucoma drainage device (GDD) with local drug delivery (LDD) system was created and characterized for safety and effectiveness after implantation into the suprachoroidal space (SCS) of rabbit eyes.

Methods: Thin films of two different polymers, Poly(3-hydroxybutyrate) (P(3HB)) and Poly(4-hydroxybutyrate) (P(4HB)), containing the drugs mitomycin C (MitC) or paclitaxel (PTX) were attached to silicone-tubes to create LDD devices. The release kinetics of these drugs were explored in vitro using high performance liquid chromatography (HPLC).

Twenty-four New Zealand white rabbits, randomly divided into eight groups, were implanted with different kinds of microstents into SCS. The intraocular pressure (IOP) was monitored noninvasively. After 6 weeks, rabbits were sacrificed and enucleated eyes were used for anterior segment optical coherence tomography (OCT), micro magnetic resonance imaging (MRI), and histology.

Results: In vitro, faster drug release from both polymers was observed for MitC compared to PTX. Comparing polymers, the release from P(3HB) matrix was slower for both drugs. MRI and OCT showed all implants maintained a proper location. An effective IOP reduction was observed for up to 6 weeks in eyes with microstents combined with a drug-releasing LDD system. Overall, the surrounding tissue revealed mild-to-moderate inflammation. No pronounced fibrosis was observed in any of the groups. However, both drugs caused damage to the neighboring retina.

Conclusions: The suprachoroidal microstent reduced IOP with mild inflammation in rabbit eyes. To avoid negative effects on the retina, it is necessary to identify novel drugs with less cytotoxicity. Future studies are needed to explore the fibrotic process over the long-term.

Translational Relevance: The presented data serve as a proof of principle study for the concept of a suprachoroidal drug eluting microstent. Future device improvements will be focused on the design of LDD systems and the use of specific anti-inflammatory or antifibrotic agents with less cytotoxicity compared to MitC or PTX. Long-term animal studies using a reliable glaucoma model will be a further step towards clinical application and improvement of surgical glaucoma therapy.

Introduction

Glaucoma refers to a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells and irreversible destruction of the optic nerve.1,2

Affecting more than 60 million people worldwide, glaucoma is one of the leading causes of visual disability.3,4 According to Quigley and Broman,5 the incidence of glaucoma will increase to almost 80 million people by 2020, resulting in blindness for 11.2 million people.

Increased intraocular pressure (IOP) caused by
impaired outflow of aqueous humor (AH) is considered to be the most significant but not the only risk factor for the development of glaucoma. Currently, the management options for lowering IOP include medication and laser and incisional surgery. Alternatively, glaucoma drainage devices (GDDs) have been gaining an increasing interest in the recent years. Unfortunately, GDDs are not without complications, such as tube obstruction or migration, encapsulation, and late failure. In spite of the success rate ranging between 60 and 90% in the first 2 years after implantation, the literature reports an approximately 10% annual failure rate in the subsequent 3 years, regardless of the type of implant used. While the implant design has been maximally optimized to overcome postoperative complications, the postoperative fibrotic processes, causing device encapsulation and failure, are still very difficult to manage.

Recently, the attention of researchers and clinicians has been shifted to the suprachoroidal space (SCS) as a compartment, in which AH can be drained. The SCS is the interface between the external surface of the choroid and the internal surface of the sclera. Investigations on the hydrostatic pressure of the SCS have shown a pressure drop between the anterior chamber and the SCS. This pressure difference was weaker in the limbal region, and increased toward the optic nerve. Regarding drainage, two possible pathways have been postulated: firstly through scleral pores and via the episcleral venous plexus, and secondly via choroidal resorption. Based on these considerations and because of the negative pressure gradient, an implant connecting the anterior chamber with the SCS should be able to drain AH and lower IOP. Therefore, we believe that implantation of a microstent to drain AH might be the most promising way to achieve sustained lowering of IOP.

Targeting the SCS with surgery is not a new idea. In 1900, Fuchs and Heine described the formation of a cyclodialysis cleft, which enabled seemingly endless fluid to escape the anterior chamber. Until the early 2000s, cyclodialysis lost favor and was almost completely outdated by trabeculectomy because of unacceptable side effects including severe intra- and postoperative hemorrhage and profound hypotony. However, more recently, this approach has started to gain increasing attention as a target site for GDD implantation.

