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Long-term prevention of capsular opacification after lens-refilling surgery in a rabbit model

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ABSTRACT.

Purpose: **To reduce capsular opacification by a peri-surgical treatment of the lens capsule with drugs in an *in vivo* rabbit model. Lens-refilling surgery is a potential therapeutic intervention to treat patients with a cataract lens. The lens material is replaced with an injectable (bio)polymer that retains the natural mechanical and optical lens properties, therewith allowing accommodation. The occurrence of capsular opacification mediated by lens epithelial cells negatively affects accommodation and vision and should be avoided in this lens restoration approach.**

Methods: **An *in vivo* rabbit animal model was used with lens replacement with a silicone-based gel-like polymer and concurrent treatment of the lens epithelium with drugs. A case-study approach was applied as both drug combinations and implantation times were varied. The following drugs were investigated for their potential to prevent capsular opacification long-term: actinomycin D, methotrexate, paclitaxel and Tween-20. All were administered in a hyaluronic acid vehicle. The rabbits were clinically followed for periods up to 4 years postimplantation. Eyes, corneas and lenses were analysed post-mortem using MRI and confocal microscopy.**

Results: **Treatment combinations containing actinomycin D generally led to the least appearance of capsular fibrosis. The use of Tween-20 or paclitaxel without actinomycin D resulted in much earlier and pronounced fibrotic responses. The aspect of capsular opacification was highly variable in individual animals. Application of the drugs in a hyaluronic acid vehicle appeared to be a safe method that spared the corneal endothelium.**

Conclusion: **The feasibility of long-term prevention of fibrosis over a period of more than 4 years has been demonstrated in lens refilling in the rabbit model.**

Key words: accommodation – capsular opacification – fibrosis prevention – intraocular lens lens epithelial cells – treatment

*Retired.

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Introduction

Cataract is a frequent disorder that leads to blindness if left untreated. Treatment involves removal of the clouded lens nucleus and cortex and nowadays replacement with a intraocular lens (IOL) made of silicone or acrylates. This IOL is placed in the emptied lens capsule. Cataract surgery can be regarded as highly successful although complications are known (Clark 2000; Saika 2004), such as the occurrence of posterior capsular opacification, which consists of the activation, proliferation and migration of lens epithelial cells that can enter a classic epithelial-to-mesenchymal transition (Saika et al. 2004). The opacification occurs on the posterior side of the implanted lens, along the lens capsule, within the visual axis and is treated by YAG laser application. The described behaviour of the lens epithelium is present on all parts of the lens capsule that is in contact with the implanted IOL. As the present IOLs are usually not designed to be accommodating lenses, and the visual axis is cleared from fibrotic tissue by the YAG laser treatment, the entire set-up is functional in terms of restored vision.

Together with vision, accommodation can potentially be restored, as the accommodating system with zonula fibres and capsule remains functional also in elderly people (Strenk et al.

1999; Stachs et al. 2002). Several strategies have emerged in the literature, one of which is the use of injectable, gel-like, lens material-replacing polymers (Kessler 1964; Haefliger & Parel 1994; Assia et al. 1999; De Groot et al. 2001; Koopmans et al. 2003; Nishi et al. 2009, 2014; Hao et al. 2010, 2012; Hettlich 2010). This method relies on a functional capsular bag in which the gel is kept. Capsular opacification cannot be resolved by laser treatment because the capsular integrity is pivotal for both accommodation and keeping the gel in place. Therefore, in order to create a functional implant, the system has to remain free of capsular opacification.

Feasibility of restoration of accommodation using injectable gel-like polymers has been shown in studies concerning *in vivo* rabbit and monkey models (Stachs et al. 2011; Koopmans et al. 2014) and in cadaver lens studies (Hao et al. 2012). At the same time, it has been shown that the use of accommodating materials can elicit a powerful fibrotic response of the lens epithelium (Hao et al. 2010; Koopmans et al. 2011), indicating that treatment of the epithelium seems to be a necessity. It cannot be ruled out, however, that choosing the proper materials for lens replacement or for interfacing the capsule may also prevent opacification (Awasthi et al. 2009). In that respect, the results demonstrated by Hao et al. (2012) seem to indicate that lens epithelial cells can proliferate in the presence of injected polysiloxane lens-refilling gels, although at the same time examination of the fundus was no longer possible after one month of implantation indicating that a fibrotic response also may be present.

In this study, the long-term opacification response in refilled lenses *in vivo* in rabbits was assessed by follow-up of lens capsule fibrosis as a function of peri-surgical treatment of the lens epithelium with different drugs. The drugs were chosen for their potential to kill cells, to prevent proliferation, to prevent fibrosis or to actively detach cells from the capsular wall. Based on *ex vivo* studies using capsulorhexis specimens and *in vivo* studies in rabbits (Sternberg et al. 2010), methotrexate (MTX) and actinomycin D (AD) were chosen to treat the lens epithelium. In the performed *in vivo* study, it was observed that the rabbit eyes treated with a mixture of methotrexate/actinomycin D showed no posterior capsule opacification at 4 months whereas without drug treatment opacification started 6 weeks postoperatively (Sternberg et al. 2010). As paclitaxel (PTX) is commonly used for drug-eluting stents and drug-coated balloons in vascular intervention (Byrne et al. 2013; Petersen et al. 2013), PTX was included regarding its antiproliferative potential to prevent posterior capsule opacification. In addition to drugs, the detergent Tween-20 was tested for its potential to lyse lens epithelial cells.

To ensure safe application, the drugs were administered by incorporating them in sodium hyaluronate (Van Kooten et al. 2006) in a hypotonic environment. A low osmolarity has been shown to assist in lens epithelial cell killing (Maloof et al. 2005). The treatment period was set at 5 min perisurgically in order to control the drug delivery and to avoid the use of a long-term drug-delivering device (Fernandez et al. 2004; Koopmans et al. 2006). Long-term implantations of 7 months and up to more than 4 years were performed using a silicone polymer that has been shown to be an excellent lens replacement material in terms of its physical, mechanical and optical properties (Koopmans et al. 2006; Norrby et al. 2006).

Materials and Methods

Experimental set-up

Rabbit eyes were implanted with a flexible silicone polymer that allows accommodation. The animal study can be considered a series of case studies as both drug combinations and implantation times were varied. Before implantation, the residual lens epithelium was treated with chemical compounds. Three treatment groups were evaluated with a total of 12 eyes. The treatments were as follows: (1) AD-based (with Tween-20 or MTX); (2) Tween-20 alone; and (3) PTX + Tween-20. All treatment drugs were administered in hyaluronic acid (Healon – HA) under hypotonic conditions. Apart from the macroscopic, clinical observations, microscopic confocal images of both lens capsules *in situ* and corneal endothelium were made post-mortem.

