

Two-photon absorption-controlled multidose drug release: a novel approach for secondary cataract treatment

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Abstract. Tens of millions of cataract surgeries are done every year and the number is increasing heavily. Posterior capsule opacification is the major postoperative complication with an incidence of 10 to 50% within 5 years, depending on the age of the patient. We present a novel approach for secondary cataract treatment in a noninvasive manner. Photochemically triggered drug release from a polymer enables repeated drug applications for cataract treatment years after implantation of the intraocular lens, just when needed. However, light in the visible spectral range must pass through the lens but must not induce drug release. We demonstrate that two-photon absorption photochemistry is a powerful tool to overcome this problem. With wavelengths in the visible regime, a photochemical reaction that requires energies in the UV is triggered. The high intensities needed for this process never occur in any lighting condition in daily lives, but may be easily obtained with focused laser beams routinely used in ophthalmology. The properties of the therapeutic system are specified and the function is demonstrated by *in-vitro* cell tests. Noninvasive multidose photochemically triggered drug release from implanted intraocular lenses carrying a drug depot may be a therapeutic as well as an economic choice to established treatments of secondary cataracts. © 2006 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2209564]

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1 Introduction

Cataract is any opacity in the crystalline lens of the human eye, which leads to reduced light transparency and may cause irreversible loss of vision. Although the problem of cataract-dependent blindness in the industrialized world has been largely solved through surgical treatments, which remove the cloudy and opaque natural lens and implant an intraocular lens (IOL) made from polymer, cataract is still by far the most common cause of blindness worldwide today. The World Health Organization (WHO) estimates the number of cataract operations worldwide to be 12 million cases in the year 2000, and predicts a rise to 32 million cataract operations by 2020.¹ The postoperative complication with the highest incidence is posterior capsule opacification (PCO), the so-called secondary cataract. PCO is caused by proliferation and migration of retained lens epithelial cells (LEC) into the optical axis, and leads to a progressive deterioration and disturbances in visual activity (Fig. 1).

Despite recent progress in cataract surgical procedures and the development of IOLs, which reduce PCO incidence, the

formation of secondary cataracts still remains as a most common problem in modern cataract treatment. The incidence of PCO varies, depending on the IOL materials and the age of patients, between 10 to 50% within 3 to 5 years after IOL implantation.²⁻⁴ In particular, in pediatric implantations, the rate of PCO is considerably higher; more or less, the occurrence of PCO is even unavoidable.⁵⁻⁹ Currently, the only effective treatment of PCO is Nd:YAG laser posterior capsulotomy, where high energy light beams are used to cause photodisruption of the opacified tissue membrane, which results in a significantly improved visual acuity and contrast sensitivity.² This procedure, however, sometimes lead to serious complications, including damages of the IOL optic,¹⁰⁻¹³ increased intraocular pressure,¹⁴⁻¹⁷ or even retinal detachment.¹⁸⁻²² To overcome these problems, in recent years extensive efforts have been made in IOL design to reduce the mitosis or migration of LEC. Improved IOL designs,²³⁻²⁹ materials,^{30,31} surface modification,³²⁻³⁶ or even combinations with a sustained drug delivery system have been explored.³⁷⁻⁴² The rapid progress in drug delivery systems attracted considerable interest during the recent decade. In numerous experimental studies, antimetabolites, immunotoxins,

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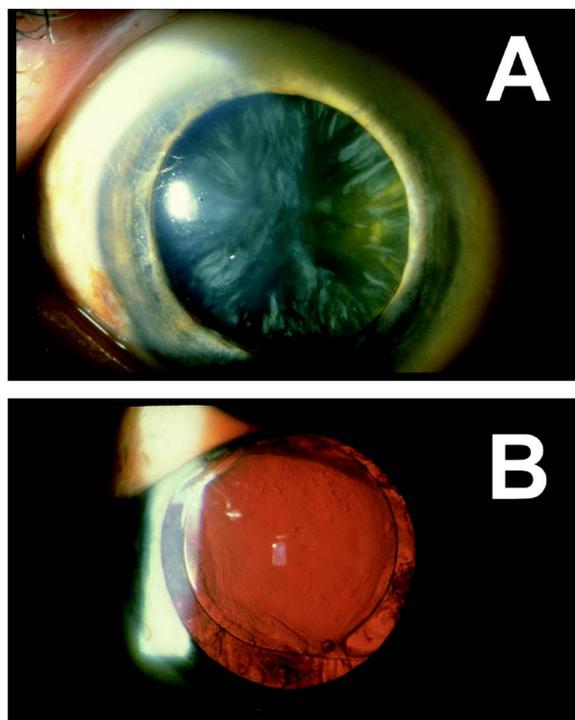