In the present study, a novel tubular microstent, draining the AH from the anterior chamber into the SCS was developed and experimentally examined in terms of safety and effectiveness in reducing the IOP (Fig. 1). Furthermore, we combined the tube with a local drug delivery (LDD) device, consisting of thin films of biodegradable polymers with incorporated antiproliferative drugs mitomycin C (MitC) or paclitaxel (PTX). Both drugs have previously been shown to inhibit proliferation, migration, and collagen production of ocular fibroblasts in vitro and in vivo. The drug-containing polymeric films were attached to the ends of the microstents as reservoirs for drug release.

Methods

Fabrication of Microstents

Microstents were manufactured from two components: silicone tube (Silastic Rx-50 Medical Grade Tubing, Dow Corning Corporation, Midland, MI; internal diameter (ID) = 0.3 µm, outside diameter (OD) = 0.64 µm, l = 10 mm) and LDD device. LDD devices were based on the biodegradable polymers Poly(3-hydroxybutyrate) (P(3HB); Helmholtz-Zentrum für Umweltforschung UFZ, Leipzig, Germany) or Poly(4-hydroxybutyrate) (P(4HB); TeDpha, Inc, Lexington, MA) in combination with the cytostatic drugs PTX (Cfm Oskar Tropitzsch e.K., Marktredwitz, Germany) and MitC (Sigma Aldrich Corporation, St. Louis, MO). Combining polymers and drugs resulted in a total number of six different LDD devices (Table 1). Polymer films were manufactured by spray coating of polymer-drug solutions on a glass substrate for MitC or by casting of polymer-drug solutions into petri dishes for PTX. Solutions were prepared in chloroform (Sigma Aldrich Corporation). The desired film thickness was approximately...
To remove residual chloroform, films were dried for 7 days at 40°C in vacuum. Afterwards LDD devices with a diameter of \( D = 3 \) mm were stamped and total mass was weighted (XP6U ultra-microbalance, Mettler-Toledo International, Inc, Greifensee, Switzerland). The incorporated initial drug mass was calculated based on the total mass of the LDD device at a polymer:drug ratio of 85/15% (w/w), respectively.

LDD devices were fixed to the distal end of the silicone tubes, and assembled microstents were sterilized by ethylene oxide for 3 hours (43°C, 2.6 bar). Venting was conducted for 5 hours (43°C, 0.05 bar) and finally for 3 days (37°C, ambient pressure).

For morphological analysis of manufactured microstents electron microscopy was performed (Philips XL 30 ESEM, Philips Electron Optics, The Netherlands) in environmental scanning electron microscopy (ESEM) mode at a vacuum pressure of 1.2 mbar and an accelerating voltage of 10 kV.

### Drug Release In Vitro

Analysis of temporal drug release of PTX and MitC from fabricated LDD devices was performed in vitro in 2 mL Dulbecco’s phosphate buffered saline (DPBS; Morphisto Evolutionsforschung und Anwendung GmbH, Frankfurt am Main, Germany) at ambient temperature. During drug release studies, specimens were stored on a rotating platform-shaking device (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 100 rpm. After a defined period \( \Delta t_i \) DPBS was exchanged and drug content \( m_i(\Delta t_i) \) was analyzed using high performance liquid chromatography (HPLC; Wissenschaftliche Gerätebau Dr. Ing. Herbert Knauer GmbH, Berlin, Germany). After \( j \) repeated exchanges cumulative released drug mass \( m_j \) was calculated as follows.

\[
m_j = \sum_{i=0}^{j} m_i(\Delta t_i)
\]

Finally, in case of stagnated drug release, the residual drug was extracted by methanol and analyzed by HPLC.

For MitC and PTX analysis, a Eurospher 100 C18, 5 \( \mu \)m, 125 \( \times \) 4 mm ID column (Knauer GmbH, Berlin, Germany) and a Chromolith FastGradient RP-18e 50-2 column (Merck KGaA, Darmstadt, Germany) were used, respectively. HPLC of MitC was conducted isocratically at 22°C, with a mixture of acetonitrile and water (15/85% v/v) as mobile phase, at a flow rate of 1.0 mL min\(^{-1}\) and an ultraviolet (UV) detection wavelength of 362 nm. For PTX, HPLC was conducted isocratically at 23°C, with a mixture of acetonitrile and PBS solution (0.005 M, pH 3.5) (50/50% v/v) as mobile phase, at a flow rate of 0.3 mL min\(^{-1}\) and a UV detection wavelength of 230 nm. Calibration was performed by using standards of MitC and PTX at concentrations of 0.1, 0.5, 1.0, 2.0, 5.0, and 10 \( \mu \)g mL\(^{-1}\).

