Improved Performances of Intraocular Lenses by Poly(ethylene glycol) Chemical Coatings

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Cataract surgery is a routine ophthalmologic intervention resulting in replacement of the opacified natural lens by a polymeric intraocular lens (IOL). A main postoperative complication, as a result of protein adsorption and lens epithelial cell (LEC) adhesion, growth, and proliferation, is the secondary cataract, referred to as posterior capsular opacification (PCO). To avoid PCO formation, a poly(ethylene glycol) (PEG) chemical coating was created on the surface of hydrogel IOLs. Attenuated total reflectance Fourier transform infrared spectroscopy, "captive bubble" and "water droplet" contact angle measurements, and atomic force microscopy analyses proved the covalent grafting of the PEG chains on the IOL surface while keeping unchanged the optical properties of the initial material. A strong decrease of protein adsorption and cell adhesion depending on the molar mass of the grafted PEG (1100, 2000, and 5000 g/mol) was observed by performing the relevant in vitro tests with green fluorescent protein and LECs, respectively. Thus, the study provides a facile method for developing materials with nonfouling properties, particularly IOLs.

Introduction

Currently, cataract surgery is a well-established ophthalmologic procedure that involves the extraction of the natural opacified crystalline lens by capsulorhesis and intraocular lens (IOL) implantation. The first poly(methyl methacrylate) (PMMA) IOL was implanted by Sir Harold Ridley in 1950. It was the starting point of a very successful and now very common surgical intervention. Nevertheless, a pending problem has been found in capsular opacification on the posterior side, usually referred to as posterior capsular opacification (PCO), that occurs in approximately half the cases 2−5 years after the surgery. PCO is mainly caused by the postsurgery proliferation of residual lens epithelial cells (LECs) inside the capsular bag.[1−13]

Neodynium:YAG (Nd:YAG) laser capsulotomy is the only practical way to treat PCO. It is however a risky treatment with possible retinal detachment, damage to the IOL, an increase in the intraocular pressure, etc.[3−6] Therefore, prevention of PCO is recommended rather than the Nd:YAgG treatment.

Several strategies have been reported for the prevention of PCO, such as optimized design of the IOL,[11,14,15] physical removal of the residual proliferative cells within the lens bag, appropriate techniques to destroy the LECs, use of pharmacological and immunological inhibitors of the LEC proliferation, etc.[6] In addition to these methods, surface modification of the IOL is a highly tolerant way for preventing PCO, because it does not require any manipulation within the eye nor the use of active and possibly harmful compounds during the IOL implantation. However, few techniques for the surface modification of the IOL have been reported. They include the surface modification of a PMMA IOL by carbon and titanium,[16] by heparin,[17] and by Teflon.[18] The surface of a silicone IOL was modified by oxygen and carbon dioxide plasma[19] or bioactive polymer containing sulfonate and carboxylate groups,[20] whereas IOLs of poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate) [poly(HEMA-*co*-MMA)] hydrogel were modified by a bioactive polymer containing sulfonate and carboxylate groups.[21] To be effective, the coatings should be not only cell-repellent but also protein-repellent because secretion of extracellular matrix proteins occurs before cell attachment.

Poly(ethylene glycol) (PEG) is a nontoxic, nonimmunogenic, and nonantigenic polymer which is known to decrease the attractive forces between surfaces and proteins as a result of high mobility in the hydrated state and related steric repulsion.[22−27] The covalent grafting of PEG onto a variety of substrates, including silicon,[24,28] polyurethane−urea,[29] glass,[30,31] fluorinated ethylene−propylene copolymers,[32] and polysulfone membranes,[33] has been reported along with a quite satisfactory protein-repellent effect. It may be anticipated that the protein-repellent properties of PEG would prevent an extracellular matrix from being formed and, thus, cells from adhering. In addition to these antifouling properties, PEG is optically transparent when hydrated, which is of the utmost importance for materials to be used as optical devices.

This work aims at coating IOLs consisting of poly(HEMA-*co*-MMA). For this purpose α-methoxy-PEG (mPEG) was reacted with the IOL surface premodified by isocyanate groups (Figure 1). Two different diisocyanates (5-isocyanato-1-(isocyanatomethyl)-1,3,3-trimethylcyclohexane, referred to as isophorone diisocyanate (IPh), and hexamethylene diisocyanate (HM)) were used to prefunctionalize the surface. mPEGs with three different molar masses (1100, 2000, and 5000 g/mol) were tested to evaluate the impact of the PEG chain length on the coating properties. The chemical composition and morphology

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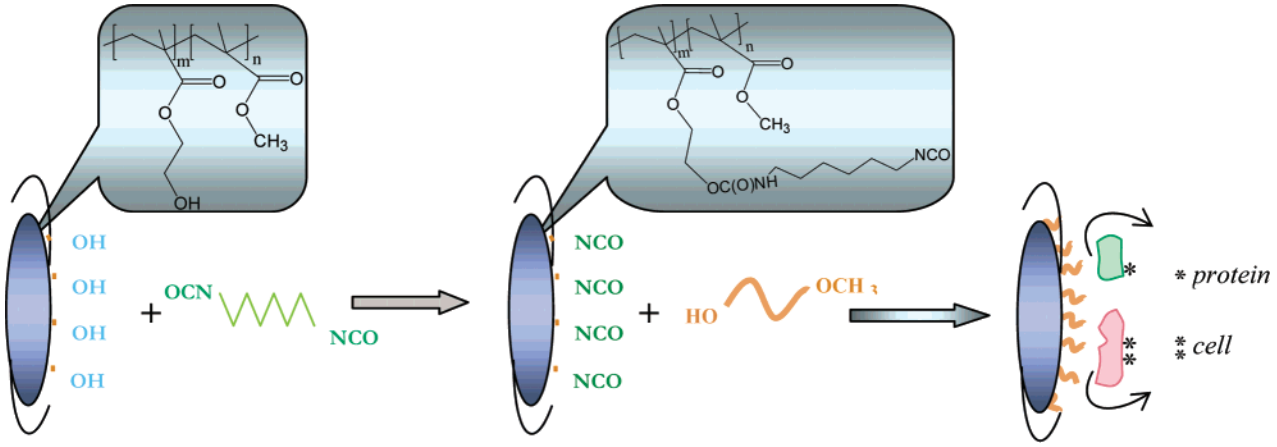

