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Changes in lens stiffness due to capsular opacification in accommodative lens refilling

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ABSTRACT

Accommodation may be restored to presbyopic lenses by refilling the lens capsular bag with a soft polymer. After this accommodative lens refilling prevention of capsular opacification is a requirement, since capsular opacification leads to a decreased clarity of the refilled lens. It has been hypothesized that capsular fibrosis causing the capsular opacification results in increased stiffness of the lens capsular bag, therewith contributing to a decrease in accommodative amplitude of the lens. However, the change in viscoelastic properties of refilled lenses due to capsular fibrosis has never been measured directly. In this study we examined natural lenses from enucleated porcine eyes and refilled lenses directly after refilling and after three months of culturing, when capsular fibrosis had developed, and determined their viscoelastic properties with a low load compression tester. Control refilled lenses were included in which capsular opacification was prevented by treatment with actinomycin D. We related lens stiffening to the degree of capsular opacification, as derived from the microscopic images taken with a confocal laser scanning microscope. Overall, the refilled lenses directly after refilling were softer than refilled lenses after three months of culturing, and refilled lenses treated with actinomycin D were softer compared with untreated refilled lenses. The degree of capsular opacification as assessed by microscopy corresponds to an increase in lens stiffness. This indicates that the viscoelastic properties of the refilled lens are influenced by capsular fibrosis and modulated by treatment of the lens epithelium. In conclusion, this study shows that the development of capsular fibrosis negatively affects the viscoelastic properties of isolated, cultured refilled lenses.

1. Introduction

In recent years, there has been an increasing interest in the prevention of posterior capsular opacification (PCO), as has been reviewed by Wormstone et al. (2009). PCO is a common side-effect of cataract surgery. With cataract surgery, the opacified lens fibers are extracted, and an intraocular lens (IOL) is placed into the capsular bag. Usually, lens epithelial cells (LECs) situated on the anterior capsule are left behind. These are the cells mainly responsible for the formation of PCO. During the development of PCO, the residual LECs proliferate, transdifferentiate, and migrate to the posterior capsule. PCO results in a decrease of vision in months to years after the initial cataract surgery and is mostly treated with a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser capsulotomy. Complications of this Nd:YAG laser therapy include damage to the IOL, elevation of the intraocular pressure, cystoid macular edema, and increased incidence of retinal detachment (Aslam et al., 2003). Beside the possible complications, the considerable high procurement costs of a Nd:YAG laser make laser capsulotomy not universally available for all patients suffering from

Abbreviations: CO, Capsular opacification; PCO, Posterior capsular opacification; IOL, Intraocular lens; LECs, Lens epithelial cells; LLCT, Low load compression tester.

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PCO. Hence there is a strong need to determine how to prevent PCO instead of performing this treatment which results in additional costs and possible complications.

The ongoing development of new intraocular lenses also results in a need for the prevention of PCO and more in general overall capsular opacification (CO) as the anterior capsule often is saved in new, proposed lens replacement strategies. Prevention is for instance important for the development of an injectable accommodating lens to restore accommodation after the development of presbyopia (Haefliger et al., 1987; Koopmans et al., 2003; Nishi and Nishi, 1998). In order to enable accommodation, the stiffened lens fibers are removed and the lens capsular bag is refilled with a soft transparent polymer. With this technique, the whole lens capsule including the anterior and posterior lens capsule remain in situ, allowing aberrant LEC growth and subsequent opacification on anterior and posterior sides of the capsular bag.

When an injectable accommodating lens is implanted, it is not desirable to destroy the capsular bag by a Nd:YAG laser capsulotomy, because then the injected material will leak out of the capsular bag into the anterior or posterior segments of the eye. Furthermore, CO after injection of a polymer in the capsular bag not only causes a decrease in lens clarity, but may also stiffen the lens due to the presence of the fibrotic tissue. The presence of this tissue has been related to the observed decrease in accommodative amplitude in monkey eyes that were refilled with a silicone polymer (Koopmans et al., 2006, 2014). The decrease in accommodative amplitude in these monkeys was probably the result of CO, although the possibility that other factors such as changes to the ciliary muscle after the implantation procedure or the reduced adhesion between lens capsule and lens refilling material as a cause cannot be completely ruled out (Koopmans et al., 2006).