Animals

The lens implantation experiments in rabbit eyes were performed at the University of Rostock, Germany. The Ethics Committee of the University of Rostock approved this study. The animal experiments were performed in compliance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Polymer lens-refilling surgery was performed in eyes of New Zealand white rabbits aged 12–15 weeks.

MRI measurements on selected rabbit eyes were performed at the University of Greifswald, Germany. Finally, post-mortem analysis of lenses and corneas was performed at the University Medical Center Groningen. Preservation details for eyes and derived tissues in the period after enucleation from the killed rabbits are given below in the Methods section for the different geographic locations in this study.

Implantation procedure

The rabbits were anesthetized with a mixture of 30 mg/kg ketamine hydrochloride and 5 mg/kg xylazine hydrochloride. Before surgery, the pupil was dilated with tropicamide (Mydrum) and phenylephrine hydrochloride ophthalmic solution (Neo-Synephrine 10.0%). A 3.0 mm clear corneal incision was created. Heparin sodium (5000 IU/0.2 ml) was injected into the anterior chamber, followed by an injection of sodium hyaluronate (1.0%, Healon). A clear corneal paracentesis was created with a 15-degree microsurgical knife. A high-frequency capsule opening device (Oertli Instrumente AG) was used to create a peripheral continuous curvilinear minicapsulorhexis with a diameter of 1.5–2.0 mm approximately 2.0–3.0 mm from the equator. Endocapsular phacoemulsification was performed using a Megatron I-Plus and a P2 handpiece (Geuder AG), with infusion through a second paracentesis into the anterior chamber. This was followed by bimanual capsule polishing. The empty capsular bag was treated with a drug-loaded solution to prevent secondary cataract development (see below). As a preparatory step, sodium hyaluronate 2.3% (Healon 5) was injected into the anterior chamber to

protect the corneal endothelium. The drug-loaded ophthalmic viscosurgical device (OVD) solution used to lyse and/or kill the lens epithelial cells (LECs) was then injected to inflate the capsular bag (see 'Capsular Opacification Prevention' section below for preparation details below). After 5 min, the drug-loaded solution was carefully aspirated bimanually using the phacoemulsification device. A purpose-designed silicone membrane plug with a diameter of 2.7 mm was inserted in the capsular bag through the capsulorhexis. The empty capsular bag was filled with the polymer through the capsulorhexis beneath the plug by inserting a 25-gauge cannula into the bag and injecting the refilling polymer until the surgeon judged that the capsular bag was completely filled. The cannula was retracted, and the plug was positioned to close the capsulorhexis. The Healon 5 was flushed from the anterior chamber with balanced saline solution via the incisions. Both incisions were sutured with 10-0 nylon, and the anterior chamber was reinflated with injected balanced saline solution.

Finally, all rabbits received a subconjunctival injection of gentamicin 40 mg/ml. Postoperatively, gentamicin eye drops (10 mg/ml, Refobacin) as well as prednisolone acetate eye drops (Inflanefran Forte) and ofloxacin eye drops (Floxal) were administered daily for 14 days. Additionally, an analgesic (novaminsulfone sodium) was given for 7 days via potable water (20 drops per 300 ml).

Follow-up procedure

Slitlamp examination and photographic documentation were performed under general anaesthesia (using the same anaesthetic protocol as described above) after 1, 3 and 6 months and then every 3 months up to 4 years postoperatively. The corneal endothelium was analysed using *in vivo* confocal laser scanning microscopy (CLSM) (Heidelberg Retina Tomograph II, Heidelberg Engineering GmbH, Germany).

Capsular opacification prevention

Prevention of capsular opacification was targeted by using OVD mixtures containing active chemical compounds dissolved/embedded in hyaluronic acid sodium salt. (HA). DL-methotrexate (MTX), actinomycin D (AD), paclitaxel (PTX) and polysorbate 20 (Tween-20) were obtained from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany. Stock solutions of 1×10^{-1} M AM and PTX in ethanol and of 1×10^{-1} M MTX in dimethylsulphoxide were prepared, which were further diluted with pure water to 1×10^{-5} M AD, PTX or MTX [corresponding to a nontoxic concentration of the organic solvent of 0.01% (Cortina et al. 1997)]. As Tween-20 can be used for lysing mammalian cells at a concentration of 0.05 to

Table 1. Lenses and corneas obtained from rabbits. Treatment and postoperative follow-up time until kill are indicated, as are the overall observations on PCO and cornea endothelium integrity. Rabbit numbers are indicated and can be related to the microscopic images and MRI data.

Treatment	Follow-up time in animal	Clinical assessment at kill	Post-mortem microscopic state of capsular bag	Post-mortem microscopic state of cornea endothelium
MTX/AD/HA Rabbit 34	4 years, 5 months	Iris bombata, polymer in the anterior chamber, lens not completely filled, low PCO	Fibrosis, strands, growth cones, plaques of cells	
MTX/AD/HA Rabbit 37	4 years, 3 months	Clear anterior and posterior capsule, no/low PCO, on posterior capsule slight fibrotic opacification, lens thickness constant, synechia	Diverse forms of fibrosis throughout capsule	
MTX/AD/HA Rabbit 40	3 years, 9 months	Clear anterior and posterior capsule, no/low PCO, slight fibrotic response, rhexis with double plug, synechia, lens thickness constant	Clear, posteriorly some strands of cells	Some EC parts gone, others normal
MTX/AD/HA Rabbit 41	3 years, 9 months	No/low PCO, clear anterior and posterior capsule, lens not completely filled	Lost capsular bag, no cells in silicone	Starting damage
AD/HA Rabbit 300	11 months	Lens material in anterior chamber, contact with cornea, vascularized cornea, hazy cornea, no capsular opacification	Clear lens with just a few cell groups present	
AD/HA Rabbit 301	1 years, 2 months	Plug not *in loco*, lens material in anterior chamber, contact with cornea, vascularized cornea, synechia, no to low capsular opacification	Some fibrosis, capsule detached from lens material	Partly fibrosis of cornea, some parts still regular, heavy influx of mononuclear cells
AD/Tween/HA Rabbit 402	7 months	Clear and well-reconstructed lens, plug in loco, cornea clear, no vascularization	Clear lens	Regular, normal
AD/Tween/HA Rabbit 403	7 months	Clear anterior capsule, plug not in loco, some polymer in the anterior chamber, cornea clear, no vascularization	Anteriorly clean, posterior active strand formation	Largely regular, normal, locally loss of cell organization
Tween/HA Rabbit 400	7 months	Plug in loco, heavy fibrosis, cornea clear	Heavy fibrosis in entire capsule	Regular, normal
Tween/HA Rabbit 401	7 months	Plug in loco, heavy fibrosis, cornea clear	Heavy fibrosis in entire capsule	Regular, normal
PTX/Tween/HA Rabbit 404	7 months	Plug in loco, polymer in the anterior chamber, synechia, capsule not completely filled	No data	EC layer largely intact, but underneath aberrant cell groups
PTX/Tween/HA Rabbit 405	7 months	Plug in loco, heavy fibrosis	Heavy fibrosis in entire capsule	Regular, normal

0.5% (v/v), it was applied at 0.025% (v/v) in the drug-containing HA.

