The Intraocular Lens as a Drug Delivery Device: In Vitro Screening of Pharmacologic Substances for the Prophylaxis of Posterior Capsule Opacification

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PURPOSE. Numerous pharmacologic substances have been proposed for preventing posterior capsule opacification (PCO). The following trial was to compare those drugs to find more suitable options. IOL should then be modified by the pharmaceuticals as a drug-delivery device.

METHODS. A systematic literature search was performed to identify published substances. FHL-124 was used to determine cell proliferation and toxicity using a dye reduction test (XTT). Prescreened substances showing a reduction on cell growth without being toxic were soaked into an IOL. Those IOL were tested for their effect on PCO in an anterior-segment model and the human ex vivo capsular bag model. Toxicity on a corneal endothelial cell line (CEC-SV40) was determined. Release kinetics of methotrexate from the IOL was measured. Toxicity testing in both cell lines was done in serum-free conditions. All growth assays were exposed to 10% fetal calf serum (FCS)-supplemented medium.

RESULTS. The substances inhibited cell growth at the following EC50: caffeic acid phenethyl ester 1.6 ± 0.9 nM, disulfiram 359 ± 33 nM, methotrexate 98.0 ± 29.7 nM, rapamycin 70.2 ± 14.0 pM, and retinoic acid 1.1 ± 0.12 nM. All but disulfiram showed an effect in the anterior-segment model when soaked into an IOL. Long-term inhibitory effects in the human capsular bag model were observed for caffeic acid phenethyl ester and methotrexate IOLs. Only methotrexate and disulfiram did not show any toxicity on endothelial cells. Methotrexate was released constantly from the hydrophilic IOL for 2 weeks.

CONCLUSIONS. We could identify caffeic acid phenethyl ester and methotrexate in vitro as potential candidates for IOL modification for PCO prophylaxis.

Keywords: posterior capsule opacification, intraocular lens, cataract surgery, pharmacological prophylaxis of posterior capsule opacification

Cataract surgery is still the most commonly performed procedure in developed countries and incidence of posterior capsule opacification (PCO) after surgery remains high.1,2 Several attempts to reduce the incidence of PCO have been introduced, for example, improvements in lens design and material10 or improvements regarding less traumatic surgical methods.4 Furthermore, extensive research has been conducted to obtain insight into molecular basis of this wound-healing reaction in order to identify a possible prophylactic biochemical or pharmacologic option.5

In the last decades, several pharmacologic substances with different cellular targets have been proposed, yet there is no clinical availability of those substances.6 In PCO, a drug needs to satisfy different requirements. Those are pharmacodynamics, the biochemical and physiologic effects of drugs, pharmacokinetics, the availability of the drug at the needed site, at a needed concentration, and the biocompatibility to exclude toxicity.7

One of the major problems is that there are very few direct comparisons of these effects in order to identify more promising substances.6 A structured literature research can be used in order to identify different substances, which have been proposed for the prevention of PCO.6 We used in vitro proliferation and toxicity assays to compare those substances for their efficacy in inhibiting growth, without showing toxic effects.

Those substances, which showed no toxicity and an antiproliferative effect in vitro, were evaluated for pharmacologic IOL modification. The IOL acts as a drug delivery device for a topical application of the drug into the eye.8 This principle has already been developed and proposed for antibiotics and anti-inflammatorials in vitro9 and in a rabbit model,10 and showed sustained release and effects on endophthalmitis prophylaxis and intraocular irritation after cataract surgery.11 In addition, pharmacologic lens modification has been proposed as an encouraging way to fight PCO,12-14 as the pharmacologic substance could mitigate pathophysiological basics of PCO directly at its anatomic origin without any additional step of surgery as a topical drug release device.15 The treatment of canine capsular bags ex vivo with celecoxib-soaked IOLs inhibited cell
growth of residual lens epithelial cells significantly.\textsuperscript{16} Rapamycin coating of IOLs using poly(lactic-co-glycolic acid) (PLGA) lead to a significant reduction of PCO formation in New Zealand white rabbits.\textsuperscript{17}

In pharmacologic PCO-prophylaxis endothelial toxicity is of high importance, especially due to the adjacent location of the endothelium to the operating field. The regenerative properties of the endothelium are under debate,\textsuperscript{18} yet clinically endothelial cell damage after cataract surgery can be permanent.\textsuperscript{19} Therefore, putative corneal endothelial cell toxicity was determined for potential antiproliferative drugs.