### Animals

All animal-related procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Altogether, 24 New Zealand white rabbits weighing 2.0 to 2.5 kg at the beginning of experiments were included. Animals were randomly subdivided into eight groups (Table 1), each of them (except for group 2) implanted with one microstent into the right eye. The animals in group 2 were a control group that was implanted with the LDD device only (i.e., no microstent).

### Surgical Procedure

One surgeon (Reto Allemann) performed all implantations. Surgery was performed under deep anesthesia with an intramuscularly administered mixture of ketamine hydrochloride (Belapharm GmbH, KG, Vechta, Germany) at a dose of 50 mg kg\(^{-1}\) and xylazine hydrochloride (Rompun, Bayer Health Care, Leverkusen, Germany) at a dose of 5 mg kg\(^{-1}\).

Surgical procedure is schematically demonstrated in Figures 2A–C. In addition, Figure 2 shows a microstent with attached LDD device (Fig. 2D) and the implantation steps (Figs. 2E–I). After incisional cut into limbus adjacent conjunctiva (Fig. 2E), a rectan-
gular lamellar scleral flap was created with hockey knife (Fig. 2F). The distal ending of microstent was inserted into the SCS, and proximal ending into anterior chamber (Figs. 2G, 2H). Finally, the scleral flap was closed (Fig. 2I). After surgery, antibiotic and anti-inflammatory eye drops (Dexa-Gentamicin, Ursa-pharm Arzneimittel GmbH, Saarbrücken, Germany) were administered to the treated eye.

**Noninvasive Measurement of IOP**

An Icare tonometer TAO1 (Icare Finland Oy, Vantaa, Finland) was used for noninvasive measurement of IOP of the right (treated) and the left (control) eye. To consider the corneal stiffness, the measured IOP values $P_{\text{Icare}}$ were corrected by a linear function as described elsewhere.\(^1\)\(^9\)

IOP measurement was conducted daily for 1 week before/after surgery and thereafter weekly, until termination of experiments. To monitor the influence of microstent implantation on IOP, the pressure difference $\Delta p$ between the right (treated) eye and the left (control) eye was calculated. In this context, the IOP values represent an arithmetic mean of $n = 10$ single measurements. Negative pressure differences $\Delta p$ indicate IOP-lowering in the right (treated) eye and vice versa. Due to error propagation, total error $\Delta \Delta p$ of the pressure difference $\Delta p$ results from summation of mean error.

For statistical analysis, IOP data for each group were summarized to a single data point per week. As a result, mean IOP difference and total error for each group was calculated for time points 1 week before surgery and for up to 6 weeks after surgery. Statistical analysis was carried out using the software IBM SPSS Statistics 19.0 (IBM Corp, Armonk, NY). Pooled variance $t$-test for independent samples yield $P$ values with regard to the comparison of IOP values after and before surgery.
respectively. Additionally, *t*-test yields probability values for the comparison between groups. The significance level was set as $P < 0.05$.

**Ultrahigh Field (UHF)-Magnetic Resonance Imaging (MRI) and Optical Coherence Tomography (OCT)**

After final examination, animals were sacrificed with an intravenous overdose of pentobarbital (Sigma-Aldrich Chemie, Munich, Germany). Immediately postmortem, high-resolution MR ocular images were acquired on an ultra-high-field MRI unit (7.1 Tesla, ClinScan, Bruker BioScan GmbH, Ettlingen, Germany) using a two-channel coil with four coil elements and T2-weighted turbo spin-echo (TSE) sequences. MRI sequences were optimized to obtain maximal contrast. For in vitro imaging, the field of view used for the T2w TSE sequences was 40 $\times$ 40 mm with a matrix of 512 $\times$ 512 pixels. The other imaging parameters were: repetition time 2420 ms, echo time 44 ms, turbo factor 7, and 15 slices with a slice thickness of 700 $\mu$m and a gap of 20% between the slices. The acquisition time was 5:12 minutes for each plane with an overall scanning time of 30 minutes.

After MRI, high-resolution information about the proximal end of the stent was obtained by performing OCT (Spectralis; Heidelberg Engineering GmbH, Heidelberg, Germany) on all study eyes.