Pure water was obtained by purification with ion-exchange resin (Ultra Clear UV Plus, SG Wasseraufbereitung und Regenerierstation GmbH). Ethanol and dimethylsulphoxide were received in analytical grade purity from VWR International GmbH, Darmstadt, Germany. The HA was dissolved in the 1×10^{-5} M drug or 1×10^{-5} M drug/0.025% (v) Tween-20 solutions to yield a concentration of 1% (w/w) HA. All treatments used are summarized in Table 1.

Lens retrieval

After diverse implantation times, the animals were killed, their eyes removed and fixed *in toto* in fixation solution [3.7% paraformaldehyde in cytoskeleton stabilization buffer – CS: 0.1 M Pipes, 1 mM ethylene glycol tetra-acetic acid (EGTA), 4% (w/v) polyethylene glycol 8000 (all Sigma), pH 6.9]. Eyes were kept refrigerated in this fixation solution until further analysis. Alternatively, eyes were removed and transported from the University of Rostock to the University Medical Center Groningen within 24 hr while kept on ice, without prior fixation. In

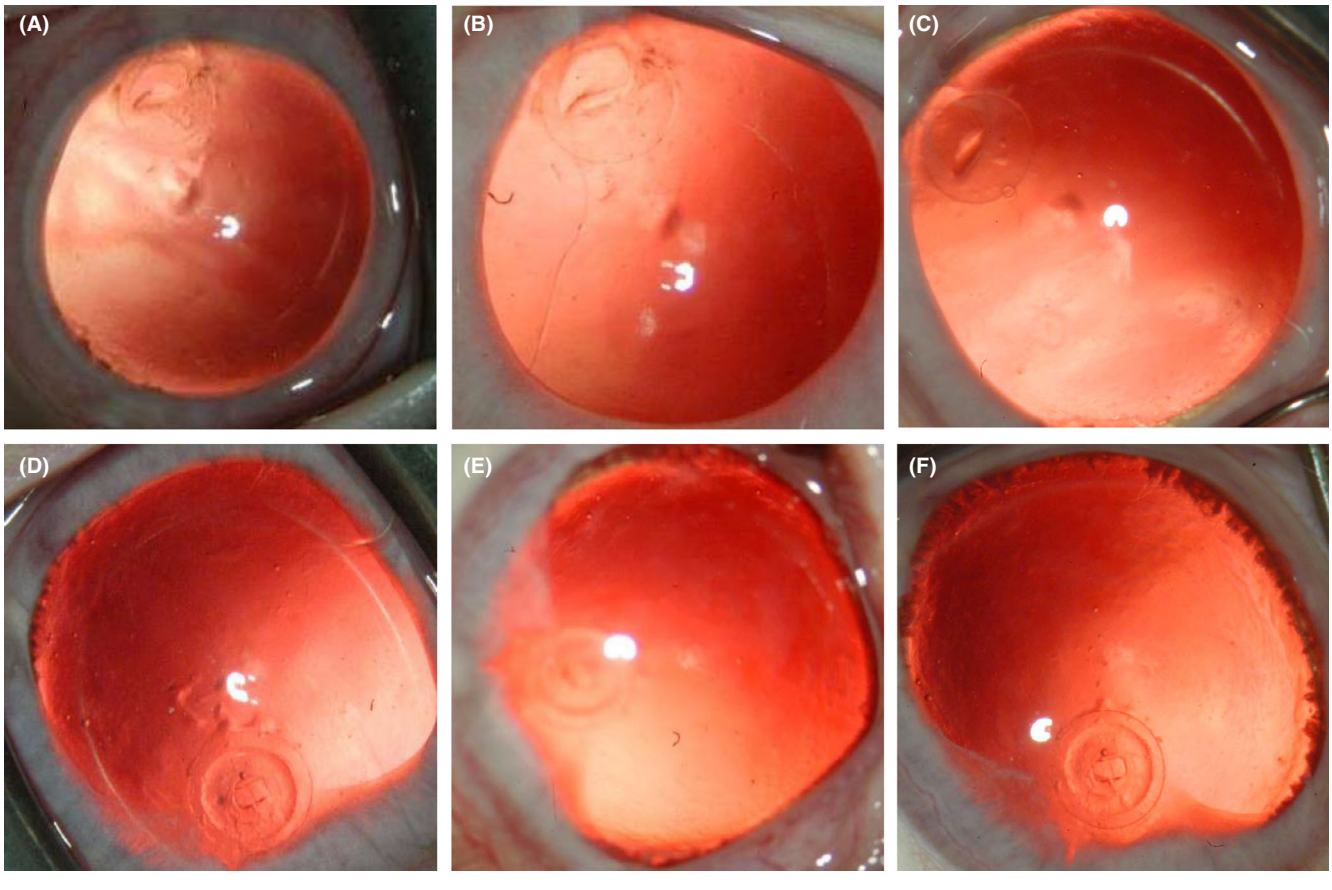

Fig. 1. Follow-up of two successfully treated rabbit lenses using an MTX/AD/HA treatment of the lens epithelium. Lenses were followed for 19 (A), 36 (B) and 42 (C) months and 15 (D), 32 (E) and 36 (F) months postoperatively, respectively [rabbits 37 (A-C) and 40 (D–F)].