METHODS

Pharmacologic Substances

A structured literature based research was conducted using the US National Library of Medicine National Institutes of Health PubMed online database. After entering the following search terms in exact search and “MESH” terms, each abstract was manually scanned for any pharmacologic substances proposed for the prophylaxis of posterior capsule opacification: posterior capsule opacification (1324 results); prevention [and] posterior capsule opacification (512 results); pharmacological prevention [and] posterior capsule opacification (60 results); and pharmacological [and] posterior capsule opacification (134 results).

As we searched pharmacologic substances for a possible midterm clinical application the following inclusion criteria were applied to the substances: drug must be approved by the Food and Drug Administration (FDA) or European Medicines Agency (EMA) for other purposes as for those drugs extensive in vitro and in vivo knowledge on action and biocompatibility is available. They needed to be safe to handle in our lab and must be feasible to use in cell culture setting. There should not be any genotoxicity or mutagenic effects and the substance must neither contain RNA nor a virus as a vehicle. The proposing article must be in English language.

Cell Culture

FHL-124 (lens epithelial cell line\textsuperscript{20}, kindly provided by M. Wormstone, Norwich, UK) and CEC-SV40 (corneal endothelial cell line\textsuperscript{21}, kindly provided by J. Bednarz Hamburg, Germany) were cultured in MEM Earle’s (Biochrom AG, Berlin, Germany). Capsular bags and human donor corneas were cultured in culture flasks and cell culture plates (NUNC, Langenselbold, Germany). The culture medium, was supplemented with 10% fetal calf serum (FCS; Biochrom AG), 50-IU penicillin/mL, 50-IU streptomycin/mL, and 2.5-μg lyophilized amphotericin B/mL (Biochrom AG). The high amount of FCS was deliberately chosen for our research on pharmacologic PCO prophylaxis, as we wanted to create an environment in which it is difficult for an inhibitor to influence growth. This might help to easier identify potential candidates. The culture medium was replaced every second day also.

Dye Reduction Assay for Drug Prescreening in Terms of Cell Growth and Viability

For the purpose of simple and reproducible prescreening of the pharmacologic substances the XTT-dye reduction colorimetric assay is commonly\textsuperscript{22,23} used to study cell viability and cell growth. This assay was first described by Scudiero et al.\textsuperscript{24} and modified for ophthalmic cell culture.\textsuperscript{25} In brief, FHL-124 and CEC-SV40 cells were incubated with different concentrations of pharmacologic substance until the maximal solubility of the substance in cell culture was reached (Table). For cytotoxicity measurement the cells were grown to full cell coverage of the plastic and starved of serum for 24 hours to further lower proliferative activity. This was followed by an exposure to the pharmacologic substance in the medium without the serum to get a stationary layer of cells.\textsuperscript{26} For analysis of cell growth, cells were allowed to proliferate by 10% FCS-supplemented medium, and 25% of the full cell confluence at the beginning of incubation with the substance. After incubation with the substance, the optical density was measured at 450 nm in an ELISA reader (Spectramax 190; Molecular Devices, Sunnyvale, CA, USA).