Figure 1. Reaction scheme for the grafting of mPEG onto the surface of poly(HEMA-*co*-MMA) IOLs.

of the coatings were analyzed by a number of techniques, such as attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), "captive bubble" and "water droplet" contact angle measurements, and atomic force microscopy (AFM). Two main properties of the coatings were investigated for their close relation to PCO prevention, i.e., (i) protein and (ii) cell repellence. The protein repellence was estimated by performing the relative in vitro green fluorescent protein (GFP) adsorption test, and the samples were observed by fluorescence microscopy (FM). The adhesion of porcine crystalline LECs was in vitro estimated, and the samples were observed by optical microscopy and environmental scanning electron microscopy (ESEM).

Experimental Section

Materials and Methods. HM and IPh (Fluka) were stored under argon and used as received. Dibutyltin dilaurate (DBTDL) (Aldrich) was used as received. Toluene (p.a.) was dried over sodium and used after distillation. Water was purified by a Milli-Q system from Millipore.

mPEG with molar masses of 1100 g/mol (mPEG 1100), 2000 g/mol (mPEG 2000), and 5000 g/mol (mPEG 5000) (Fluka) were dried by three azeotropic distillations with toluene just before use.

Commercially available poly(HEMA-*co*-MMA) hydrogel IOLs (equilibrium water content of 26%) were extracted in a Soxhlet extractor with Milli-Q water for 5 days to eliminate any residual monomer and oligomer. After extraction, the IOLs were dried at 50 °C for 2 days and stored in a vial until use. Under these storing conditions, the IOLs can absorb water from the atmosphere. Therefore, before reaction with moisture-sensitive isocyanate, the lenses were carefully dried (80 °C, 0.05 mmHg, overnight) in the reaction flask.

Coating of the IOLs. The flask with the dry hydrogel samples was purged with argon, and the liquid diisocyanate was added until the hydrogel was fully immersed. The reaction proceeded at 80 °C for 24 h in the presence of a catalytic amount of DBTDL (0.1 mol %) with respect to the isocyanate.[34,35] Then the excess of unreacted diisocyanate was drawn out through a capillary under argon pressure. A toluene solution of mPEG (1/2, g/mL) was added also until complete immersion of the hydrogel. The reaction proceeded at 80 °C for 48 h in the presence of a catalytic amount of DBTDL (0.1 mol %) with respect to the mPEG.

The hydrogel was washed with toluene and water. Finally, it was either dried in a vacuum oven at 50 °C for 2 days or kept in water until characterization.

Characterization. The surface of dried IOLs was analyzed by ATR-IR with a Nicolet spectrophotometer (Ge crystal) in the 800−1800 nm range.

Captive bubble and water droplet contact angle measurements were performed on the surface of hydrated and dried IOLs, respectively, at a constant time of 30 s with a DGD Fast/60 contact angle meter and WINDROP++ software at an average deviation of ± 2°.

The transparency of the samples hydrated with Milli-Q water was measured with a Perkin-Elmer UV/vis spectrophotometer, Lambda 14P, in a quartz cell. Diopter measurements were performed on an ROTLEX IOLA instrument with shaped IOLs conditioned in physiological solution before and after modification.

Tapping-mode atomic force microscopy (AFM) was performed with a Nanoscope IIIa microscope from Digital Instruments/Veeco (operating in air at room temperature). Microfabricated silicon cantilevers were used with a spring constant of ca. 30 N m^{-1}. Different areas of one sample were analyzed, and the images were collected with the maximum number of pixels (512) in each direction and analyzed with the Nanoscope image processing software.

Green Fluorescent Protein Adsorption in Vitro Test. The IOLs were conditioned by immersion in a physiological solution, SERAG BSS, at pH 7.3 for 24 h and finally washed with PBS (pH 7.2). They were then immersed in a PBS solution of GFP (0.022 mg/mL) and maintained for 1 h in an incubator. They were gently washed with PBS just before observation by fluorescence microscopy.

Images of the IOL surface were recorded with a cooled AxioCam MRm (Zeiss) mounted on a Zeiss Axio Imager Z1 microscope through an EC Plan-NEOFLUAR 100×, 1.3 oil immersion objective with a 400−500 nm band-pass excitation filter and a 460−560 nm band-pass emission filter. AxioVision Rel 4.5 (Zeiss) software was used.

LEC Isolation and Culturing. The eyes of pigs (sort Pietrain-Landrace) were provided by the "Abattoir communale de Liége", stored at 0−4 °C (ice) in iso-Betadine (5%) containing phosphate buffer (pH 7.2) (PBS), and dissected up to 30 h postmortem.