**Histology**

Following UHF-MRI and OCT, the eyes were enucleated and fixed overnight in 3.7% formaldehyde at room temperature. After dehydration in graded solutions of ethanol, samples were embedded in paraplast, and serially cut into sections of 6-$\mu$m thickness. For general histological observation, the sections were routinely stained with hematoxylin-eosin (H&E).

**Results**

**Fabrication of Microstents**

Average total and drug mass of manufactured LDD devices are summarized in Table 2. Representative images of an assembled microstent of group 5 are shown in Figure 3A. Patching the LDD device to the distal ending of silicone-tube caused no negative effects such as narrowing of tube lumen. Scanning electron microscopy (SEM) yielded smooth surface of silicone-tube and MitC-crystals visible at high magnification (Figs. 3B, 3C).

**Drug Release In Vitro**

Drug release was faster for MitC compared to PTX for both, P(4HB)- and P(3HB)-based LDD devices and faster from P(4HB)- compared to P(3HB)-based matrix for both drugs (Fig. 4). After an initial burst release of 55% MitC at a rate of $4.1 \times 10^{-5}$ g d$^{-1}$ from P(4HB) and 54% MitC at a rate of $3.5 \times 10^{-6}$ g d$^{-1}$ from P(3HB) within the first day, there was a continuous slow release of 100% MitC at a rate of $3.5 \times 10^{-6}$ g d$^{-1}$ from P(4HB) and 91% MitC at a rate of $1.8 \times 10^{-6}$ g d$^{-1}$ from P(3HB) within 10 days. For PTX, there was an initial burst release of 3% at a rate of $2.4 \times 10^{-6}$ g d$^{-1}$ from P(4HB) and 0.4% at a rate of $7.7 \times 10^{-7}$ g d$^{-1}$ from

![Figure 3](https://www.tvst.org/content/4/3/Article14/F3.large.jpg)
P(3HB) within the first day followed by a continuous slow release of 14% at a rate of 5.2E-7 g d^-1 from P(4HB) and 1% at a rate of 6.8E-8 g d^-1 from P(3HB) within 25 days.

Noninvasive Measurement of Intraocular Pressure (IOP)

For animals of all groups, the IOP before surgery in the left and right eye were almost equal (p_le = 12.9 ± 1.7 mmHg versus p_re = 12.7 ± 1.7 mmHg) so that the resulting pressure difference was negligible. After surgery, the IOP in the left (control) eye remained unchanged (p_le = 12.0 ± 2.0 mmHg). Therefore, any change in pressure difference between right (treated) and left (control) eye after surgery was considered the result of microstent implantation into the right eye.

The mean pressure difference between right and left eye and total error for each group is shown in Figure 5. Asterisks indicate level of significance referring to the corresponding value before surgery.

Tendencies with regard to the influence of LDD device material (P(4HB) or P(3HB)) and applied drug (MitC or PTX) could not be clearly proven in statistical analysis.

Noninvasive Imaging

Micro-MRI

Anatomically correct placement and lumen of microstents were analyzed for at least two animals per group. Overall, 22 and 17 eyes were examined with MRI and OCT, respectively. Due to the fact that micro-MRI was conducted ex vivo, seven eyes were extensively collapsed, which led to a poor image quality. The remaining eyes exhibited a regular spherical shape, enabling a sufficient quality of obtained images.

Micro-MRI revealed a proper localization of the microstents at the implantation site. Figure 6 shows representative micro-MRI results of a microstent with a P(3HB)/PTX LDD device (group 8). There were no obstructions in any of the microstents. Minor dislocation of microstents towards the anterior chamber occurred in four eyes.

Optical Coherence Tomography

OCT is the most suitable tool to inspect the microstent positioning in the anterior chamber. Figure 7A shows a representative microstent with a P(3HB)/PTX LDD device. The proximal end of microstent is located freely in the anterior chamber and does not contact the cornea. Contact between the proximal end of microstent and the cornea was observed in a total of 11 cases, as shown in Figure 7B. Additionally, OCT allowed visualization of the distal end including the LDD device (not shown).

Histology

Six weeks after implantation, the histological examination demonstrated the full length of the microstent (Fig. 8), stretching from the anterior chamber (Fig. 8A), through the sclera (Figs. 8B, 8C) to the SCS (Fig. 8D). In the distal part, the LDD device (with or without drug) could be easily identified (Fig. 8D).