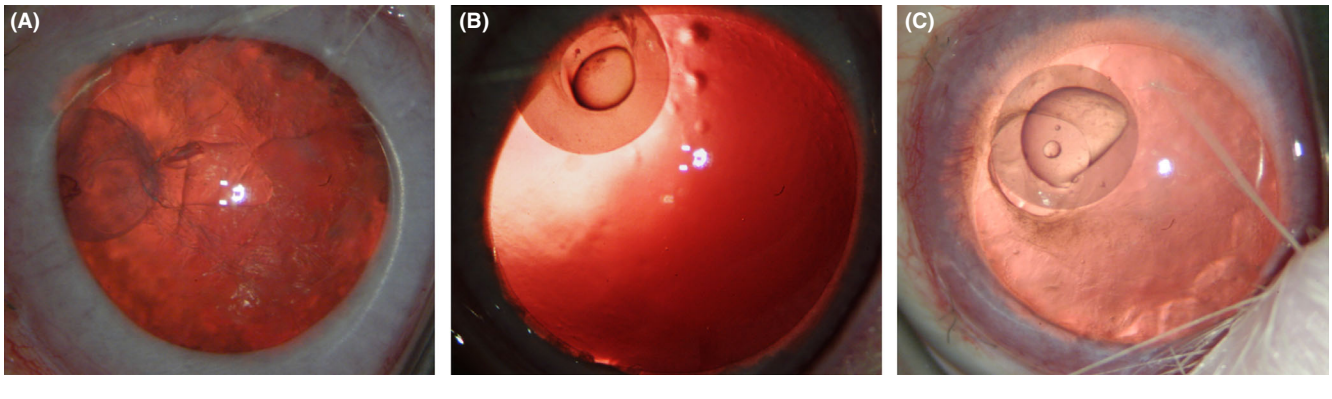

Fig. 2. 'Clinical' imaging of various stages of PCO in treatment groups Tween/HA (A), Tween/AD/HA (B) and Tween/PTX/HA (C), all 3 months postoperatively. Only (B) represents a clear lens.

the latter case, lenses were taken out directly after receipt and fixed. Corneas were treated according to the same procedure. Selected eyes were enucleated in Rostock, subsequently brought to Greifswald for MRI measurements on the same day (see below) and directly after MRI measurements fixed before they were transported to the University Medical Center Groningen.

T magnetic resonance imaging

In selected cases, axial globe cross sections of whole eyes were acquired *ex vivo* using a 7.1 T magnetic resonance scanner (ClinScan, Bruker BioScan GmbH) according to Stachs (Stachs et al. 2011). Eyes were measured within 12 hr of enucleation from the killed rabbits. Eyes were imaged using a phased array surface coil (rat brain) with two channels and two coil elements for each channel. After exploratory T2-weighted turbo spinecho images to localize the globe within the orbit, a high-resolution scan was performed. A field of view of 40 mm × 40 mm with a matrix of 320 pixels × 320 pixels provided an in-plane resolution of 125 mm × 125 mm. The other imaging parameters were repetition time 2420 milliseconds, echo time 44 milliseconds and 15 slices with a slice thickness of 700 mm and a gap of 20% between slices. The acquisition time was 4:43 min for the exploratory scan and 16:04 min for the final high-resolution data set scan, with an overall scanning time of approximately 30 min per rabbit eye. Eyes were subsequently put in fixation solution and transported to the University Medical Center Groningen for further microscopic analysis.

Microscopic assessment of lenses and corneas

For fluorescence microscopy, complete lenses and corneas were permeabilized for respectively 15 and 3 min in 0.5% Triton X-100 in phosphate buffer saline (PBS: NaCl 137 mM, KH_2PO_4 1.47 mM, Na_2HPO_4 8.10 mM, KCl 2.68 mM) and stained for nuclei (DAPI, 4 μg/ml) and the cell cytoskeleton (TRITC-Phalloidin, 2 μg/ml). Specimens were observed with confocal laser scanning microscopy (LEICA TCS SP2) using a fully water-immersed LEICA 40X objective with NA 0.80. Lenses were observed intact, with the refill material still in position in the lens capsule. Images were taken from both the anterior and posterior sides. Corneas were sliced into four quarters using a scalpel in order to be able to adequately approach the concave endothelial cell layer. Two images were taken from each quarter, giving a total of eight images.

Results

Surgery and macroscopic follow-up

Lens-refilling surgery was performed in 12 eyes of 12 rabbits in three different treatment groups and resulted in successfully refilled lenses in all cases. No complications occurred during surgery. During the follow-up, the occurrence of capsular opacification was monitored up to 4 years after surgery using slit-lamp examination. Animals were killed when opacification was observed to a significant extent. Postoperative complications also resulted in animal kill. Posterior synechiae were detected in the plug area in some cases. Examples of clear lenses more than 3 years after lens refilling are shown in Fig. 1, whereas in Fig. 2 examples of lenses showing different grades of PCO are shown three months postoperatively. The influence of the treatment on PCO formation is clearly illustrated by the absence in the Tween/actinomycin D treatment and the abundant presence in the treatment groups with Tween-20 alone or Tween-20 and paclitaxel together. In Fig. 3, MRI images are shown demonstrating the contours of the refilled lens relative to the lens shape in the control eye. These images were made after implantation and presence in the eye for 3 years and 9 months and may not represent the original shape directly after refilling.

Lens epithelial cell response

Lens epithelium was fluorescently labelled in the intact lens and observed with confocal microscopy. Representative images are shown in Figs 4 and 5 for refilled lenses in the different treatment groups and in Fig. 6 for control lenses. Furthermore in Fig. 7, a detailed composite image is shown of a fibrotic tissue

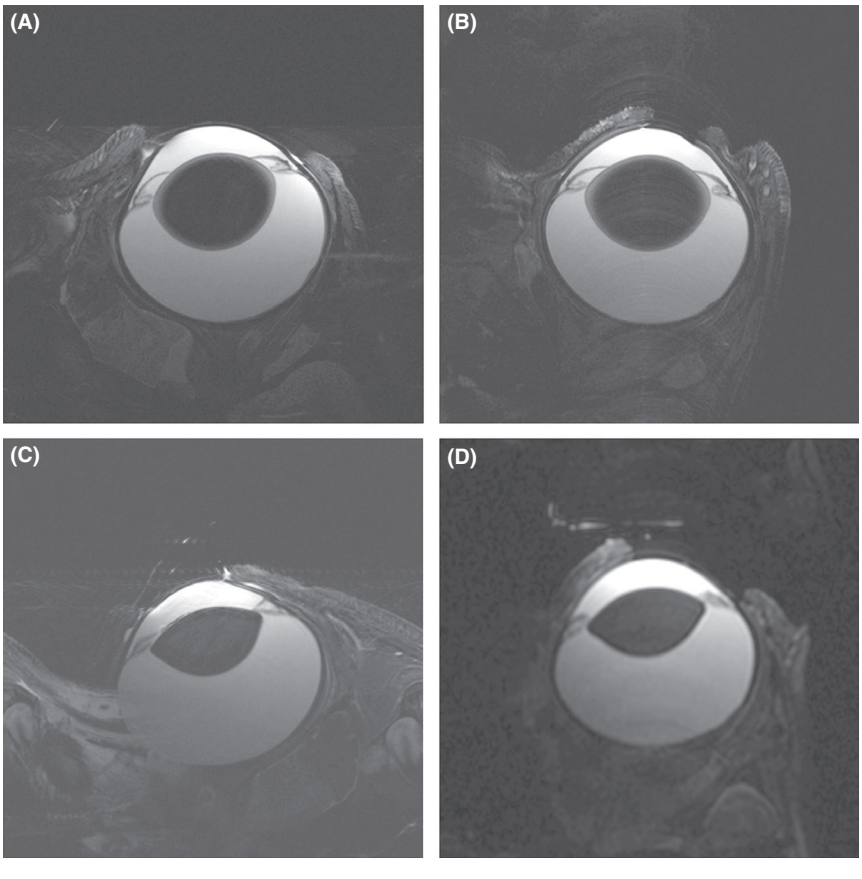

Fig. 3. Transversal and sagittal MR images exemplifying the lens shape of the crystalline, original (above) and the refilled lens (below) treated with MTX/AD/HA at 3 years and 9 months postoperatively (rabbit 40).