Pharmacologic Modification of Intraocular Lenses

The IOL were modified as described before.\textsuperscript{8,26} In brief, one type of hydrophilic (Raysoft 574R; Rayner Intraocular Lenses Limited, Worthing, UK) and one type of hydrophobic (YA-65BB; Hoya Surgical Optics GmbH, Frankfurt am Main, Germany) of the same lot were used for all experiments. The IOL included in the study is currently used in modern cataract surgery and reflects the clinical standard of care. It was a hydrophilic acrylic IOL with an optic diameter of 6.0 mm (square-edged design) and a C-loop haptic and one hydrophobic IOL with an optic diameter of 6.0 mm (square-edge design) and V-haptic. The IOLs were coated separately in 1-mL coating solution with disulfiram, methotrexate, rapamycin, retinoic acid, and caffeic acid phenethyl ester for 72 hours at room temperature. All coating solutions were supersaturated in order to allow enough pharmacologic substance to bind to the IOL. The coating solution did not contain linker molecules.

The same IOL was incubated in PBS and served as control. The IOLs were controlled for transparency under a phase-contrast microscope (Axioskop \times100; Carl Zeiss Meditec AG, Oberkochen, Germany). All IOL were rinsed of all crystals by PBS, which formed inside the coating solution and washed in 3 mL of PBS for 30 seconds.

Human Capsular Bag Preparation

There were 60 cadaver eyes from 30 human donors (at the age of 21–77 years; mean age, 56 years) obtained from the Munich University Hospital Eye Bank and processed within 12 to 24 hours of death. None of the donors had a known history of eye disease. Methods for securing human tissue were humane, included proper consent and approval, complied with the Declaration of Helsinki, and were approved by the local ethics committee (Ethic ID 73416).

The capsular bags were prepared as described by Liu et al.\textsuperscript{17} with some modifications. After removing the corneal scleral ring for transplantation purposes, open-sky cataract surgery was performed. First the iris was removed by dissection at its base, followed by a continuous circular capsulorhexis, nuclear hydroexpression, aspiration of residual lens cortex, and polishing of the posterior capsule. The IOL was implanted before cutting the zonulas. Afterward, the capsular bag was pinned to the culture flask using sterile thin entomologic pins and kept under 10% FCS-supplemented medium.

Anterior Segment Model

The anterior segment model was conducted as first described by Gotoh et al.\textsuperscript{26} with some modifications. Imitating the anterior segment of the eye, the experimental setup is divided into two chambers: one for the anterior chamber and the
second for the capsular bag. The one simulating the capsular bag is a cell culture insert (BD Biosciences, Franklin Lakes, NJ, USA) carrying the modified IOL fixed with a small aluminum weight (Haefner, Oberrot, Germany) on a semipermeable membrane. This insert was placed in a tissue-culturing well containing 1 mL of MEM Earle’s, so that there was a free flow between the two chambers. FHL-124 cells (3 × 10^4 cells/mL) were seeded on top and to the edges of the cell culture insert. The cells then had to proliferate and migrate on the membrane below the IOL into the center, analogous to the in vivo formation of PCO. Cells were incubated for 72 hours and were stained with 0.1% citrate crystal violet solution afterward to visualize the area covered with cells. The membrane was cut out of the insert and fixed on microscope slides.

In order to analyze cell growth, pictures were taken with a distance fixed Canon 5D Mark III camera (Canon, Inc., Tokyo, Japan). To measure the actual cell growth, cells were counted by the semiautomated color threshold feature of ImageJ software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). The acquired pixel count representing the stained area was used for further statistical analysis.

### Human Capsular Bag Cell Coverage Assay

Both capsular bags of each donor were used in one experiment: one served as control the other was exposed to the previously modified IOL. An unmodified IOL, which was treated equally as the modified IOL except no drug was added to the coating solution served as control. The capsular bags were retained under 10% ECS-supplemented medium, and observed regularly under a stereo microscope (Stemi 508; Carl Zeiss, Jena, Germany). The amount of days upon confluence of the cell layer was recorded. The experiment was repeated three times for each concentration. The mean was taken of each experimental sample and displayed graphically. The medium (2 mL) was changed every second day.