![Image](https://tvst.arvojournals.org/pdfaccess.ashx?url=/data/journals/tvst/933932/)
Evaluation of histological images revealed no significant fibrosis or capsule formation along the silicone tube in any of the groups. There was an infiltration of lymphocytes around the microstent tube (Figs. 9A, 9B).

There was no fibrous capsule formation in the groups around the LDD device (Figs. 9C–E). The overall inflammatory response to the foreign body was mild to moderate. Importantly, the intensity of inflammation did not correlate with the polymer type used.

The morphology of the retina exhibited abnormality in the eyes implanted with drug-incorporated LDD system. The severity of retinal destruction was not related to the LDD device biomaterial or to the particular drug used.

Figure 10 demonstrates representative histological images of the retina of two rabbits, implanted with PTX-containing LDD device (P(3HB) or P(4HB)). The pathological changes encompassed the entire retinal thickness around the LDD device (Fig. 10A), revealing a certain vacuolization of cells within the...
internal granule layer (Fig. 10D). In close proximity to the LDD device, the retina still contained vacuolized cells, but exhibited a better layer organization (Fig. 10E, black outline).

The same observation was made for eyes with MitC-containing LDD devices (Fig. 11). Also here, a dramatic retinal thinning was observed, independently of the LDD device biomaterial (Fig. 11A). At higher magnification (Fig. 11B), it becomes evident that these changes were almost exclusively related to the region of LDD device placement. Distally from the LDD device, the retina displayed a normal appearance.

Histological evaluation of samples with “empty” LDD devices demonstrated a much better retinal architecture, when compared to drug-eluting LDD devices (Figs. 11C, 11D). Thus the abnormal retinal architecture of the drug loaded LDD devices suggest the morphological changes were related to the drugs used, rather than the device design or type of biopolymer used in the film.

Figure 6. 7.1-T-MRI proves the anatomically correct placement of microstent from group 8 consisting of silicone tube (T) and P(3HB)/PTX LDD device (L). Proximal end, located in anterior chamber and distal end in the SCS; longitudinal (A) and cross section through microstent (B).

Figure 7. OCT of a microstent from group 8 (A) and 5 (B) in longitudinal and cross section through microstent. Proximal ending located in the anterior chamber without contacting (A) or contacting (B) the cornea.
Figure 8. Microstent correct localization exemplarily shown for Glau44 (silicone tube plus P(4HB) LDD device without drug). Sections perpendicular to the longitudinal axis of the microstent, visualizing the tube in the anterior chamber (A), followed by Sclera (B, C). Further distally, both tube and LDD device can be seen in one section (D). Abbreviations: ac, anterior chamber; L, LDD device; scl, sclera; T, tube. Scale bar represents 500 μm.

Figure 9. Microscopic examination of tissue surrounding the tube (A, B) or LDD device (C–E). Around the tube, there was a comparable reaction in all groups. A and B demonstrate the tissue reaction in two randomly chosen animals. (A) Glau49: silicone tube plus P(3HB)/MitC. (B) Glau43: silicone tube plus P(3HB). Around the LDD device, made of P(4HB), a considerable amount of inflammation was observed (D, Glau44: silicone tube plus P(4HB)), as opposed to the P(3HB) device, which yielded a less pronounced tissue reaction (C; Glau43: silicone tube plus P(3HB)). (E) Giant cell around the LDD device, enlargement from the box in C (Glau49: silicone tube plus P(3HB)/MitC). Abbreviations: L, LDD device; scl, sclera; T, tube. Scale bar 500 μm (A–D) and 100 μm (E).
Figure 10. Microscopic examination of H&E-stained histological cross-sections revealed a severe morphological impairment of retina, as shown exemplarily in two randomly chosen rabbits, implanted with microstent equipped with PTX-containing LDD device. (A) Glau54: silicone tube plus P(3HB)/PTX: transition from normal to damaged area is indicated by arrowheads. (B) Detail (from A) of intact retina with an almost normal layering of the retinal neuroepithelium. (C) Detail (from A) of impaired retina: the clear layering of the retina is completely lost. Instead, there is some apparent lymphocytic invasion, increased connective tissue reaction, and neovascularization. (D) Glau58: silicone tube plus P(4HB)/PTX. (E) Better retinal structure and organization distally from LDD device. Abbreviations: L, LDD device; T, tube. Scale bar = 100 μm.
In the last years, surgeons have tried to modulate the postoperative fibrotic response after GDD implantation by applying antifibrotic drugs. However, no clear effect of antifibrotic agents has been demonstrated. While some experimental publications have claimed considerable success of MitC application following GDD implantation in rabbits, prospectively randomized clinical studies have failed to demonstrate MitC advantages.