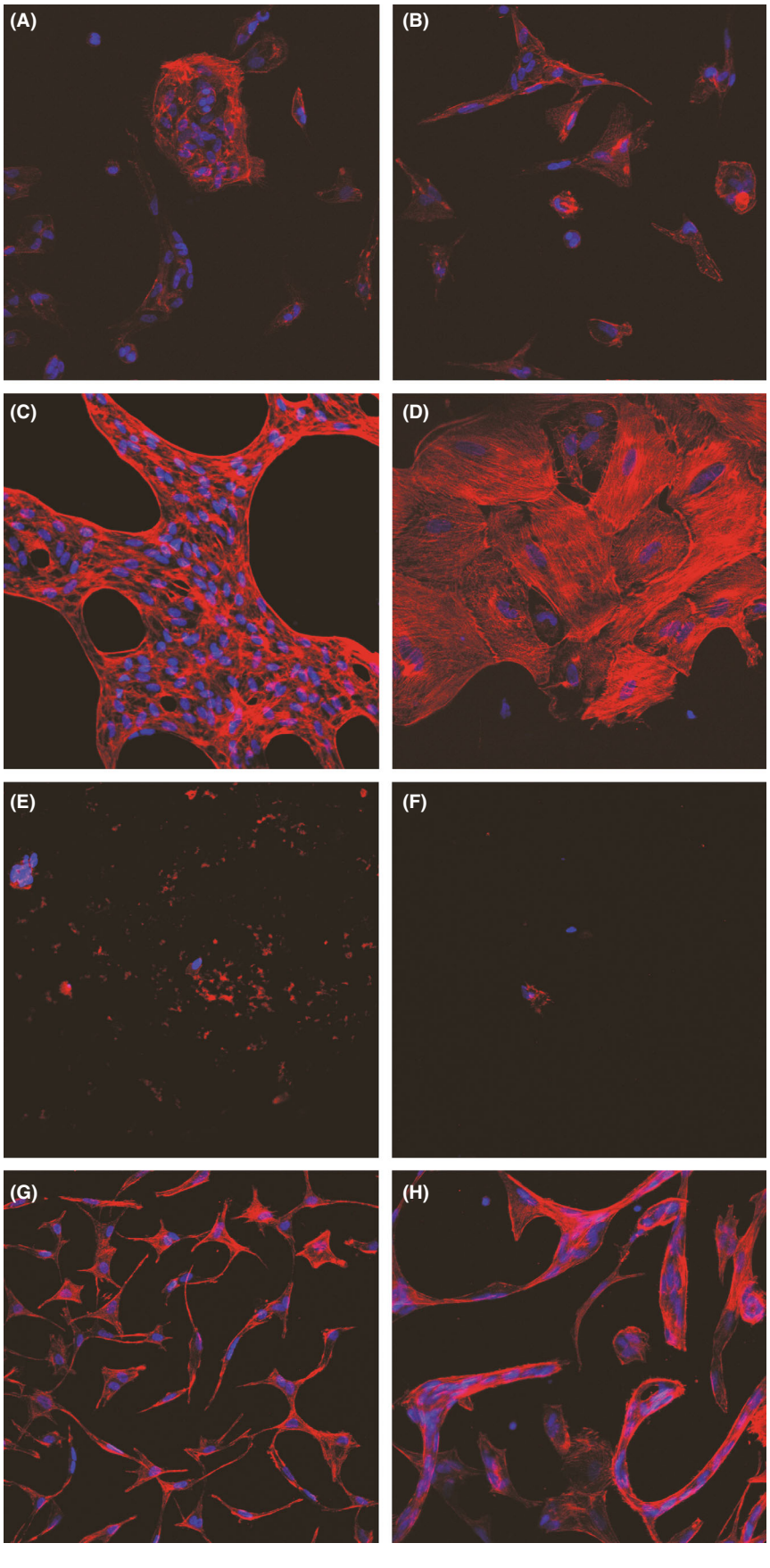

Fig. 4. Representative CLSM images of lens epithelium in the AD /HA treatment groups, combined with either Tween or MTX. Long-term implantation gave little cell proliferation and transformation as seen in (A) and (B) representing rabbit 40 on the anterior side towards the equator. Posteriorly, some cell aggregates were formed also located in the periphery (C: rabbit 37 and D: rabbit 40). Note the difference in morphology and cell size between both micrographs. With shorter implantation duration, the presence of cells on the anterior side was very limited (E: rabbit 300; F: rabbit 301), whereas posteriorly network formation could be seen in diverse stages (G: rabbit 301, near the equator; H: rabbit 403). The images in G and H together with those in C and D show the broad range of possibilities lens epithelial cells have in responding to the presence of the silicone. Each image represents 375 × 375 μm^2.

strand in the MTX/AD/HA group after 4 years and 3 months of implantation. Results are summarized in Table 1. A large diversity in the presence of capsular opacification and characteristics has been observed.

Corneal endothelial cell response

Corneas were fluorescently labelled and observed with confocal microscopy. When the endothelial layers are considered that can be counted, in general the number and distribution of corneal endothelial cells were comparable in refilled eyes and control eyes, as shown in Fig. 8, without significant differences. Representative confocal images are shown in Fig. 9 for treated and control eyes. From these figures, it can be seen that most corneas demonstrate a homogeneous layer of endothelium without signs of damage. Only three corneas are damaged. Results are summarized in Table 1.

Discussion

In this study, rabbit lenses were refilled with a silicone polymer that potentially allows accommodation (Koopmans et al. 2006). Apart from its translational use towards clinical applications, it may also serve as an excellent model to study the process of fibrosis (Eldred et al. 2011). In order to control opacification of the lens capsule by lens epithelium, the interior capsular wall was treated with different drug combinations during a peri-surgical time window of 5 min. Results demonstrate the feasibility of a long-term prevention of fibrosis, that is over a period of more than 4 years in the rabbit *in vivo* eye lens model.