The manipulations were carried in a class II laminar-flow hood, as described elsewhere.[6,8] Briefly, the ocular globe was opened by a circular incision with a scalpel close to the corneoscleral junction. The iris was incised into five pieces which were sewn onto the sclera and the zonules were delicately removed. The lens was taken from the aqueous humor and placed in a Petri dish. An incision was made on the posterior side of the capsule, and the lens crystalline substance was delicately removed. The capsule was then placed in a plate (six-well), and 0.5 mL of culture medium (CM) [89% Dulbecco's modified Eagle's medium (Cambrex), 10% bovine fetal serum (Gibco), and 1% antibiotics (penicillin/streptomycin, 10000 U/10000 μg/mL)] was added. After 30 min in an incubator, the capsule was attached to the bottom of the well, and an extra 2 mL of CM was added. The plate was kept at 37 °C and 5% CO_2 in the incubator, and the CM was changed 1 day after dissection and every 3−4 days afterward. The tissues were washed with PBS containing antibiotics (1% penicillin/streptomycin, 10000 U/10000 μg/mL) at each step of the dissection.

After tissue extraction, cells started to grow and proliferate. A 70% confluence was observed within 10−12 days. The cells were then

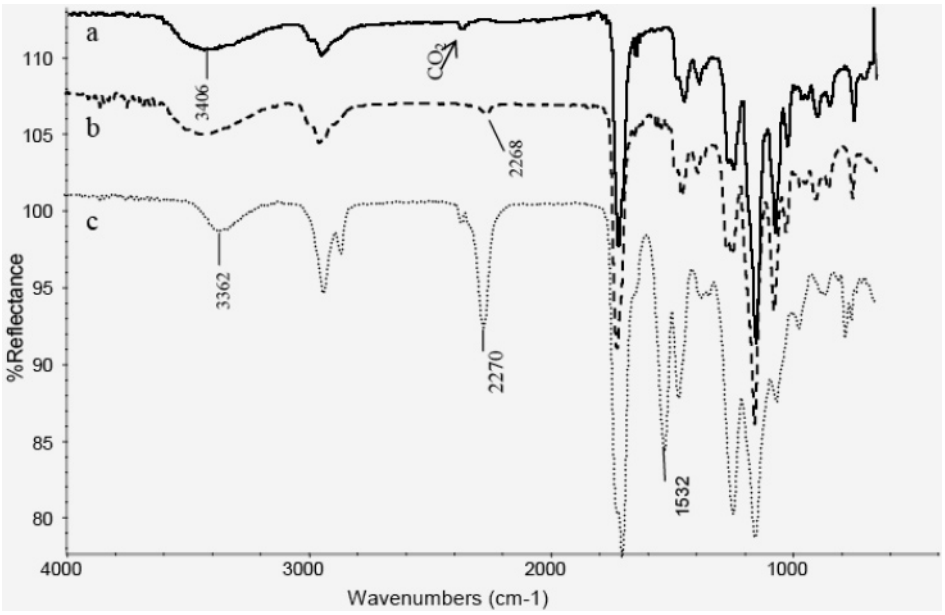

Figure 2. ATR-IR spectra of (a) unmodified and (b) IPh- and (c) HM-functionalized surfaces.

dissociated with trypsin/EDTA (Gibco), centrifuged, resuspended, and counted on a Thoma slide.

The growth and proliferation of the LECs in primary cultures were observed with inverted phase contrast microscopy (IPCM; Nikon).

LEC Adhesion (in Vitro Test). The samples were preconditioned by immersion in SERAG BSS physiological solution (pH 7.3) for 2 days and cut as disks with a 6 mm diameter fitting the test wells. They were rinsed and finally sterilized in physiological solution at 1.5 bar and 120 °C for 21 min. They were then fixed on the bottom of a 96-well plate and conditioned with CM in the incubator for 2 h to adjust the pH. After removal of the conditioning CM, 13000 cells/200 μL of suspension were added to each well.

The hydrogel coatings were put in contact with the LEC suspension in the incubator for 3 days. Then the culture medium was removed, followed by a careful washing with 1 mL of PBS to eliminate the nonadhered and dead cells. The cells were fixed with 2.5% glutaraldehyde for 30 min at 4 °C, washed three times with 0.2 M sodium cacodylate, and finally kept in 0.2 M sodium cacodylate at 6 °C.

The sample surface was observed by IPCM (Nikon microscope), environmental scanning electron microscopy (Philips XL 30 with an FEG electron source), and optical microscopy (Olympus BX60). Each sample was analyzed in triplicate.

Results and Discussion

Covalent bonding of PEG onto solid substrates is an efficient way to create a permanent PEG coating. The techniques known for covalently attaching PEG with different architectures onto surfaces can be subdivided into "grafting from" and "grafting onto" techniques. A "grafting from" technique consists in surface-initiated polymerization of functional PEG monomers, usually resulting in a "brushlike" coating. Any coupling reaction of preformed PEG derivatives with the surface belongs to the "grafting onto" techniques that allow PEG with predetermined architectures to be immobilized.

In this work, preformed mPEG−α-methoxy,ω-hydroxypoly(ethylene oxide) was grafted onto the surface of poly(HEMA-*co*-MMA) IOLs, which was previously modified by reaction with an excess of diisocyanate, as shown in Figure 1.

Surface −NCO Functionalization. To make −NCO groups available on the surface for further grafting of mPEG, we selected two isocyanates of different reactivities, IPh and HM. Their chemical structures are different, as well as the reactivities of their −NCO groups. Although HM contains two identical −NCO groups,[36] one primary −NCO group coexists with a less reactive secondary one in IPh. Therefore, depending on the diisocyanate used, the surface −NCO functionalization might be modulated, if necessary. Clearly, the IR absorption of the −OH groups of the untreated surface (Figure 2a) at 3406 cm^{-1} persisted when IPh was the diisocyanate (Figure 2b). In sharp contrast, this absorption was no longer observed upon reaction with HM (Figure 2c). Moreover, strong absorptions were observed at 3362 and 1532 cm^{-1}, assigned to the −NHCO− from the urethane group, and at about 2270 cm^{-1}, typical of the expected −NCO groups when HM was the reagent, which was not the case with IPh. It thus appears that HM is more effective than IPh in reacting with the hydroxyl groups of the hydrogel. A reason might be a difference in the penetrating depth within the hydrogel and/or in the conformational structure.[37] The two diisocyanates expectedly react by the more reactive of the two isocyanate groups, thus a primary isocyanate, which cannot explain the observed difference.

mPEG-Grafted Surfaces. mPEGs with different molar masses (1100, 2000, and 5000 g/mol) were reacted with the isocyanate groups available at the surface of the poly(HEMA-*co*-MMA) IOLs to provide them with protein- and cell-repellent properties.