### Toxicity on Corneal Endothelial Cells

To exclude any putative toxicity of the proposed pharmacologic substances on the corneal endothelium, CEC-SV40 were grown to full cell coverage on the plastic and starved of serum for 24 hours. This was followed by incubation with different concentrations of pharmacologic substance until the maximal solubility of the substance in cell culture was reached. Furthermore, cells in the same setup were exposed to the different modified IOLs. The IOLs were placed into a cell culture insert on a porous membrane as described in the anterior segment model section. The culture insert was then suspended above the CEC-SV40, which were seeded to the cell culture well below. Therefore, the investigated substance could freely diffuse to the CEC-SV40. Unmodified IOLs and cells exposed to solvent only served as controls. An XTT-dye reduction assay as detailed above was performed to determine viability.

### Release Kinetics of Methotrexate From the IOL

To clarify for the amount released by the IOLs we have placed both the hydrophilic and hydrophobic IOLs modified with methotrexate in 1-mL cell culture medium and replaced the medium by putting the IOL in a new tissue well with the same amount of medium every day. The whole setup was always kept under standard cell culture conditions. The concentration of methotrexate in the resulting solution was determined using a methotrexate assay (Siemens EMIT; Siemens AG, Munich, Germany) as detailed in the manufacturer’s descriptions.

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**Table. Overview of the Pharmacologic Substances Used and a Comparison of Solubility, Toxic Concentration, EC_{50}, and Maximum Inhibitory Effect**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Maximum Solubility in Culture</th>
<th>Toxicity From Concentration FHL-124</th>
<th>Efficient Concentration 50% (EC_{50})</th>
<th>Maximum Inhibition (% of Control)</th>
<th>Toxicity From Concentration CEC-SV40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betamethasone</td>
<td>100 μM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>Caffeic acid phenethyl ester</td>
<td>100 μM</td>
<td>50 μM (P &lt; 0.05)</td>
<td>1.6 ± 0.3 nM (r^2 = 0.06)</td>
<td>93.3 ± 2.3%</td>
<td>100 μM (P &lt; 0.05)</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>1 mM</td>
<td>10 μM (P &lt; 0.05)</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Curcumin</td>
<td>50 μM</td>
<td>5 μM (P &lt; 0.05)</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>500 μM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1 mM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Disulfiram</td>
<td>10 μM</td>
<td>Not detected</td>
<td>359 ± 33 nM (r^2 = 0.86)</td>
<td>77.4 ± 3.4%</td>
<td>Not detected</td>
</tr>
<tr>
<td>EDTA</td>
<td>5 mM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Heparin</td>
<td>50 μM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Indometacin</td>
<td>5 μM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>10 mM</td>
<td>5 mM (P &lt; 0.05)</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Lithium</td>
<td>10 mM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>750 μM</td>
<td>Not detected</td>
<td>98.0 ± 29.7 nM (r^2 = 0.61)</td>
<td>33.1 ± 1.4%</td>
<td>Not detected</td>
</tr>
<tr>
<td>Mibefradil</td>
<td>500 μM</td>
<td>10 μM (P &lt; 0.05)</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>1 mM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Octreotide</td>
<td>100 μM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>5 mM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Ramipamycin</td>
<td>1 mM</td>
<td>50 μM (P &lt; 0.05)</td>
<td>70.2 ± 14.0 μM (r^2 = 0.94)</td>
<td>57.3 ± 1.6%</td>
<td>50 μM (P &lt; 0.05)</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>250 μM</td>
<td>50 μM (P &lt; 0.05)</td>
<td>1.1 ± 0.12 μM (r^2 = 0.64)</td>
<td>35.3 ± 7.8%</td>
<td>50 μM (P &lt; 0.05)</td>
</tr>
<tr>
<td>Trypan blue</td>
<td>100 μM</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not investigated</td>
</tr>
</tbody>
</table>


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Statistical Analysis

Statistical comparison between multiple groups was performed by ANOVA with a Bonferroni post hoc test. If there were only two groups, a nonparametric Mann-Whitney $U$ Test was used. A $P$ value $< 0.05$ was considered statistically significant with a 95% confidence interval (CI). Graphs were plotted in Microsoft Excel (Redmond, WA, USA) showing the standard deviation (SD). Statistical analysis was performed by SPSS 24.0 (IBM, Armonk, NY, USA).