Fig. 1 Cataract and secondary cataract-related opacification of the human eye lens. (a) Cataract and (b) posterior capsule opacification (PCO, secondary cataract).

and antiinflammatory agents such as 5-fluorouracil, daunomycin, thapsigargin, indomethacin, and others have been reported to significantly inhibit LEC growth in cell cultures.^{41,43–52} In view of these results, several attempts to prevent PCO using the IOLs themselves as sustained drug delivery devices have been reported.^{37–39,42} However, a major disadvantage of such preprogrammed passive devices is the lack of any dynamic response. Further, the release of any substances affecting the wound healing after cataract surgery is not desirable. A drug-loaded IOL that offers noninvasively triggered drug release would overcome all the current limitations.

In this study, we present a novel photocontrolled drug delivery device for PCO treatment, an IOL with a drug chemically attached to the polymer backbone. A challenging demand is that daylight needs to pass through the implanted IOL for years without triggering the drug release. Two-photon absorption (TPA)-induced photocleavage of the linker site is employed to release the immobilized drug, a photochemical event that is extremely unlikely to occur even in bright sunlight, but is easy to obtain by short laser pulses.

2 Concept for Photocontrolled Drug Release Polymers for Intraocular Lenses

An IOL material has to meet a wide variety of properties to make it suitable for secondary cataract treatment (Table 1). First of all, it should be a fully transparent polymer having an index of refraction high enough to not make the IOLs thicker than they are today for the same refractivity. The material properties should not be significantly different from those ma-

Table 1 Figure of merit for intraocular lenses with phototriggered drug release.

Polymer material	Transparency	High from 400 to 800 nm
	Index of refraction	Medium to high
	Biocompatibility	Noncytotoxic, before irradiation
	Flexibility	Low T_g , foldable, injectable lenses
Drug delivery	Processability	Compression molding, preferred
	Noninvasive	Photochemical
	Triggerable	Photochemical
	Multidose capability	2 to 5 doses
Drug		Release of chemically nonmodified drugs

terials currently in use, mainly acrylic and silicone polymers. Photochemical triggering of the drug release needs to be noninvasive, an important aspect if ambulant treatments are considered in the future. The implanted IOL should not release any drug until triggered from the outside, which may be years after implantation. Further, a single IOL should carry a sufficient amount of drug to enable more than a single secondary cataract treatment. The challenge is to release the drug in a chemically nonmodified form. The solubility of common drugs in polymers is poor, and in turn, their diffusion constants are quite low. The concept employed should not be restricted to a single chemical compound, but should be suitable for a variety of drugs. Last but not least, the linker system employed needs to have a high TPA coefficient. The cornea effectively blocks UV radiation to reach the implanted photocontrolled drug delivery IOL (PCDD-IOL) but light in the green, at the double wavelength required for the photochemical reaction, passes easily (Fig. 2). Due to the geometry of the laser beams employed for TPA-triggered drug release inside a small volume of the PCDD-IOL, the required high intensities are reached.

2.1 Functional Building Blocks and Polymer Synthesis

For the photocleavable linker system, which attaches the therapeutic drug 5-fluorouracil (5FU) to the polymer backbone, a coumarin molecule is chosen (Fig. 3). The acrylic polymer serves two functions; first it is the IOL material, and second it is the drug reservoir. For this purpose, a copolymer (PC) from a nonmodified monomer (P_{mono}) and a monomer with a coumarin side group (C_{link}) is used. The drug employed is 5FU (D_{drug}), which is effective for secondary cata-