Undiluted mPEG 1100 and mPEG 2000 were used as liquids at the reaction temperature (80 °C). The high viscosity of mPEG 5000 at 80 °C prevented the reaction from being conducted properly. Therefore, IOLs were coated by each of the three mPEG samples (mPEG 1100, mPEG 2000, and mPEG 5000) in toluene (1/2, g/mL) with the other conditions the same. All the samples were extensively washed with water to eliminate noncovalently attached mPEG before characterization.

The ATR-IR analysis of the IOLs grafted in toluene solution (Figure 3) confirmed the success of the grafting. Indeed, the absorption of the isocyanate at 2270 cm^{-1} disappeared, whereas a shoulder typical of the C−O−C grouping of mPEG was observed at 1105 cm^{-1}. The relative intensity of this absorption expectedly increased with the molar mass of the grafted mPEG.

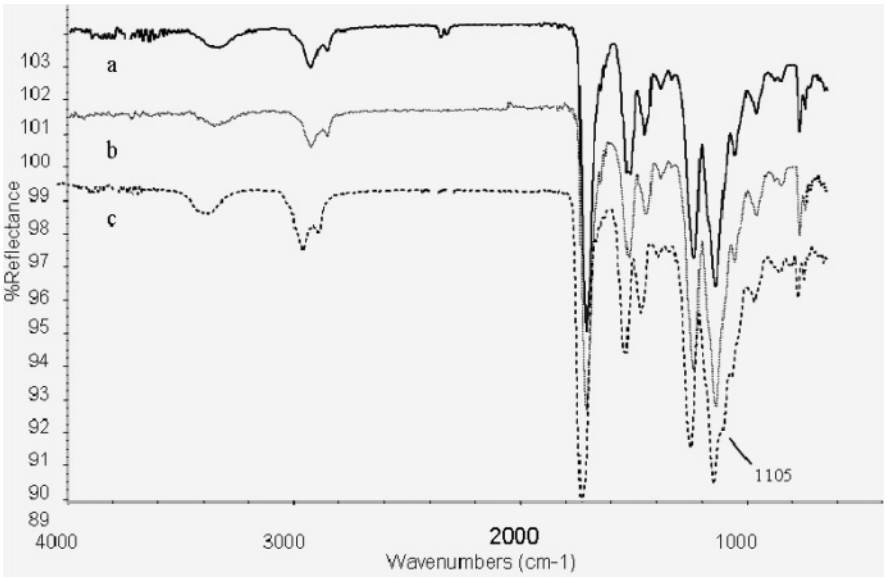

Figure 3. ATR-IR spectra of IOLs modified by mPEG (a) 1100, (b) 2000, and (c) 5000 in toluene solution.

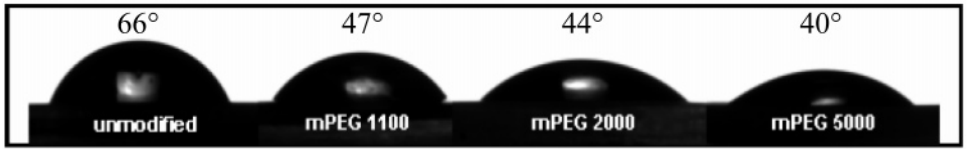

Figure 4. Contact angle of water on the surface of unmodified and mPEG-modified (in toluene solution) poly(HEMA-*co*-MMA) IOLs.

The ATR-IR spectra of IOLs grafted in the bulk are not represented because they are similar to those shown in Figure 3.

The contact angles of these surfaces (IOLs untreated and modified in toluene solution with mPEG 1100, mPEG 2000, and mPEG 5000) were measured in the hydrated state by the captive bubble technique. The contact angles of air-in-water were 141°, 147°, 154°, and 139°, for untreated and mPEG 1100-, mPEG 2000-, and mPEG 5000-modified IOLs, respectively. We concluded that the grafting of low molecular weight mPEG (mPEG 1100 and mPEG 2000) made the surface more hydrophilic, whereas no significant change of the contact angle was observed for the surface modified by mPEG 5000. These results do not completely agree with the contact angles of water-in-air, which decreased from 66° for an unmodified IOL to 47°, 44°, and 40° for the lenses grafted in toluene with mPEG 1100, mPEG 2000, and mPEG 5000, respectively (Figure 4). When mPEG 1100 and mPEG 2000 were grafted in the bulk, the contact angle (CA) was 51°. It may be noted that the coating consisting of the low molar mass PEG (mPEG 1100) is more effective when carried out in solution (CA(water) = 47°) rather than in the bulk (CA = 51°).

A reasonable explanation for the difference observed in the two series of contact angles could be found in the interaction of the grafted mPEG chains with the environment used in the measurements (air vs water). Indeed, in contact with water, all the grafted chains are hydrated and dangling in the water phase. The apparently lower hydrophilicity imparted to the surface by mPEG 5000 more likely results from a lower grafting density compared to those of mPEG 1100 and mPEG 2000. When the contact angles of water-in-air were measured, the hydrophilic mPEG chains tried to restrict their contact with hydrophobic air and to penetrate the more hydrophilic hydrogel, which is easier for the shorter chains. Therefore, the surface should be more hydrophilic when the mPEG chains are longer, as was observed.