RESULTS

Literature Research

In total, 62 different substances have been identified, which have been proposed to influence PCO. There were 41 substances that did not fit into the above-described selection criteria and are summarized in the Table. The 21 substances fulfilling the above-described criterion and are listed in the Table. Erlotinib was not evaluated further as we have already published this data.

Cell Toxicity in FHL-124

A reduction in viability was regarded significant when the $P$ value in comparison to the untreated control reached a value of $P < 0.05$. The following substances reached this predefined level of toxicity in FHL-124: caffeic acid phenethyl ester at a concentration of 50 $\mu$M, celecoxib at 10 $\mu$M, curcumin at 5 $\mu$M, lidocaine at 5 mM, mibefradil at 10 $\mu$M, rapamycin at 50 $\mu$M, and retinoic acid at 50 $\mu$M.

Cell Proliferation

Proliferation was investigated within the nonviability reducing concentrations of the substances. Caffeic acid phenethyl ester, disulfiram, methotrexate, rapamycin, and retinoic acid were the pharmacologic substances, which showed an antiproliferative effect on human lens epithelial cells in our screening test (Fig. 2). The $EC_{50}$ concentrations on growth inhibition were calculated by a best fitting sigmoidal curve as follows: caffeic acid phenethyl ester $1.6 \pm 0.9$ nM ($r^2 = 0.60$), disulfiram $359 \pm 35$ nM ($r^2 = 0.86$), methotrexate $98.0 \pm 29.7$ nM ($r^2 = 0.61$), rapamycin $70.2 \pm 14.0$ pM ($r^2 = 0.94$), and retinoic acid $1.1 \pm 0.12$ nM ($r^2 = 0.64$). The following agents could not inhibit the cell proliferation in our model: betamethasone, celecoxib, curcumin, dexamethasone, diclofenac, EDTA, heparin, indomethacin, lidocaine, lithium, mibefradil, minoxidil, octreotide, prednisolone, and trypan blue (Supplementary Fig. S2 and Table). All substances, which could not inhibit the proliferation while being nonviability reducing to the cells, were not investigated further in IOL modification.

Anterior Segment Model

Both hydrophilic and hydrophobic IOLs soaked with either caffeic acid phenethyl ester (phob $P = 0.02$, phil $P = 0.02$), rapamycin (phob $P = 0.02$, phil $P = 0.02$), retinoic acid (phob $P = 0.02$, phil $P = 0.02$), and methotrexate (phob $P = 0.02$, phil $P = 0.02$) showed a significant capability of reducing human lens epithelial cell growth compared with the control. Disulfiram-soaked IOLs could not show a significant difference in the anterior segment model (phob $P = 0.44$, phil $P > 0.999$). Due to the insignificant difference between disulfiram modified IOLs and the control group in the anterior segment model, we decided to pass on further investigations in the human capsular bag model (Fig. 3).

Human Capsular Bag Model

After preparation and adding either pharmacologically treated or control IOLs, the number of days until a continuous cell...
layer could be observed, was counted. Methotrexate led to a significant and the most pronounced increase in time until full cell coverage of the posterior capsule for both hydrophilic and hydrophobic IOL (\( P = 0.46 \), \( P = 0.46 \)). Caffeic acid phenethyl ester could also increase the time significantly (\( P = 0.046 \), \( P = 0.046 \)). Both retinoic acid (\( P = 0.068 \), \( P = 0.105 \)) and rapamycin (\( P = 0.068 \), \( P = 0.261 \)) did not show a significant effect against PCO in the human capsular bag model when soaked into an IOL (Fig. 4).