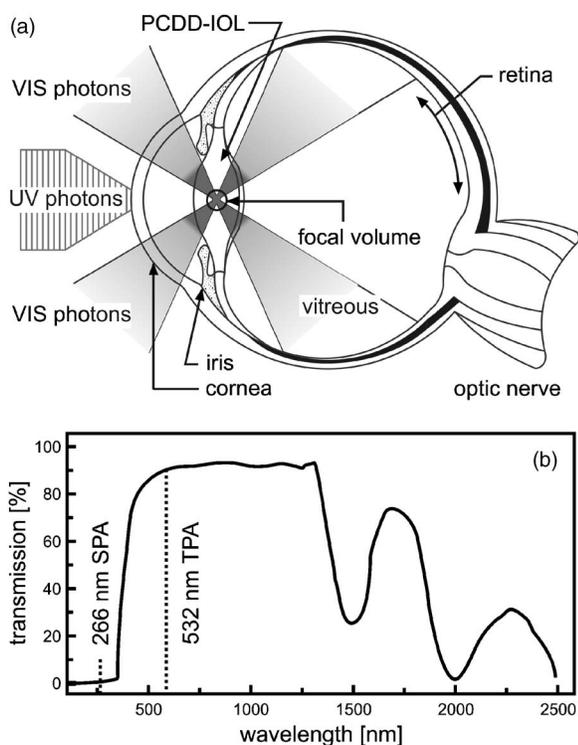


Fig. 2 Scheme of two-photon absorption (TPA)-triggered drug release from a polymeric intraocular lens that contains a photocontrolled drug depot (PCDD-IOL). (a) Visible light from natural sources does not reach the intensities inside the PCDD-IOL so that any TPA-dependent process must be considered, exposure to short laser pulses focused in or on the PCDD-IOL pass the intensity thresholds for TPA-dependent photoreactions easily. The UV absorbing cornea blocks UV photons effectively to reach the PCDD-IOL. (b) Visible wavelengths pass the cornea with only few losses and are focused in the PCDD-IOL to obtain the intensities required for TPA-induced release of the drug from the polymer.

tract treatment, but has a very low solubility and diffusion constant in the used PC polymer. To overcome this problem, a side group (D_{diff}) is attached that improves both parameters. The formed linkage in the formed drug-diffusion modulator complex (DD) is hydrolysable, and upon contact with chamber water the 5FU is released in its original form. In a photochemical reaction, the DD complex is attached through a [2+2] cycloaddition reaction to the coumarin side group, and a polymer with photocontrolled drug delivery (PCDD) capabilities is obtained.

The photoreversibility of cyclobutane systems is well known.^{53–58} UV photons as a trigger cannot be employed here due to the strong UV absorption of the cornea, which limits the penetration depth of the light. We employ a TPA-induced process that enables a cleavage of the UV-active linkage behind the cornea as a photochemical barrier. The required intensities are achieved by focusing a pulsed laser system to a spot inside the PCDD material. Since the probability for a TPA-dependent reaction is relatively low, the process requires considerably high photon densities. This is because the device is not affected by ordinary light.^{59–62} Compared with conventional sustained-release intraocular drug delivery devices based on biodegradable polymers, this novel photomodulated

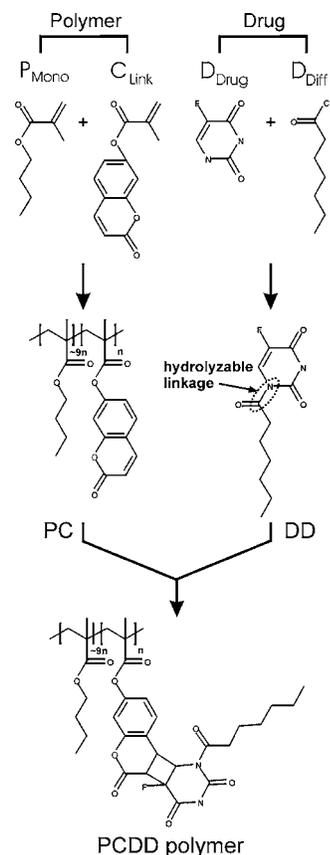


Fig. 3 Functional building blocks for photocontrolled drug delivery intraocular lenses (PCDD-IOL). The polymer backbone is a copolymer (PC) obtained from a polymer monomer (P_{mono}) and a polymer monomer with a photosensitive linker molecule attached (C_{link}) at a molar ratio of about 9:1. The drug complex (DD) comprises the drug molecule (D_{drug}) and an optional diffusion modulator molecule (D_{diff}), which is attached through an easily hydrolysable linkage. D_{diff} is required, as the solubility and diffusion constant of the nonmodified drug in the polymer is often very poor. The photocontrolled drug delivery polymer (PCDD) is finally obtained by coupling the drug complex (DD) to the copolymer (PC) by means of a photocleavable linkage.

delivery device offers temporal and spatial control over the release of the drug. The required or desired drug levels can be easily controlled by dosing the irradiation energy.