Among the papers dealing with the hydrophilicity of PEGylated surfaces, one of them reported data that can be compared to our results (same PEG molar mass and same contact angle measurement techniques). Otsuka et al.[38,39] reported a contact angle of water-in-air of 47°, to be compared to 40° in this work, for a PEG 5000 coating.

According to Yamakawa et al.,[40] not only the surface hydrophilicity but also the surface roughness has an impact on the cell adhesion. They studied the adhesion of rat cells (splenocytes) on PMMA IOLs with different roughnesses, and they observed that cell adhesion increased with increasing surface roughness. Thus, nonpolished lenses were used in this study for testing the protein adsorption and cell adhesion, assuming that surface polishing, which is a common industrial practice, will improve the protein and cell repellence.

The surface of the dried unmodified and nonpolished lenses was observed by AFM and expectedly showed the grooves imprinted by the cutting diamond (Figure 5a,f). It is not surprising that the grafting of a monolayer of PEG did not erase the grooves, whatever the mPEG molecular weight (Figure 5f). Actually, the grafting of mPEG 1000 and mPEG 2000 made the surface more granular (Figure 5c,d), more likely as result of PEG crystallization. Nevertheless, when the grafted chains were longer (mPEG 5000), the homogeneity and flatness of the surface were improved (Figure 5e). It must be stressed that the surface morphology shown in Figure 5 is not representative of the hydrated material and thus not of the IOLs ready for use. Upon swelling by water, the lens surface should be smoother due to hydration of the PEG coating.

It is of critical importance that the transparency of the original IOLs is maintained after the PEG grafting. This was not the case for the PEG-modified lenses in the dried state because of the surface crystallization of PEG. The opacity however disappeared upon hydration, and visually transparent hydrogels were obtained, as shown in Figure 6. This visual observation was confirmed by UV−vis analysis (Figure 7). Indeed, grafted

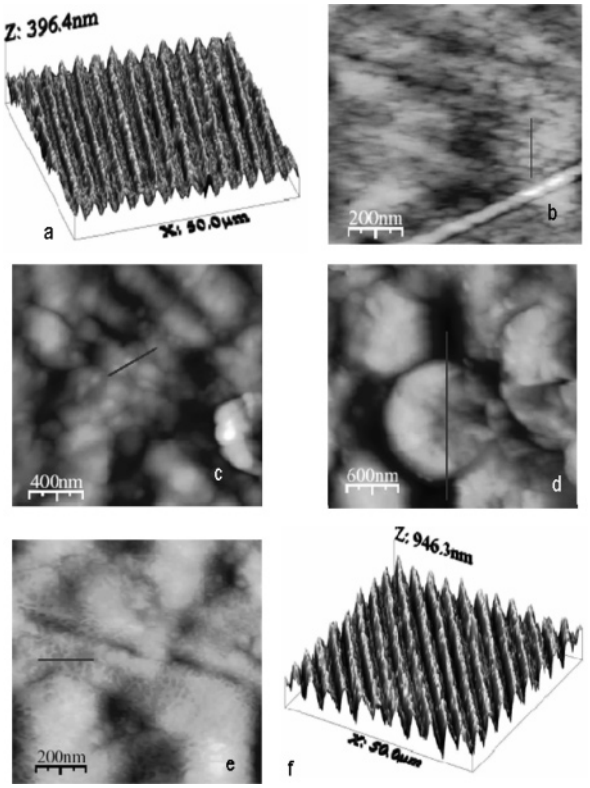

Figure 5. AFM images of (a, b) unmodified and (c) mPEG 1100-, (d) mPEG 2000-, and (e, f) mPEG 5000-modified (in toluene solution) IOL surfaces.

or not by PEG, the visible light transmission by the hydrated lenses is higher than 90%. The strong UV absorption was expected because a UV blocker is systematically added to the hydrogel to protect the eye afterward. The transparency of the IOLs was thus unaffected by the PEG grafting.

The refractive index of a hydrogel depends on its composition. In the case of poly(HEMA-*co*-MMA) it lies in the 1.44−1.46 range.[41] The refractive index of poly(ethylene glycol) is 1.4537,[42] thus falling within the range of that of the IOL substrate. The refractive power of the shaped IOLs remained unchanged upon modification, as observed by performing diopter measurements.

In Vitro Protein Adsorption. Proteins contained in the eye liquids in contact with the implanted lenses are expected to adsorb very rapidly onto the surface of the hydrogel. This nonspecific adsorption of proteins is uncontrolled and is thought to trigger deleterious reactions of the body, such as foreign-body response and fibrous encapsulation. As a rule, the adsorption of proteins, which are the major constituents of the extracellular matrix, is the preliminary step of cell adhesion. Therefore, in vitro protein adsorption was tested to estimate the protein repellency properties of the coated IOLs in relation with the molar mass of the grafted mPEG.

From an extensive study of PEG−protein interactions, Abbott et al.[43] concluded that PEG coils effectively exclude from the space they occupy proteins of a comparable or greater size. Consistently, Jeon et al.[44] proposed that the surface attraction was as high as the protein was large, resulting in a higher compression of the PEG chains and thus in a more effective repulsion. This is the reason why PEGs with a rather low molar mass (1100−5000 g/mol) were grafted onto the lens surface. For the same reason, the protein repellency by the modified IOLs was tested with a model protein (GFP) of a molar mass (28.8kDa) considerably smaller than those of the proteins present in the aqueous humor of the eye, i.e., fibrinogen (340 kDa), albumin (67 kDa), and fibronectin (460−500 kDa).