The concept of replacing the stiff presbyopic lens with a material or lens design that simulates the young

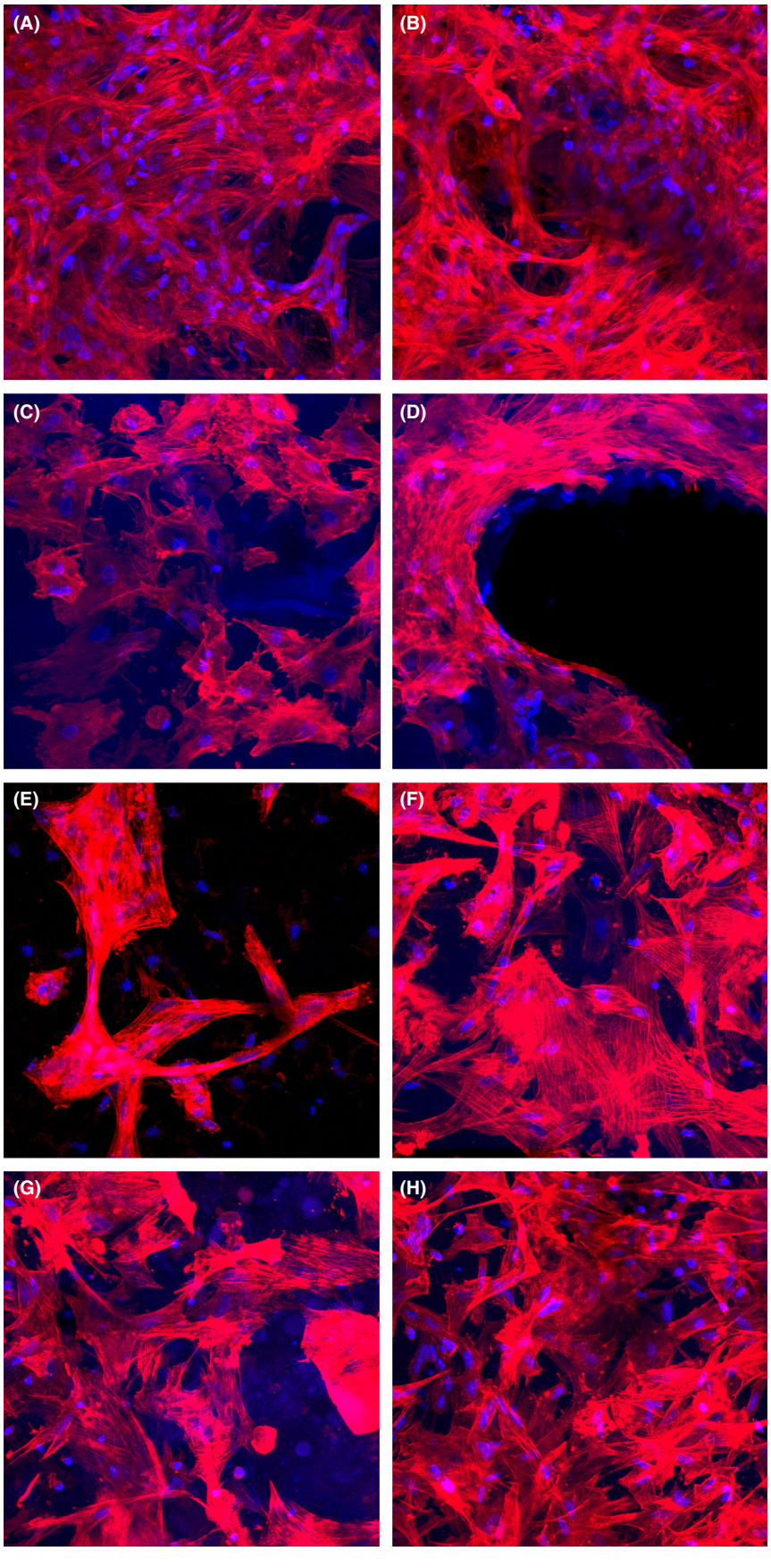

Fig. 5. Representative CLSM images of lens epithelium in the Tween/HA and PTX/Tween/HA group. A thick, clearly fibrotic tissue is present on the anterior side in the central region, often with large cells that indicate epithelial-to-mesenchymal transition (A, B: rabbit 400; E, F: rabbit 405). Also posteriorly, cells have advanced to the central region (C, D: rabbit 400; G, H: rabbit 405). Each image represents 375 × 375 μm^2.

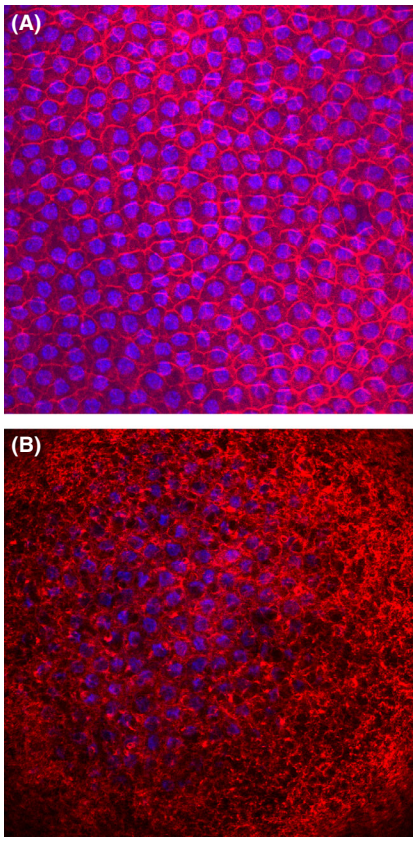

Fig. 6. Representative CLSM images of lens epithelium in control lenses. A perfectly organized lens epithelial cell monolayer is present (A). Imaging near the apical surface of the epithelium demonstrates the highly irregular cytoskeletal network underneath the cell membrane (B). Each image represents 375 × 375 μm^2.

crystalline lens to restore accommodation is not new. Details of several techniques have been published (Nishi et al. 2009). Results reported here are derived from a number of case studies of accommodating lens implantations in rabbit eyes, that were treated with different drug regimes, and were followed for different lengths of time. The implanted silicone-based accommodating lenses were present in the animals between 7 months and more than 4 years. Despite these variations in implantation time, a clear picture emerges from these experiments. Treatment with drug combinations including actinomycin D can result in the prevention of fibrosis, although some variation exists. Treatment with

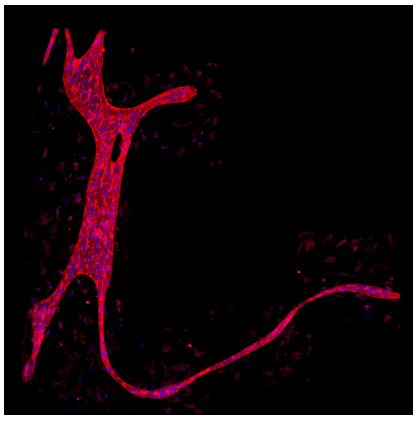

Fig. 7. Composite CLSM image of lens epithelium in the MTX/AD/HA treatment group after 4 years and 3 months of implantation in rabbit 37. The image represents 1.50 × 1.50 mm^2. The transformed cells have built strands of tissue with active growth cones into the silicone lens-refilling material.