Toxicity on Corneal Endothelial Cells

To exclude any putative toxicity all five substances, which reduced growth in the prescreening, were added to CEC-SV40 cells in different concentrations. A significant reduction in viability was defined as when the \( P \) value in comparison to the untreated control reached a value of \( P < 0.05 \). The following substance therefore showed a reduction in viability in CEC-SV40: caffeic acid phenethyl ester at a concentration of 100 \( \mu M \), rapamycin at 50 \( \mu M \), and retinoic acid at 50 \( \mu M \). The following substances did not show a reduction in cell viability within the soluble concentrations in cell culture: methotrexate and disulfiram (Table and Fig. 5). Both hydrophilic (\( P < 0.001 \)) and hydrophobic (\( P = 0.009 \)) IOLs soaked with retinoic acid and hydrophilic (\( P = 0.01 \)) IOL soaked with rapamycin showed a significant reduction of reducing human corneal endothelial cell viability compared with the control. All other comparisons were not statically significant reduced (Fig. 6).

Release Kinetics of Methotrexate From the IOL

After an initial release of a higher amount of methotrexate the hydrophilic IOL showed a constant release of substance over the 2 weeks. Furthermore, the drug delivery was higher than the determined \( \text{EC}_{50} \) at all time points over the 14 days. The hydrophobic IOL did also show a high initial peak but no release above the detection limit after day 3. The total amount of methotrexate accumulated on the hydrophobic IOL was 23.2 \( \mu g \pm 10.8 \) SD and 76.3 \( \mu g \pm 30.6 \) SD for the hydrophilic IOL, respectively (Fig. 7).

DISCUSSION

Several pharmacologic substances have been proposed for the prophylaxis of PCO. Yet, there is no clinical application of a pharmacologic option to reduce the incidence of PCO until now. We suggest that one of the reasons for this might be there are no direct in vitro comparisons of those substances to identify the more promising agents. In researching the literature, we could identify a vast amount of potential pharmaceutical substances. As a future clinical application should be pursued, we applied certain inclusion criteria to the substances (e.g., missing mutagenic potential, FDA/EMA approval, no viruses or RNA). Furthermore, to reduce possible adverse events of the drug, we focused on a nontoxic, specific, and selective way to inhibit lens cell growth. The toxic concentration should lay a few orders of magnitude higher than the efficient concentration. Using lens cell toxicity poses a higher risk to surrounding tissues. Yet, we acknowledge, that cell toxicity is a worthy approach for PCO mitigation, which of course needs to be evaluated scientifically. The resulting drugs were then tested by a simple and commonly used drug/action screening test on lens epithelial cells. Using this test we could identify five promising candidates (i.e., caffeic acid phenethyl ester, disulfiram, methotrexate, rapamycin, and retinoic acid).

After identifying those promising substances a method of application to the eye needs to be determined. The use of a drug-delivering device is well established in medicine, for example, the cardiac drug eluting stent,\(^{30}\) Ozurdex,\(^{31}\) an intravitreal dexamethasone implant or the NUVA ring,\(^{32}\) a local hormone releasing device. Even more convenient for both the surgeon and the patient is the use of the IOL as a drug delivery device. The topical release to the capsular bag secures an application to the location of action. A topical drug reduces systemic adverse effects.\(^{33}\) Furthermore, the surface of the eye...
is not used to transport drugs, as it is in case of eye drops with high concentrations of the drug due to necessary diffusion. No patient adherence is needed for the drug application. Additionally, the principle is convenient for the surgeon as no altering in surgical procedure is required to apply the substance to the capsular bag and it can be safely integrated into cataract surgery routine.14