The material properties should be equivalent or superior to polymethylmethacrylate (PMMA), which is currently one of the standard materials for IOL manufacturing. As a first example, we have chosen n-butylmethacrylate (NBMA) as a polymer backbone, which provides the unique mechanical and optical properties of the acrylic polymers. The fabrication of the device consists of three different steps, as sketched in Fig. 2. The copolymer matrix bearing photoreactive moieties (C_{link}) was obtained by copolymerization of NBMA (P_{mono}) and monomeric methylmethacrylate modified with coumarin (C_{link}). The coumarin side group acts as a molecular linker due to its capability for reversible [2+2] photocyclizations/photoreversions in dependence on the wavelengths of light used.^{63–65} We have shown that coumarin photodimers used in these experiments can be cleaved by two-photon absorption.⁶⁶ The model drug, 5-fluorouracil (5FU), was modified with hep-

tanoic acid (D_{diff}) resulting in the drug precursor (DD), 1-heptanoyl-5-fluorouracil (H5FU). While possibilities of direct photochemical crossdimerization of coumarin derivatives and 5FU were reported in several studies,^{67–69} the utilization of alkylated 5FU provides some advantages such as improved solubility, miscibility, and diffusion properties in organic solvents and hydrophobic polymers, respectively. The diffusion modulator (D_{diff}) is attached to the drug by a hydrolysis-labile ester bond that is cleaved on exposure to water at the target site. Finally, the polymer-drug conjugate (PCDD) was obtained using photochemical [2+2] cycloaddition reaction. Analysis by UV/VIS spectroscopy shows that the typical absorption band of coumarin at 314 nm corresponding to the double bond besides the carbonyl group,⁷⁰ is missing in the absorption spectrum of the obtained polymer-drug conjugate. This indicates a complete cyclodimerization of all coumarin moieties to [2+2]-photoproducts. In the spectrum, no absorbance in the visible region is observed, which guarantees the excellent transparency of the polymer for its use in IOLs. Because the temperature at which 5% of the polymer decomposed (244 °C) is much higher than the glass-transition temperature $T_g=83$ °C, it is possible to fabricate IOLs via compression molding. The high value of T_g guarantees that the IOL is stable after implantation. With the data from elemental analysis, we can calculate a drug loading in the polymer of about 6% (wt/wt).

2.2 Phototriggered Drug Release

Both single-photon absorption (SPA) and TPA-induced processes were investigated. Polymer films for testing were fabricated from a blend of the PCDD polymer-drug conjugate and PMMA (for SPA 3:1 wt/wt and for TPA 1:1 wt/wt, respectively) by the solvent casting technique. During the irradiation of the obtained films with 254-nm UV light, the absorption band with a maximum at 312 nm, which corresponds to the coumarin moieties, increased [Fig. 4(a)], which indicates the cleavage of the cyclobutane ring and the related drug release. TPA-induced photocleavage of the PCDD material by intense 532-nm pulses is observed at much higher energies. The spectral changes during the course of irradiation with an average pulse intensity of 43.5 mJ/pulse are shown in Fig. 4(b). The observed spectral changes of SPA- and TPA-induced processes are similar.

The dependence of the initial photocleavage rate on the incident intensity was determined to confirm the TPA nature of the process induced by the 532-nm pulses. The initial rates of photocleavage in the model IOL were derived from the changes in the absorption at 312 nm, and the incident intensities range from 14 to 43.5 mJ/pulse at a repetition rate of 20 Hz were employed. The experimentally obtained slope of 1.93 in the log-log plot, shown in Fig. 4(c), corresponds nicely to the theoretically expected value of 2,^{59,61,62} and indicates that the cleavage of the PCDD conjugate is solely induced by TPA.