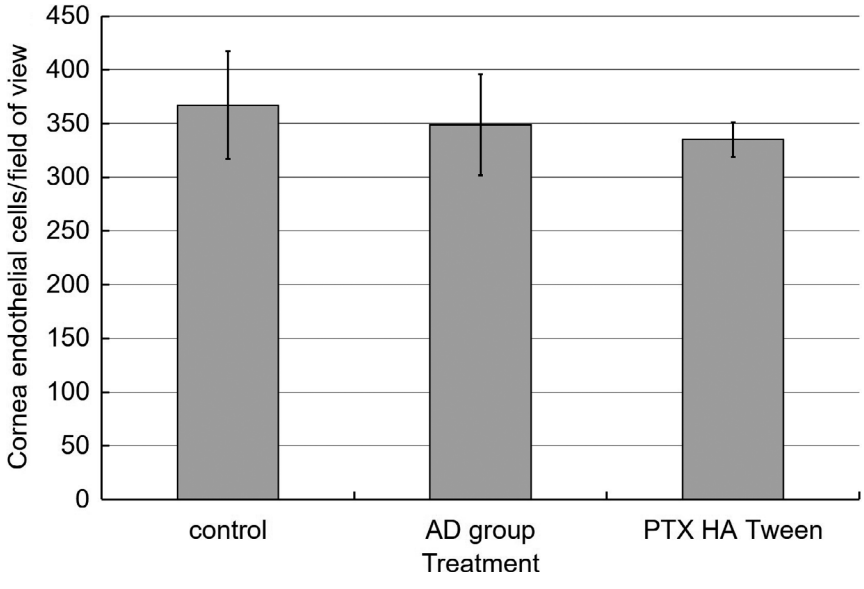

Fig. 8. Effect of lens capsular treatment on the density of cornea endothelium per field of view. Cell densities were calculated for those corneas that possessed a reasonably intact endothelial layer. No corneas were obtained for the Tween/HA treatment group.

Tween-20 alone does not prevent the occurrence of fibrosis in any way. The same is true for the combination of paclitaxel and Tween-20. Within the treatment groups with actinomycin D, lenses were observed that were still clear at the end of the follow-up period. Other rabbits in these groups showed signs of fibrosis ranging from initial stages to more mature stages. In this respect, the implantation time is important for the interpretation given to the data. The MTX + AD lenses showed signs of fibrosis in two out of three lenses, but this fibrosis was minor when compared with the Tween-20 group. The MTX + AD rabbits walked around with the implants for over 4 years, whereas the Tween-20 rabbits had to be killed 7 months after implantation based on the clinical assessment of level of fibrosis at that point in time. Another actinomycin D group showed signs of beginning fibrosis in two out of three lenses after one year, at which point it was decided to kill this group in order to compare the response with that of the Tween-20 group. Despite the beginning fibrosis, the result of AD-containing treatment is far better than the result of Tween-20 or the PTX treatment. Taken together, the data indicate that AD-containing treatment strategies have the potency to prevent fibrosis in rabbits. As pointed out by Wormstone, the rabbit *in vivo* model is often chosen due to the rapid onset and PCO severity in this species (Wormstone & Eldred 2016). At the same time, it is realized that drugs that are efficient against PCO in rabbits may not act accordingly in other species (Koopmans et al. 2014; Wormstone & Eldred 2016).

In the literature, a plethora of methods has been proposed to prevent opacification from occurring, both in classical intraocular lens implantations and in accommodating lens strategies. Proposed and investigated methods include the use of mitomycin C, 5-fluorouracil, EDTA, acetic acid, ultrapure water, Triton X-100, mibefradil, dexamethasone, diclofenac, RGD peptides, alkylphosphocholines and also drugs successfully used in other implant systems such as the drug-eluting stents (Beck et al. 2001; Inan et al. 2001; Fernandez et al. 2004; Maloof et al. 2005; Abdelwahab et al. 2006; Joner et al. 2006; Lüscher et al. 2007; Eibl et al. 2009). Many aspects of cell adhesion, growth and signalling have been targeted. Altogether, it can be expected that only a few drugs may be able to permanently stop the EMT process when administered during a 5-min peri-surgical treatment window, and actinomycin D seems to have this ability.

Regeneration of a capsule-like structure as shown by Saika et al. (2001) was not observed in the experiments reported in this study. Fibrosis seems to be easily introduced when lens epithelial cells interact with silicone chemistry (Saika 2004; Hao et al. 2010), although the stiffness of the material can be of influence. In his early demonstration of the feasibility of lens refilling, Kessler used non-medical grade silicone oil in combination with silastics and observed lower rates of PCO (Kessler 1964). The intrinsic material stiffness may combine with the extent of filling and therefore the capsular tension. These mechanical influences may be crucial for epithelial cell behaviour, as demonstrated by several recent studies on cell–material interactions (Kim & Asthagiri 2011). Fibrosis also appears when lenses in rabbits are refilled with sodium hyaluronate, although the fibrosis then can occur together with lens regeneration (Fernandez et al. 2004). It is interesting to note that filling with hyaluronic acid resulted in the fastest fibrosis response and that prior treatment with distilled water resulted in well-organized lens regrowth. This is in contrast with the conclusion in the 2014 JCRS publication from Koopmans that no capsular bag fibrosis occurred in the monkey model in the presence of hyaluronate after chemical pretreatment with AD/MTX/Cape/HA (Koopmans et al. 2014). From the results, it is not possible to conclude whether a subpopulation of lens epithelial cells is responsible for transition towards fibrosis. Quiescent, germinative and differentiating lens epithelial cell populations all possess some amount of telomerase activity (Colitz et al. 1999)