There are different methods for IOL drug modification, for example, soaking, coating, drug reservoir in the haptics, and hypercritical fluid impregnation.15 For this paper, drug soaking/coating in a supersaturated solution was used. It is proposed that, depending on the physicochemical properties of the pharmacologic substances they will bind to the surface and the high water content of the polymer.16 After implantation, it can be postulated that they will diffuse into the capsular bag. In material science, this process is referred to as dip coating.34 Potential advantages in comparison to other drug-loading processes are, that no additional form of energy like heat, pressure, or electricity is needed, which could damage the IOL. Furthermore, no linker molecules with potential side effects and toxicities are required to bind the substance.35 Yet, this drug-loading process is highly dependable on many of the drugs chemical properties, for example, polarity, reactivity, or the biological EC50. The drug must be able to bind to the polymer in sufficient concentration. In our experiments, in some drugs their effect varied depending on whether hydrophobic or hydrophilic IOL material was soaked. Disulfiram did show a strong inhibitive effect on lens epithelial cells in culture but no effect was observed when coated to the lens, this might be connected to the higher EC50 in comparison to the other substances and a potential inability to bind to the IOL material. To study the effect of which chemical properties are needed for a substance to bind the IOL material future studies are needed.

The prescreening test showed several substances, which showed no effect in our model. As those are all drugs, which have been suggested for the prevention of PCO in peer reviewed manuscripts, we compared those articles with our work, and why we could not reproduce this effect. Some of the substances were already proven ineffective in other studies, for example, heparin,36 prednisolone, 37 dexamethasone, and diclofenac.38 Some other substance were used at higher concentration in order to cause toxic lens epithelial cell death, for example, trypan blue,39 lidocaine, 40 or EDTA. 41 By inclusion criteria, we excluded toxic effects causing cell death in our work. Furthermore, authors have applied different IOL drug-loading principles.42 In addition, some of the proposing authors have used different models, for example, different

- **FIGURE 3.** Graphic display of the area covered by cells on the membrane below the IOL in the anterior segment model. Both hydrophilic and hydrophobic IOLs soaked with either caffeic acid phenethyl ester, rapamycin, retinoic acid, and methotrexate showed a significant capability of reducing human lens epithelial cell growth compared with the control. Disulfiram had no effect when soaked to an IOL. Caffeic acid phenethyl ester: $P = 0.02$ (phob); Co-phob 100% (95% CI: 90%–110%), CA-phob 22% (95% CI: 4.6%–39%); $P = 0.02$ (phil); Co-phil 100% (95% CI: 91%–109%), CA-phil 5% (95% CI: –2% to 12%), rapamycin: $P = 0.02$ (phob); Co-phob 100% (95% CI: 49%–151%), RE-phob 49% (95% CI: 25%–75%); $P = 0.02$ (phil); Co-phil 100% (95% CI: 76%–124%), RE-phil 11% (95% CI: –5% to 27%), retinoic acid: $P = 0.02$ (phob); Co-phob 100% (95% CI: 96%–104%), RE-phob 64% (95% CI: 36%–92%); $P = 0.02$ (phil); Co-phil 100% (95% CI: 92%–108%), RE-phil 11% (95% CI: –4% to 26%); methotrexate: $P = 0.02$ (phob); Co-phob 100% (95% CI: 90%–110%), MT-phob 25% (95% CI: 15%–35%); $P = 0.02$ (phil); Co-phil 100% (95% CI: 96%–104%), CA-phil 26%; (95% CI: 20–32), disulfiram: $P = 0.44$ (phob); Co-phob 100% (95% CI: 86%–115%), DS-phob 104%; (95% CI: 101%–107%); $P > 0.999$ (phil); Co-phil 100% (95% CI: 92%–108%), DS-phil 104%; (95% CI: 88%–106%). *Statistically significant.
Current data demonstrate that the human capsular bag model is effective for PCO research, and might be superior to other nonhuman models. This applies to basic research on PCO pathogenesis as well as to relevant clinical questions, such as the evaluation of novel IOL technology or the feasibility of PCO prophylaxis. For pharmacologic approaches to PCO prevention in particular, there is a need for human models because animal models might differ in regulation mechanisms like signal transduction pathways and growth factor secretion.