We analyzed the identity and quantity of the delivered H5FU by HPLC analysis (Fig. 5). From solutions of PCDD in chloroform, samples were analyzed before and after irradiation with the energies given. In both cases, the obtained HPLC profiles are very similar and show only two products, which are H5FU and its hydrolysis product 5FU, released

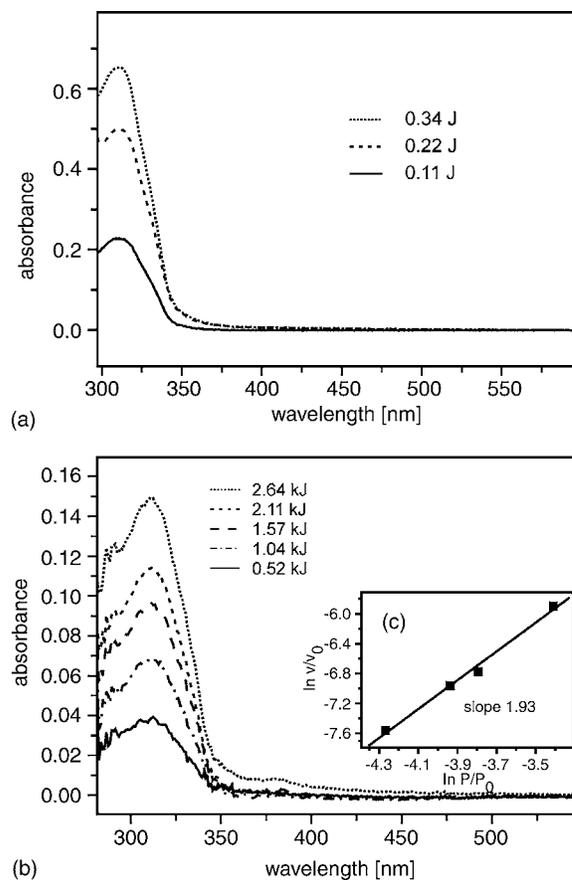


Fig. 4 Single-photon and two-photon absorption-dependent drug release from PCDD-IOL material. Difference absorption spectra of a PBMAOCH5FU film during photocontrolled drug release. (a) Single-photon absorption: UV light ($\lambda=254$ nm) is used to trigger release. (b) Two-photon absorption: a frequency-doubled Nd:YAG-laser ($\lambda=532$ nm, 43.5 mJ/pulse, 20 Hz) is employed. (c) The initial photocleavage rate is plotted as a function of the incident intensity at 532 nm. A slope of about 2 is derived, which corresponds to a two-photon reaction.

during photocleavage without any side products. The total amount of delivered H5FU and 5FU, respectively, correlates exactly with the absorption changes at 312 nm measured by UV/VIS spectroscopy.

2.3 Multidose Drug Delivery

Secondary cataract is observed many months even years after IOL implantation. Even after treatment, secondary cataract may reappear. Due to this, a multidose capability of the PCDD IOL is desired. To demonstrate an external triggered repeated drug release, the model IOL was irradiated in a step-wise manner, and the release pattern of 5FU was monitored using UV/VIS spectroscopy. The result depicted in Fig. 6 shows the cumulative amount of the discharged 5FU after three consecutive steps of irradiation with a constant energy dose of 112 mJ. During each irradiation step, approximately 2 μ g of 5FU were released from approximately 30-mg PCDD material. The amount of released drug was strictly proportional to the applied energy dose. This was also confirmed by HPLC analysis. In view of the low volume inside the capsular

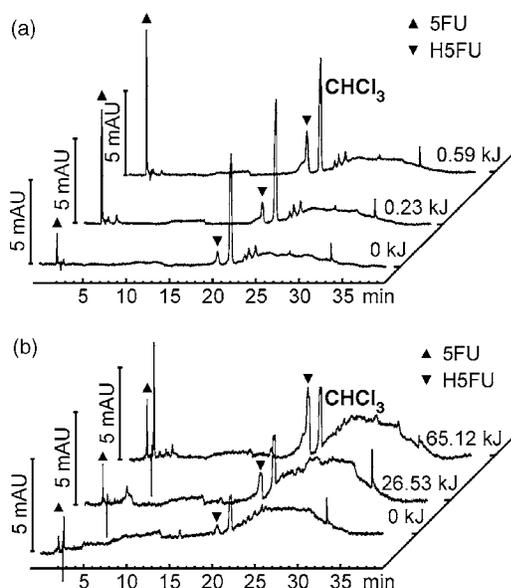


Fig. 5 HPLC analysis of phototriggered released compounds. (a) PBMAOCH5FU solution on irradiation with UV light ($\lambda=254$ nm). (b) PBMAOCH5FU solution on irradiation using frequency-doubled Nd:YAG-laser ($\lambda=532$ nm, 67 mJ/pulse, 20 Hz). The solvent peak (chloroform) is marked.

back, the LD50 of 5-fluorouracil, which was reported for rabbit lens epithelial cells (RLEC) to be $0.58 \mu\text{g}/\text{ml}$,⁷¹ can easily be obtained.