Figure 6. Visual transparency of hydrated unmodified and mPEG-modified (in toluene solution) poly(HEMA-*co*-MMA) IOLs.

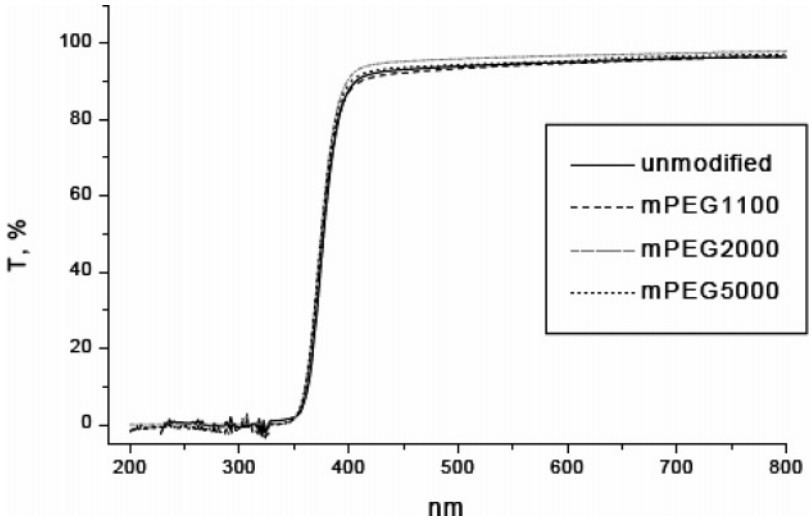

Figure 7. UV−vis spectra of hydrated unmodified and mPEG-grafted (in toluene solution) poly(HEMA-*co*-MMA) IOL hydrogels (sample thickness 0.7 mm in the dry state).

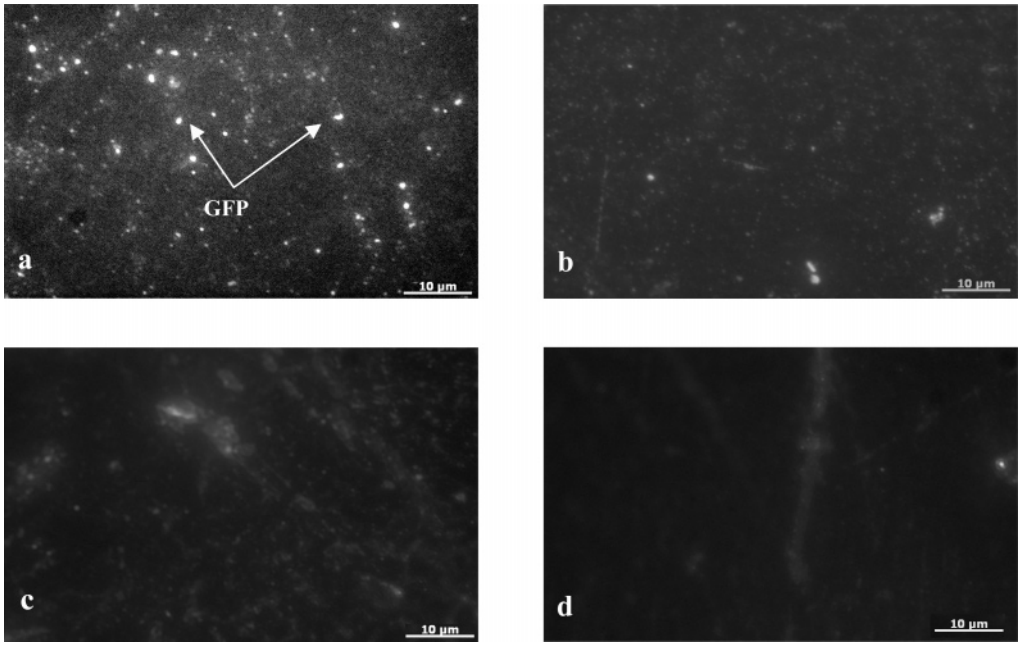

Figure 8. Fluorescent microscopy images of (a) unmodified, and (b) mPEG 1100-, (c) mPEG 2000- and (d) mPEG 5000-modified (in toluene solution) surfaces after 1 h of interaction with GFP.

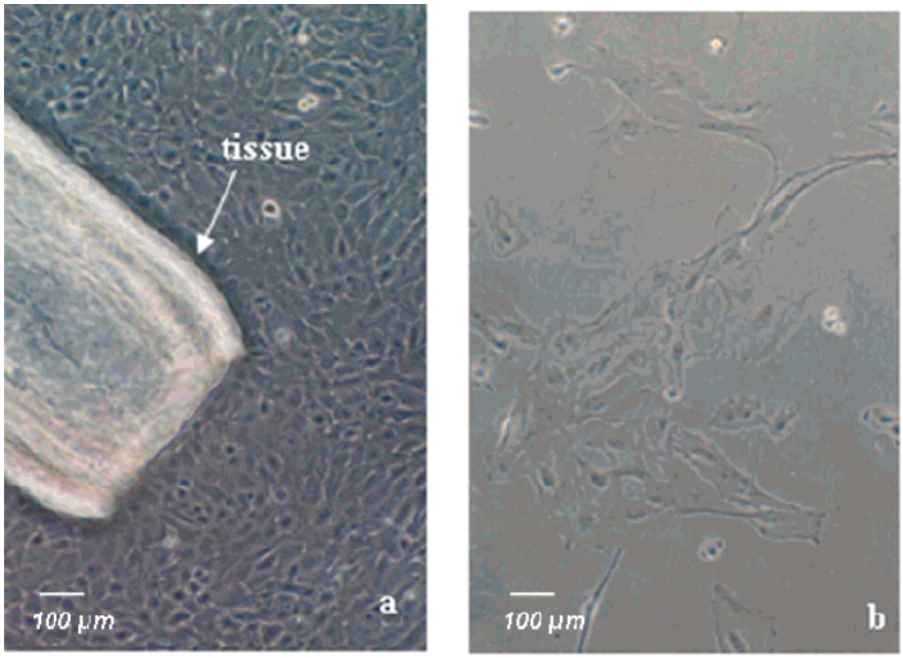

Figure 9. IPCM observation of living pig crystalline epithelial cells: (a) migration out of the tissue; (b) proliferation on the surface of the flask.