Some of the five more promising substances identified in the prescreening test did show an effect in the anterior segment model, but did fail to inhibit cell growth in the long-term in the human capsular bag model. This might be due to the described effect of different chemical abilities of the drugs to bind the IOL, with missing long-term release or possibly a too low concentration of substance. Furthermore, the anterior segment model uses the lens epithelial cell line, whereas the human capsular bag model might differ in biological response. In addition, some of the substances might cause a long-term effect, even if they are present in culture for a short period. Regarding PCO pathogenesis, an early therapeutic hit might be effective enough even for a long-term therapeutic effect. The mean aqueous flare after routine cataract surgery measured by a laser flare-cell meter increased significantly from preoperatively to 1-day postoperatively, other studies show an increase of different growth factors and cytokines directly after surgery. It might be interesting to inhibit this early response in order to reach a long-term inhibition of growth.

Wormstone et al. was able to show that the effect of a growth factor added to in vitro capsular bags over 24 hours after preparation was able to induce long-term responses after 28 days. In our study, we could observe a long-term therapeutic effect over 20 to 60 days for methotrexate and caffeic acid phenethyl ester in the human capsular bag organ culture in comparison to 8 to 10 days in the control. In doing so, the hydrophilic IOL elutes methotrexate at a concentration higher than the EC50 for 14 days and the hydrophobic IOL does not give away any measurable amount of substance after day 3. Although this is rather a short-time exposure of the capsular bag directly after sham cataract surgery, the biological effect of methotrexate led to a long-term deceleration of cellular growth. The observed effect might be of interest in the clinical situation as a relatively short exposure, for example, through a coated or soaked IOLs is more feasible than a long-term exposure.

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lum is of high interest regarding toxicity. In this study, both IOLs modified with rapamycin and retinoic acid showed a reduction of cell viability of CEC-SV40. Both substances could not produce an antiproliferative effect in the human capsular bag model anyway. Caffeic acid phenethyl ester did not produce a significant reduction in endothelial cell viability, when soaked to an IOL but reduced viability when added to media at concentrations higher than 100 μM. Of note, all three substances had a high therapeutic margin from five to six orders of magnitude in comparison to their measured EC₅₀. Further studies are underway, how different linker molecules, different soaking solutions, and concentration of drug in the soaking solution influence the amount of substance given away. This will help to control toxicity.

Methotrexate was the substance, which showed the most pronounced effect in our in vitro study, without being toxic to endothelial cells. As there are alternative and convenient treatment options for PCO like the Nd-YAG-capsulotomy, toxicity is an important issue. Methotrexate has been FDA-approve for several diseases since 1950. There is extensive clinical and preclinical knowledge on action and biocompatibility available. In ophthalmology the drug is used as an off-label intravitreal injection for lymphoma. There were clinical studies on an intravitreal slow release device for treatment of uveitis and chorioiditis. Furthermore, it seems to have a positive effect on other fibrotic reactions in the eye comparable to PCO. There have been clinical studies showing promising results for the prevention of proliferative vitreoretinopathy and fibrosis in AMD. Another group injected methotrexate for diabetic macular edema and found that after a 6-month follow-up study, intravitreal injection of 400-μg methotrexate had no significant effect on corneal endothelial cell.

The experimental data of this study is limited to the nature of an in vitro setting. Limitations include the chosen amount of serum conditions to determine the toxicity and growth inhibiting effects. Further studies are needed to determine the effect of lower concentration of FCS in the cell culture medium. Furthermore, the capsular bag model, does not guarantee the effect of the sharp edge. As we could not determine the effect of the sharp edge we refrained from comparing hydrophilic with hydrophobic IOLs and only determined the difference between modified and unmodified IOLs.
In summary, this is the first study presenting a direct comparison of pharmacologic substances for the prevention of PCO using IOL modification. Modified IOLs appear easily applicable in daily routine cataract surgery. Methotrexate seems to be a promising substance for IOL modification, which is well characterized in human and ocular use, without being toxic. Thus, the technique has the potential to improve cataract surgery outcome and might become an antifibrotic treatment strategy in the future. Further studies, experimental and clinical, are warranted to determine the clinical potential of the findings of this manuscript.

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