2.4 In Vitro Test

In *in-vitro* cell tests, IOLs were tested to show that the drug-loaded material itself is nontoxic to the cells, but as soon as it is photochemically activated, the drug release is triggered and the cell count is reduced significantly. PCDD polymer disks of 8 mm radii with a thickness of 0.5 mm carrying approximately $800 \mu\text{g}$ of 5FU each were used for the tests. Such samples were incubated with pancreatic carcinoid cells (line Bon 1) and the proliferation of the cells was analyzed (Fig. 7).

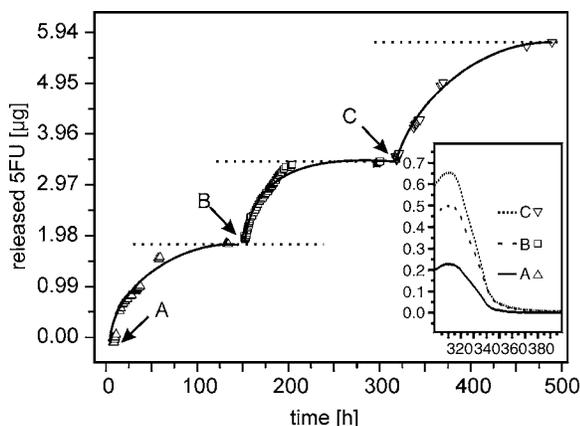


Fig. 6 Multidose drug delivery triggered by repeated irradiation. The cumulative amount of the 5FU released was determined from the absorbance changes observed at 267 nm. At the points A, B, and C, the film was irradiated with 254-nm light for 10 min. The insert shows corresponding absorbance changes.

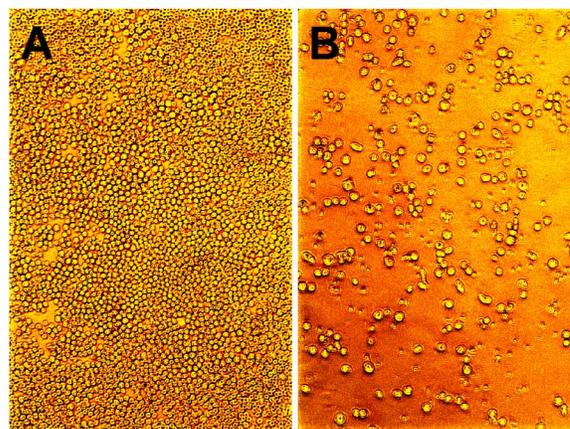


Fig. 7 Cell tests. Proliferation of (a) control cells and (b) cells treated with the activated IOL, both after 7 days in culture. The treated cells show a significantly lower cell density.

The data obtained clearly indicate that IOLs that were not photochemically activated have no significant influence on the cell proliferation. However, photoactivated IOLs cause a significant reduction of 29% of the cell mass compared to an untreated control group (Table 2). It should be mentioned that the *in-vitro* tests are for screening purposes, as *in-vivo* lens epithelial cells may show a different sensitivity toward 5FU.

2.5 Summary and Conclusion

We have developed a photocontrolled multidose drug delivery device that may be applied in the form and having the function of an IOL. This combination we named PCDD-IOL. The drug delivery is photochemically triggered by TPA, which enables ambulant treatment of readily available laser sources. The multidose capability of the PCDD-IOLs was demonstrated *in vitro*. Cell tests confirmed that the PCDD-IOL material itself shows not cytotoxicity until photochemical stimulation. Such materials will be tested in rabbits in the near future. The presented materials and concept are a new improved route in secondary cataract treatment.

3 Methods

Chemicals were purchased from Fluka (Taufkirchen, Germany) (5-fluorouracil HPLC grade), Acros (Nidderau, Germany) (heptanoyl chloride 99%, hydroxycoumarin 99%), Lancaster (Frankfurt, Germany) (methacryloyl chloride 97%), Riedel de Haen (triethylamine purum), Fisher Scientific (Nidderau, Germany) (acetonitrile HPLC grade), Aldrich (Taufkirchen, Germany) (butyl methacrylate 99%), and Merck (Darmstadt, Germany) (silica gel 60), and used as received. THF was dried over sodium and stored under argon until used. Azobisisobutyronitrile (AIBN) (BASF, Ludwigshafen, Germany) was recrystallized from ethanol.