Adsorption of GFP was examined on the following surfaces: neat poly(HEMA-*co*-MMA) (control) (a) and poly(HEMA-*co*-MMA) grafted by mPEG 1100 (b), mPEG 2000 (c), and mPEG 5000 (d) in toluene solution.

Figure 8a shows that more GFP was adsorbed onto unmodified IOLs as a result of strong hydrophobic interactions. In contrast, the highly hydrated grafted PEG chains minimized these interactions and decreased the extent of the protein adsorption (Figure 8b−d). This beneficial effect was actually as strong as the length of the grafted PEG chains was high. For the longer chains used in this work (mPEG 5000), GFP was not adsorbed except onto surface defects (scratches). This size effect might be explained by the larger number of conformations that longer polyether chains can adopt. The accordingly higher mobility also minimizes the time of interaction of the proteins and is thus deleterious to their adsorption.[24]

Although based on only three molar masses, the aforementioned observations do not contradict the conclusion by Gref et al.[45] that the plasma protein adsorption onto PEG-coated polylactide nanoparticles was minimized for PEG with M_n in the 2000−5000 range.

Therefore, the in vitro GFP adhesion tests showed that the mPEG-modified IOLs are repellent for the model protein used in this work. As discussed by Abbott et al.[43] and Jeon et al.,[44] if these surfaces are repellent for the small sized GFP, they should be even more repellent for the bigger proteins present in the aqueous humor of the eye. Less protein adsorption should result in no or poor extracellular matrix formation and, thus, no or loose cell adhesion. This preliminary conclusion will be supported by the in vitro LEC adhesion tests discussed hereafter.

In Vitro Lens Epithelial Cell Adhesion. Because LECs are commonly observed in the pathology of the secondary cataract,

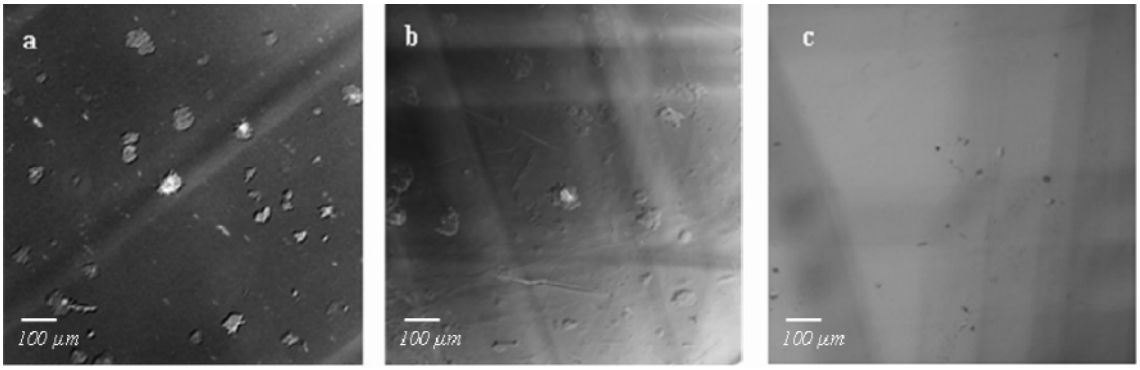

Figure 10. Optical microscopy of the surface of (a) an unmodified IOL and (b) mPEG 1100- and (c) mPEG 2000-modified IOLs after 3 days of interaction with LECs.

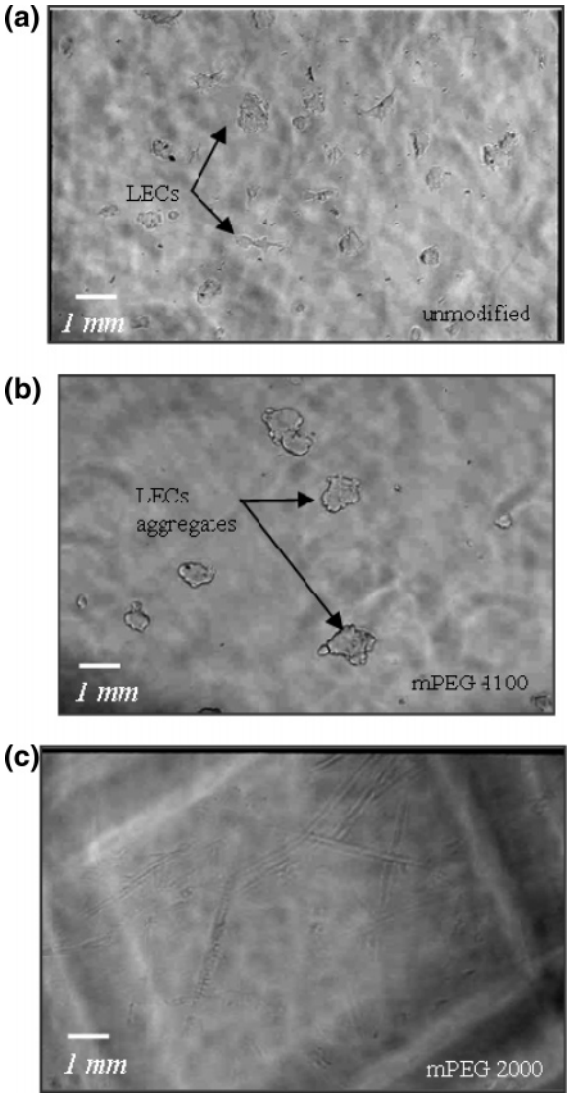

Figure 11. IPCM images of the surface of (a) unmodified and (b) mPEG 1100-, and (c) mPEG 2000-modified IOL hydrogels after 3 days of interaction with LECs.

they were selected for the in vitro testing of the cellular response of the surface-modified IOLs. A primary culture of LECs extracted from a porcine crystalline lens was used because they are supposed to be free of genetic modifications, which is not the case for cell lines.[6]

Morphology and proliferation of the cells were analyzed in the primary culture to learn about their behavior under *in vitro* conditions.