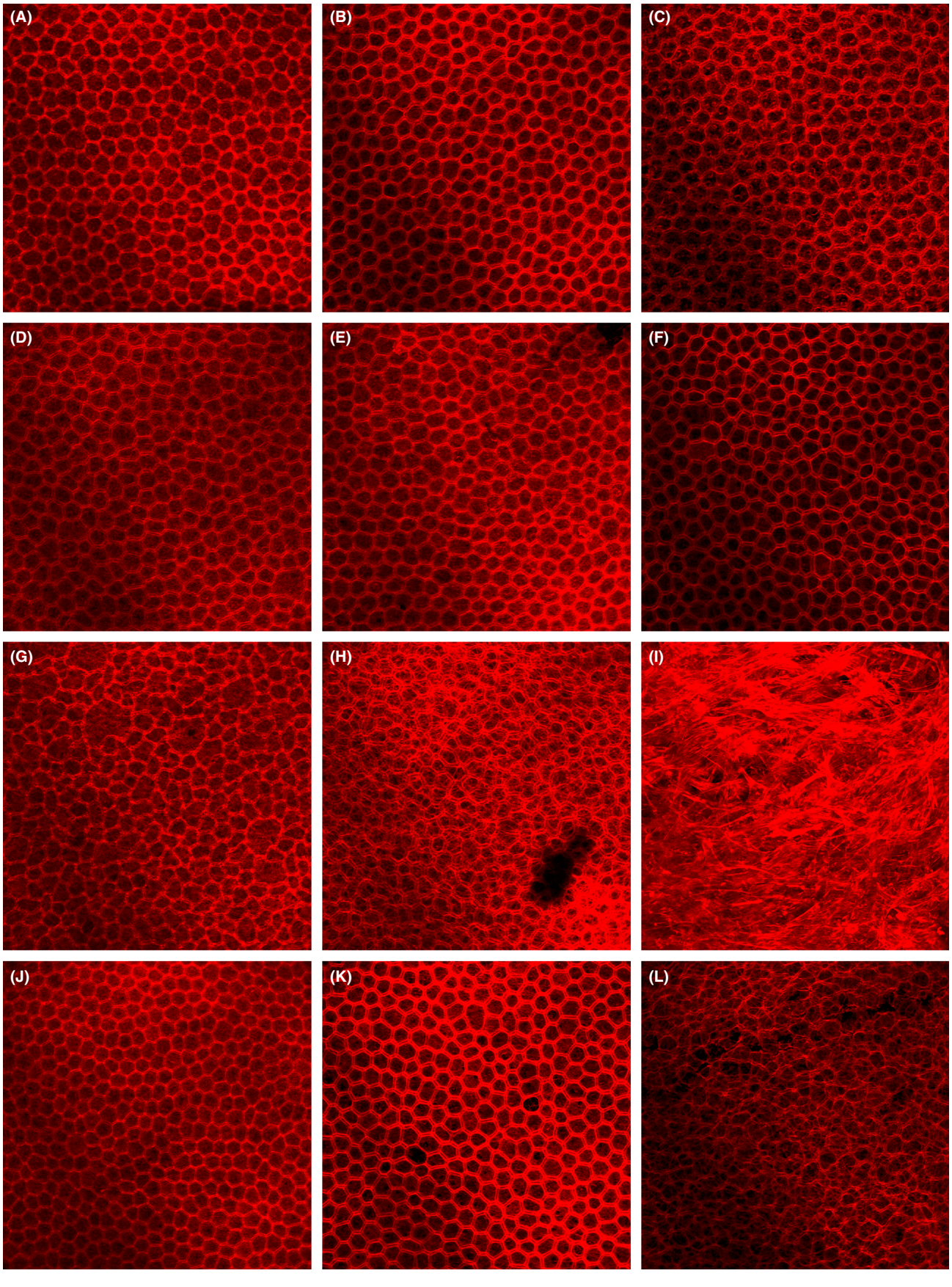

Fig. 9. Cornea endothelium of eyes with treated and filled lenses (A–I) and of control eyes (J–L). Damage to various degrees can be observed in images G-I of AD-containing treatment groups. Damage is also observed in one of the control eye corneas (L). Each image represents $375 \times 375\ \mu m^2$.

indicating that all have regenerative potential. Furthermore, the cells have been shown to be able to grow on the collagen capsule (Wormstone et al. 1997; Burger et al. 2008) provided they can migrate into the space between capsule and silicone gel. In this study, examples are shown of cells migrating into the silicone, indicating that they are able to push the gel aside. The use of a gel may impede the proposed no space–no cells hypothesis reported in the literature (Assia et al. 1999). This is corroborated by observations that space filling with Healon GV after IOL implantation is not associated with a lower rate of PCO. In this context, it can be noted that in the refilling experiments in rabbits the natural lens volumes could not be achieved due to incomplete refilling of the lens capsule intra-operatively despite silicone-plug sealing of the microcapsulorhexis. Using 7.1 T MRI, we have shown that further efforts are necessary to optimize the intraoperative refilling of the lens capsule, at least in rabbit eyes. The variation in the degree of refilling may have contributed to variation in the observed fibrotic responses, but cannot explain the observed differences in fibrotic responses associated with the use of the different treatments. It most likely contributes to variations in fibrosis observed within the different treatment groups.

A successful prevention of fibrosis must be combined with a safe application of the treatment regime. The corneal endothelial cells are a sensitive marker of potential damage either by surgical intervention in the anterior eye chamber or by administration of toxic compounds in the capsular bag that may leach to other structures in the anterior eye. Corneal endothelium is clinically inspected to assess potential damage as a result of ophthalmologic intervention. Macroscopic signs of damage include corneal clouding, which results from or is associated with a destruction of the endothelial cell layer (Koopmans et al. 2011). Microscopic inspection of the endothelial layers can indicate early signs of damage in the endothelial layer. In the treatment groups in this study, the corneas generally were not damaged by the treatment and surgery. Starting damage was seen in one case with MTX + AD treatment and one case with Tween-20 + AD. In two other cases, the influx of aberrant cell groups into the corneal tissue was observed, one of which was due to an inflammatory response. Strangely, one of the control eyes demonstrated a heavily disturbed corneal endothelium. This concerned a rabbit with a three-year follow-up. Data demonstrated that drug administration through the Healon vehicle can be a safe procedure for treating just the lens epithelial cells.

Summarizing, in this study the feasibility of a long-term prevention of fibrosis, that is over a period of more than 4 years in the rabbit model, has been demonstrated for the use of a peri-surgical treatment of the inner lens capsular bag with actinomycin D incorporated in hyaluronic acid. The use of Tween-20 and/or paclitaxel does not result in reduced capsular fibrosis. This creates opportunities for replacing the stiff presbyopic lens with a biomaterial that simulates the optical and biomechanical properties of the young crystalline lens to restore both vision and accommodation.

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