3.1 Syntheses

3.1.1 1-Heptanoyl-5-fluorouracil (H5FU)

5.2-g (40-mmol) 5-fluorouracil (5FU) and 2.24-g (39-mmol) potassiumhydroxide (KOH) were dissolved in methanol and stirred at room temperature (RT). After 1.5 h, the methanol was removed in vacuum and the residue was

Table 2 Cell test: The protein content after 7 days was analyzed with the RC-DC Protein Assay. The statistic correlation performed with a Turkey multicomparison test shows a significant difference between nonactivated and activated PCDD-IOLs (p less than 0.001).

Device	Protein content				Mean	%
Control	0.520	0.485	0.450	0.448	0.476	100
Nonactivated	0.490	0.551	0.497	—	0.513	108
Activated	0.277	0.322	0.397	0.320	0.329	69

suspended in dry acetonitrile. 5.6-ml (36 mmol) heptanoylchloride was added at 0 °C to the solution. After the addition, the reaction was warmed to RT and stirred for a further 20 h. The solvent was removed and the crude product was dried under vacuum. Extraction with ethylacetate returned the H5FU product (yield 7.26 g, 75%).⁷²

3.1.2 7-Methacryloyloxycoumarin (MAOC)

To a solution of 16.2-g (0.1-mol) 7-hydroxycoumarin, 50.6-g (0.5-mol) triethylamine and 500-ml dry tetrahydrofuran (THF), 15.7-g (0.15-mol) methacryloylchloride was added under argon gas and heated for 2 h to 55 °C. After cooling to RT, the solution was stirred for another 20 h. The crude product was purified by flash chromatography on silica gel using CHCl₃:MeOH (40:1) as an eluent. MAOC was eluted at Rf=0.6. An amount of 35.05-g MAOC was obtained (yield 65%).⁷³

3.1.3 Poly(*n*-butylmethacrylate-co-7-methacryloyloxycoumarin) (PBMAOC)

42.66-g (0.3-mol) *n*-butylmethacrylate, 6.9-g (30 mmol) MAOC, and 30.0-mg (0.18-mmol) azobisisobutyronitrile (AIBN) were dissolved in 50-ml dry THF and reacted for 21 h at 60 °C. After precipitating the crude product twice in methanol, 34.8-g PBMAOC was collected (yield 70%).⁷³

3.1.4 H5FU loaded polymer (PBMAOCH5FU)

In a mixture of 9-ml chloroform-acetone (1:2 v/v) 0.2-g PBMAOC, 1.16-g (4.8-mmol) H5FU and 0.18-g (0.96-mmol) benzophenone were dissolved and irradiated in a Rayonet-type photoreactor (12 Eversun L40W/79K, Osram, Munich, Germany, concentrically installed) under continuous stirring for 20 h. Precipitating the crude product twice in methanol returned 0.18 g of the PBMAOCH5FU polymer (yield 91%).⁷⁴

3.2 Preparation of Polymer Films

Polymers or polymer blends were dissolved in chloroform to the highest concentration possible by stirring overnight. The solutions were filtered through 0.45- μ m Teflon filters (P819.1, Roth, Karlsruhe, Germany). A few milliliters of the filtered chloroform solution were pipetted on a glass plate. By means of a coating knife, a wet film of 0.4 or 0.8 mm thickness was obtained (=solvent casting). After solvent evaporation (typically for 20 h), a polymer film was obtained. Samples were prepared by either punching out or cutting out the desired pieces from the prepared polymer films.

3.3 Phototriggered Drug Release: Single-Photon Absorption

Single-photon absorption (SPA)-induced drug release was analyzed in solutions. Samples were prepared by dissolving 32.8 mg of PBMAOCH5FU in 4.5-ml CHCl₃. The solution was through a 0.45- μ m Teflon filter to remove any scattering particles. Excitation light of 266-nm wavelengths from a fluorescence spectrometer (RF-1502, Shimadzu, Duisberg, Germany) was used to induce SPA-dependent drug release in the test solutions directly in the spectrometer, where the resulting absorption spectra were recorded. The test samples were continuously stirred (Telemodul 20P and mini, H+P Labortechnik, Oberschleißheim, Germany), but during recording of the absorption spectra, the stirring was switched off. The intensity of the 266-nm light in the fluorescence spectrometer was 196 μ W/cm².