In order to investigate the independent role of CO on the viscoelastic properties of lenses, we examined natural and refilled porcine lenses directly after the refilling procedure and after three months of culturing, after which capsular fibrosis had developed. The viscoelastic properties were measured with the low load compression tester (Sharma et al., 2011). After *in vivo* implantation of an accommodative lens, measurements of the changes in lens viscoelastic properties during the development of CO are not possible since the lenses are then situated in an eye, thus allowing indirect measurements only. In a set-up using enucleated porcine lenses in culture we were able to examine the process of lens stiffening due to capsular fibrosis directly. Additionally, we analyzed the viscoelastic properties of refilled lenses treated with the cytotoxic agent actinomycin D, in order to prevent the development of CO, and compared these measurements with untreated refilled lenses.

2. Material and methods

2.1. Lenses

Eyes from approximately six month old pigs (*Sus domesticus*) were obtained from the local slaughterhouse, lenses were extracted, and the viscoelasticity was measured within eight hours postmortem. The lenses were divided into five groups. The first group of nine natural lenses served as a control, the lenses were extracted and the viscoelasticity was measured (natural, N). In the following group of seven lenses, the lens fibers were removed, the lenses were refilled with a silicone polymer and the viscoelasticity was measured (refilled, R). The next group consisted of ten lenses which were refilled and cultured for three months before the viscoelasticity was measured (refilled cultured, RC). The last two groups of eight and seven lenses underwent respectively mild (10 μg/ml for 5 min) and strong treatment (50 μg/ml for 1 h) with actinomycin D dissolved in purified water (milli-Q, EMD Millipore Corporation, Billerica, USA) before the lenses were refilled (treatment cultured mild and strong, TC mild and TC strong). Subsequently, these lenses were cultured for three months and were then measured for viscoelasticity. All groups started with ten samples but due to either fungal contamination or filling errors (under or overfilling) some lenses had to be left out from further statistical analysis.

2.1.1. Surgical refilling procedure

The following surgical procedure is based on the method previously described by Koopmans et al. (2003, 2006). To refill the lens, a 0.8 mm clear cornea paracentesis was made with a single edged diamond knife and an anterior chamber maintainer was inserted. This maintainer was connected to an infusion bottle containing phosphate buffered saline (PBS). Then, a 2.0 mm clear cornea tunnel incision was made using a diamond phaco knife. On approximately 1/4 of the diameter of the lens, a small peripheral puncture of the anterior lens capsule was made by a 25G needle. The capsule opening was torn into a 1.0–1.5 mm diameter circular capsulorhexis with a micro-incision capsulorhexis forceps. Subsequently, a 25G blunt cannula connected to a 10 ml syringe with a polyethylene tube was used to extract the lens fibers by manual aspiration. After extraction of the lens fibers, the anterior chamber maintainer was removed and the anterior chamber was filled with sodium hyaluronate (Healon 10 mg/ml; Abbot Medical Optics, Uppsala, Sweden). A 3.0 mm diameter capsular plug (Terwee and Koopmans, 2007) was inserted through the capsulorhexis into the capsular bag, and the capsular bag was filled with a silicone polymer (Abbot Medical Optics, Groningen, The Netherlands). To inject this polymer, a 25G blunt cannula was inserted into the bag. The silicone polymer was injected until it started to leak around the plug and the capsular bag was completely filled. The cannula was removed, and the plug was positioned to close the capsulorhexis.

2.1.2. Treatment with actinomycin D

In the treatment cultured groups, the lens capsules received a mild (10 μg/ml for 5 min) or strong (50 μg/ml for 1 h) treatment with actinomycin D (Sigma–Aldrich Corporation, St. Louis, USA) dissolved in purified water (milli-Q, EMD Millipore Corporation, Billerica, USA). After the removal of the lens fibers and the injection of sodium hyaluronate in the anterior chamber, the capsular bag was filled with the actinomycin D solution. The solution was left in the capsular bag for five minutes (TC mild) or one hour (TC strong). Then the actinomycin D was aspirated and the bag was flushed with PBS. Subsequently, the refilling procedure was continued with the injection of the silicone polymer.

2.1.3. Extraction of the lens from the eye

To enable viscoelasticity measurements, culturing, and microscopy, the natural and refilled lenses were extracted from the eye. First, the cornea was cut off with scissors with sharp curved blades. Then the iris was removed and the zonules were cut using Westcott scissors. The attached vitreous was removed by lifting the lens, and the lens was placed in the culture medium.

2.1.4. Culturing method

The lenses were kept in culture medium consisting of minimal essential medium (MEM) supplemented with 12% fetal bovine serum (FBS), 2 mM GlutaMAX™-I, 1 mM sodium pyruvate, and 500 Units/ml penicillin – 500 μg/ml streptomycin – 1.25 μg/ml amphotericin B (all Life Technologies Ltd, Paisley, UK). The refilled cultured lenses and the treatment cultured lenses were cultured for 3 months in a 37 °C 5% CO_2 incubator. The medium was changed twice a week.