When the cells migrated out of the original tissue (within 2−3 days after dissection), they were more compact and dense (Figure 9a). Upon proliferation, they colonized on the plate surface, being more elongated and spread out (Figure 9b). In the primary culture they typically reached 70% confluence within 10−12 days, while initial cell migration, attachment, growth, and proliferation took 1−1.5 days. When confluent, the cells were trypsinated and used in the first passage in the in vitro cell adhesion test.

A 3 day time of interaction was chosen, because it was long enough for the cells to adhere to the surface (and possibly to proliferate), while being short enough for the culture medium to remain unchanged, which hides a risk of aspiration of nonadhered or loosely adhered cells.

The observations of the IOL surface by optical microscopy confirmed the cell repellency of the PEG coating (Figure 10). A high number of well-spread LECs (Figures 10a, 11a, and 12a) were observed on the unmodified surface, in contrast to cells that formed aggregates on the mPEG 1100 coating (Figures 10b, 11b, and 12b) predominantly close to surface defects (scratches), which indicates that they have poor affinity for the PEG-grafted surfaces due to high hydrophilicity. Moreover, increasing the length of the PEG chains decreases further this affinity for the surface, to the point where no adhering cells were observed on the surface grafted by mPEG 2000 (Figures 10c and 11c). In line with air-in-water contact angle measurements, no better results could be expected for surfaces modified by mPEG 5000, and thus, no observation was reported in this case.

It is well-known that the cells need to attach and spread out on the substrate before starting to grow and to proliferate.[46] Whenever the cells retain a rounded shape, they do not adhere or they adhere very loosely to the surface, and they are not prone to growth and proliferation. Therefore, anytime the LECs did not adhere to the surface, they were eliminated with the culture medium before fixation (mPEG 2000 coating). Whenever LECs were loosely adhering, they were observed on the surface with however a rounded shape and no substantial proliferation occurred (mPEG 1100 coating). In contrast, well-spread cells that grow and proliferate onto the unmodified surface would lead to PCO formation.

These observations are in good agreement with the in vitro protein adhesion tests and the nonfouling properties of the modified surfaces.

Conclusions

A novel and feasible method for covalently grafting mPEG onto the isocyanate-functionalized surface of poly(HEMA-*co*-MMA) hydrogel IOLs was developed. Coatings of grafted

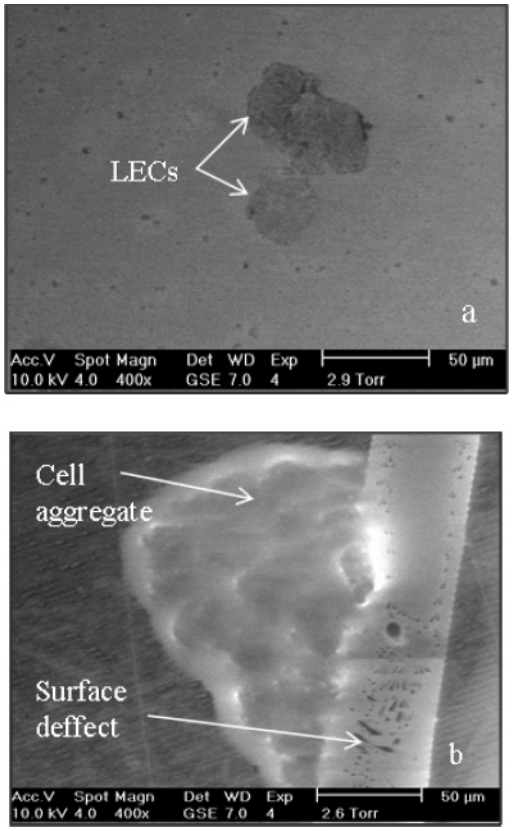

Figure 12. ESEM images of the surface of (a) unmodified and (b) mPEG 1100-modified IOL hydrogels after 3 days of interaction with LECs.

mPEG of different lengths were prepared and extensively characterized. The chemical composition and hydrophilicity of the modified surfaces were analyzed by ATR-IR and captive bubble and water droplet contact angle measurements. The hydrophilicity of the modified surfaces was as high as the grafted hydrophilic polyether chains were long. The surface modification was also confirmed by AFM, which showed changes in the morphology of the previously dried surface, which is additional evidence for the grafting of mPEG chains. The optical properties of the hydrated IOLs were maintained after coating.

The main purpose of the PEG coating is to restrict and hopefully to eliminate PCO formation on the posterior side of the IOL. This action was tested by in vitro protein and cell adhesion. The adsorption of the model protein, GFP, onto the mPEG coatings was significantly reduced, particularly in the case of higher molecular weight PEG. Cell repellency was indeed effective in the case of IOLs grafted with mPEG with a molar mass equal to 2000 g/mol.

The herein reported technique is extendable to any material, with $-OH$ or $-NH_2$ groups available on the surface, to which proteins and cells should not adhere.

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