Polymer films for SPA-induced drug release were made from a mixture of PBMAOCH5FU and PMMA (3:1 wt/wt) as described before. A wet thickness of 0.8 mm was used. Light of 254-nm wavelength (MinUVIS, Desaga, Heidelberg, Germany, 187 μ W/cm²) was used to induce the drug release by light-induced cycloreversion of the coumarin linker system.

3.4 Phototriggered Drug Release: Two-Photon Absorption

Solutions for two-photon absorption (TPA)-induced drug release tests were prepared by dissolving 51.7-mg PBMAOCH5FU in 4-ml CHCl₃. The solutions were filtered through 0.45- μ m Teflon filters before use. An Infinity 40-100 mode-locked Nd:YAG laser (Coherent, Rödemark, Germany) emitting 3-ns pulses at 532 nm at a repetition rate of 20 Hz was used to excite the samples. The pulse energy was 67 mJ and the beam diameter was 5.5 mm.

Polymer films for TPA-induced drug release were made from PBMAOCH5FU and PMMA (1:1 wt/wt) blends. A wet thickness of 0.4 mm was used. To induce the cycloreversion of the coumarin system via two photon absorption, the Infinity 40-100 system described earlier was used. Pulse energies ranging from 14 mJ/pulse to 67 mJ/pulse were used.

3.5 Quantitative Analysis of Released Drug

HPLC analysis of the released drug was done on a Hewlett-Packard Model 1050 system equipped with a diode array detector. The 260-nm trace was used for quantitative determinations. A reversed phase column (Nucleosil, 3 μ m, RP18, 250 \times 4 mm, Bischoff, Leonberg, Germany) equipped with a

precolumn (20×4 mm, Bischoff) was employed for trapping the polymer content. A water / acetonitrile gradient was used.

3.6 Multistep Drug Release

A polymer film comprising PBMAOCH5FU and PMMA at a ratio of 1:3 (wt/wt) was prepared from a chloroform solution as described earlier. A suitably cut piece of the polymer film was mounted on one side of the inner walls of a fluorescence cuvette (101 QS, Hellma, Müllheim/Baden). The cuvette was filled with 3 ml of water. The film was irradiated with 254-nm light from a MinUVIS (Desaga, 187 $\mu\text{W}/\text{cm}^2$) until the desired energies were reached. After light-dependent release of 5FU from the polymer matrix, the drug diffuses from the polymer film into the aqueous solution. The UV/VIS spectra of the aqueous solution were recorded in a UVIKON 922 (Kontron, Munich, Germany) spectrophotometer. Accompanying HPLC analyses of small samples taken from the cuvettes were done as described before.

3.7 Cell Test

Polymer films for cell tests were prepared from 1:3 (wt/wt) mixtures of PBMAOCH5FU and PMMA dissolved in chloroform. A 0.8-mm coating knife was used to prepare the films as described earlier. Test disks having 16 mm diam and a thickness of 0.5 mm were punched out of the polymer foil and used for the cell tests. The drug release was triggered by light with 254-nm wavelength having a total energy of 0.504 J/cm².

2000 Bon 1 cells in 1.2-mL Dulbecco's modified Eagle's medium and Ham's F-12 medium containing 10% fetal calf serum (PAA, Cölbe, Germany) and 1% penicillin/streptomycin were seeded in 24 well plates (Greiner Bio-One, Frickenhausen, Germany). The polymer disks were added after 4 h in a way that any contact of the polymer disks with the Bon cells was omitted. No polymer disks were added to the control samples. The cell cultures were incubated at 37 °C in a saturated H₂O atmosphere containing 5% CO₂ (HS incubation cabin, Heraeus, Hanau, Germany). The cells were observed for 7 days. Then the cells were washed three times with phosphate buffered saline (PBS). Then, 1-mL 20-mM 3-(N-Morpholino) propanesulfonic acid containing 0.1% Triton-X-100 was added to each well to homogenize the cells. 50 μL of the suspension was analyzed with the RC-DC Protein Assay (Bio-Rad, Hercules, California) to determine the protein content following a modified Lowry method.⁷⁵ The protein content correlates to the total cell count. The statistic correlation was performed with a Turkey multicomparison test.

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