An attractive option for long-term reduction of fibrosis would be the creation of drug eluting GDDs, which would enable a slow drug release over time. Previously, antimetabolic drugs have been loaded in devices consisting of nondegradable, permanent polymers. However, degradable biomaterials have gained popularity in recent years. The advantage of biodegradable polymers is that they degrade into nontoxic compounds in the body, thus helping to overcome long-term complications. Here, we incorporated antimetabolites into the end-plate of a GDD microstent by attaching drug containing polymer films consisting of P(3HB) or P(4HB). Both of these polymers are biodegradable polyhydroxyalkanoates based on hydroxybutyric acid. In our previous in vitro studies, P(4HB) was shown to completely degrade within 1 year, whereas only 60% degradation of P(3HB) occurred during the same time. Also in vivo, P(3HB) exhibited a longer degradation, when compared to P(4HB).

When comparing the release kinetic for MitC and

Figure 11. Comparable to the results seen in Figure 10, also the retinas of rabbits implanted with microstent MitC-containing LDD device demonstrated a dramatic thinning and loss of architecture (A, B; Glau49: silicone tube plus P(3HB)/MitC). (B) Relative normal retinal appearance distally from LDD device. Arrowhead indicates the transition from destructed to the normal area. (C, D) When implanted without antimetabolites, the LDDs did not lead to significant retinal damage as exemplarily shown for the rabbit Glau49: silicone tube plus P(4HB). Abbreviations: L, LDD device; T, tube. Scale bar = 500 μm.
PTX in vitro, we observed an appreciably faster release for the former, and this from both, P(3HB) and P(4HB), polymers. Within the first 10 days, 91% of MitC was released from P(4HB), while the release of the same amount of PTX took twice as long. Similarly, a complete drug release from another polymer (poly2-hydroxyethyl methacrylate) could be shown for MitC within 1 to 2 weeks, while 82% release at 21 days was reported for PTX. Faster release of both drugs from P(4HB)- compared to P(3HB)-based matrix can hardly be explained by minor deviations of contact angle ($0_{P(4HB)} = 67.7^\circ$ versus $0_{P(3HB)} = 69.3^\circ$) or degree of crystallinity ($k_{P(4HB)} = 71.7\%$ versus $k_{P(3HB)} = 68.0\%$) and the resulting differential water uptake. The different release kinetic cannot be attributed to the different degradation behavior of both polymers, because in the present case the drug release occurs primarily by diffusion mechanism. Higher glass transition temperature of P(3HB) ($T_g = 1^\circ$C) compared to P(4HB) ($T_g = -51^\circ$C) is a more likely explanation for observed differences.

Foreign body reaction and fibrotic capsule formations are well known consequences of GDD implantation. Overall, we observed a moderate lymphocytic infiltration 6 weeks postoperatively. Keeping in mind implantation studies, which revealed a peak of inflammation by 2 weeks and its absence by 15 weeks, we believe that by 6 weeks the inflammatory reaction had started to decline. No direct correlation between the degree of inflammation and the amount of residual polymer material could be shown, although our previous study demonstrated that P(3HB) was more biocompatible than P(4HB).

Importantly, the absence of pronounced fibrosis cannot be explained by use of antiproliferative drugs, because even in the eyes, with drug-lacking LDD devices, no fibrotic tissue could be observed. This contrasts with experimental studies utilizing subconjunctival GDDs, most of them reporting on strong postoperative fibrotic tissue reaction. A pronounced fibrotic capsule consisting of collagen was seen 8 weeks after implantation of Ahmed glaucoma valve, accompanied by fibroblasts proliferation and transformation to myofibroblasts. Additionally, Jacob et al. reported on a fibrotic encapsulation for 3 to 6 weeks after Baerveldt GDD implantation.

We proposed that absence of encapsulation could be ascribed to the site of implantation; that is, suprachoroidal versus subconjunctival space. In our previous research, we have demonstrated that scleral and choroidal fibroblasts, both bordering the SCS, differed from conjunctival fibroblasts in terms of cell proliferation and expression patterns of extracellular matrix proteins and chemokine receptors, suggesting that SCS could be less prone to fibrosis, compared to subconjunctival space. There are clinical results and experimental studies demonstrating fibrotic reaction following suprachoroidal implantation of GDDs. This, however, may be due to specific properties of gold and polypropylene used as biomaterials in these studies. Medical grade silicone, used in our study, exhibited a biocompatibility superior to polypropylene in a study comparing both materials.

The good biocompatibility of silicone combined with a relative low elastic modulus of 2.1 N mm$^{-2}$ (as compared to 77,200 N mm$^{-2}$ and 1500 N mm$^{-2}$ for gold and polypropylene, respectively) might explain the total absence of fibrosis observed in our experiments. This statement, however, needs a further approval in animal trials with a follow-up of longer than 6 weeks.

Previously, 5-FU-, MitC-, or diclofenac-eluting GDDs have been implanted in subconjunctival and suprachoroidal compartments of rabbit eyes. However, the retinal safety has not been addressed yet. Here, we found at the site of implantation a severe retinal damage, visualized as cell vacuolization, layer disorganization, and disruption of normal architecture, which with high probability was related to the presence of antimetabolite drugs used. In spite of the fact that this observation was exclusively made in the GDD-surrounding area, the long-term consequences, like choroidal detachment or scleral thinning, as well as progression of degeneration to the central retina, should not be underestimated. This finding raises a serious concern about applicability of MitC or PTX in drug-eluting GDDs. In this regard, studies are needed to focus on the identification and application of other drugs with lesser cytotoxicity. Optimal drugs are expected to exert anti-inflammatory/antifibrotic effects and inhibit fibroblasts activation without impairing the vitality of surrounding cells.

Regarding IOP, we observed a more pronounced decrease in eyes with drug-eluting LDDs. However, no clear tendency could be found in regard to the particular LDD material or applied drug. Nevertheless, we cannot ascribe this difference to the drugs’ presence and their antiscarring properties, because, no clear fibrotic capsule formation, which would impair the draining function, could be seen in any of the
groups. A similar observation was made at the early postoperative period, revealing a greater IOP drop in eyes with GDDs supplied with 5-FU-releasing plug. This difference, however, appeared diminished by the end of the experiments.30

Proper positioning of drainage devices after implantation is critical for functionality. In clinical practice, the anterior segment OCT has been used to visualize morphological changes following trabeculectomy or nonpenetrating glaucoma surgery.36 More recently, OCT examination performed after microstent implantation in glaucoma patients revealed a correct localization and a preferential accumulation of AH posterior to the implant.37 This longitudinal study did not show any significant changes in stent position or patency over time. In our study, OCT employed on rabbit eyes ex vivo, enabled visualization of the microstent and surrounding tissues in high quality, emphasizing feasibility for experimental studies.

The main limitation of our study is that experiments were performed on healthy nonglaucomatous rabbits. We tried several approaches, which have been previously described in the literature, to induce increased IOP in rabbits. Nevertheless, no sustained increase could be obtained, and sometimes, as in cases with corticosteroids, we terminated experiments because of severe side effects.38 A reliable glaucoma model would allow mimicking of human disease conditions and more exact analysis of IOP changes upon implantation. Additionally, for exploring the tissue reaction, long term studies and models with elevated cytokine levels in AH might be more appropriate; namely, the absence of pronounced fibrosis observed here, can be ascribed not only to the biocompatibility of polymers, but also to the absence of ongoing pathologic stimuli.

**Acknowledgments**

The authors thank Anja Meyer and Heike Brückmann for histological processing, Günther Kundt for helpful assistance in statistical analysis, Günther Ritschel for schematic drawing of Figures 1 and 2, and David P. Martin for helpful comments and suggestions.

This study was supported by the German Research Foundation (DFG Transregio 37, Micro- and Nano-systems in Medicine-Reconstruction of Biological Functions).

**Disclosure:** M. Hovakimyan, None; S. Siewert, None; W. Schmidt, None; K. Sternberg, None; T. Reske, None; O. Stachs, None; R. Guthoff, None; A. Wree, None; M. Witt, None; K.-P. Schmitz, None; R. Allemann, None

* Marina Hovakimyan and Stefan Siewert contributed equally to this work.

**References**

13. Chiou AG, Mermod A, Hédiguer SE, Schnyder CC, Faggioni R. Ultrasound biomicroscopy of


