STUDIES OF THE DEVICE PERFECT CAPSULE IN AN ANIMAL MODEL TO REDUCE LENS EPITHELIAL CELL PROLIFERATION

Mahmoud Taie Abdelwahab
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To my father
ABSTRACT

The sealed-capsule irrigation device, the Perfect Capsule, is a surgical device designed to target lens epithelial cells (LECs) during cataract surgery and prevent posterior capsule opacification (PCO). This is especially important in cases in which the posterior capsule must stay intact after cataract surgery, such as when implanting accommodative lenses. It also can be used when PCO is expected to be extensive, such as in pediatric cataract surgery. This thesis was created to evaluate the use of the Perfect Capsule in small young eyes with very proliferative lens epithelial cells in an animal model with young rabbits.

In the first study, 30 4-week-old rabbits had bilateral clear lens extraction. The Perfect Capsule was implanted in one eye and sealed-capsule irrigation was performed with either balanced salt solution (BSS), distilled deionized water (DDW), or 5-fluorouracil (5-FU) 50 mg/ml for 5 minutes. The capsule then was flushed with BSS to wash out the residual substance. The contralateral eye did not undergo sealed-capsule irrigation. The after-cataract was evaluated clinically and in images taken at 5.5 weeks after surgery and histologically after the endpoint 6 weeks postoperatively. Only 5-FU effectively prevented the development of after-cataract.

The second study evaluated the safety of irrigation with 5-FU in 30 8-week-old rabbits. Clear lens extraction was performed in one eye and the Perfect Capsule was implanted in three of the four groups. In one group, the capsule was irrigated for 5 minutes with BSS, in the second group with 5-FU 50 mg/ml, and in the third group with 5-FU 50 mg/ml and then BSS to wash out the residual substance. In the fourth group, the Perfect Capsule was not used; to mimic leakage in the device 0.2 ml of 5-FU 50 mg/ml was instilled in the capsule, left for 30 seconds, and washed out with I/A. Safety was evaluated by comparing pachymetry, endothelial cell count, and histologic findings after the endpoint of 24 or 48 hours postoperatively (half of each group at each time point). There was no difference in pachymetry or endothelial cell count among the groups. Histology showed no damage in the central or peripheral retina or the trabecular meshwork due to the substance used. We concluded that it is safe to use the Perfect Capsule with 5-FU even if leakage occurs.

The third study evaluated the possibility of lowering the 5-FU concentration and maintaining a sufficient preventive effect on LEC proliferation. We also evaluated irrigation with thapsigargin, which was reported to be effective in in vitro human studies. Thirty 4-week-old rabbits had clear lens extraction in one eye. The Perfect Capsule was implanted and the capsule was irrigated for 2 minutes with either BSS, 5-FU 50 mg/ml, 5-FU 25 mg/ml, or thapsigargin and then BSS for 10 seconds. The after-cataract was evaluated clinically and in images taken 5 weeks postoperatively and histologically at 6 weeks postoperatively. The 50 mg/ml concentration of 5-FU successfully prevented after-cataract formation. Thapsigargin was ineffective in this animal model.

In the fourth study, we used transmission electron microscopy (TEM) to determine if the posterior capsule is damaged by 5-FU or thapsigargin or just by the use of the Perfect Capsule. Clear lens extraction was performed in 4-week-old rabbits. We studied two eyes that were irrigated for 2 minutes with BSS, two eyes with 5-FU 50 mg/ml, two eyes with 5-FU 25 mg/ml, and two eyes with thapsigargin. The
substances were washed out with BSS for 10 seconds. We also included two control eyes that had not had surgery. At 6 weeks postoperatively, the capsules were extracted and fixed for TEM. The analyses showed no ultrastructural damage to the posterior capsules in any group.


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<tr>
<td>CCC</td>
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<td>LEC</td>
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<td>Nd:YAG</td>
<td>Neodymium-yttrium aluminium garnet</td>
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1 INTRODUCTION

1.1 HISTORY

A Carthaginian monk, Constantinus Africanus (AD 1010-1087) introduced the term *cataract*, meaning waterfall or blockage of flow (Johns et al. 2003). Surgery for cataract has been performed for more than 2,000 years. In India, couching was practiced as early as 800 BC. An assistant held the patient’s head still. The doctor then plunged a pointed needle through the cornea or 4 mm temporal to the limbus, a pars plana approach. A blunted needle then was used to push the lens down or backward to loosen it from the zonules. In the 10th century, an Iraqi ophthalmologist, Ammar, described an aspiration method through a hollow needle. This method was used by the Syrians for a few centuries.

In the 18th century, a Frenchman, Jacques Daviel, described extracapsular cataract extraction (ECCE) through the pupil. This was beneficial since the lens then was removed from the eye. An incision was made in the inferior cornea and enlarged with scissors. An incision was then made in the capsule and the nucleus was expressed and removed. The cortex was removed by curettage. The method had considerable complications, such as endophthalmitis, chronic inflammation, glaucoma, posterior capsule opacification (PCO), and uveal prolapse.

Since many complications were due to incomplete lens removal, intracapsular cataract extraction (ICCE) was developed, in which the lens capsule was removed together with the lens. Different methods were introduced to break the zonular fibers. ICCE was practiced successfully in the 18th, 19th and the beginning of the 20th century. In 1961, Tadeusz Krwawicz in Poland developed a cryoprobe that formed an iceball comprised of the capsule, cortex, and nucleus. This diminished the risk of capsule rupture during the procedure. In the middle 1950s, 85% of patients who underwent ICCE had a postoperative best-corrected visual acuity of 0.65 decimal. However, 5% of the patients had potentially blinding complications.

In 1967, Charles Kelman, from the USA, developed phacoemulsification. The instrument emulsifies the nucleus with ultrasound, which diminishes the need for a large incision size. This technique is the method of choice for departments that can afford the apparatus. When phacoemulsification cannot be used, small-incision ECCE is most often used, and the cortex is manually removed with a Simco cannula.

Cataract surgery was performed earlier without anesthesia. Retrobulbar anesthesia was described in 1884, the modern technique of which was described in 1945. In Sweden and many other countries, topical anesthesia with eye drops supplemented with an intracameral injection is most widely used. General anesthesia is used in children, mentally challenged patients, and those with dementia or other problems.

Correction of aphakia has been a large problem in the past. The inventor of the intraocular lens (IOL), Harold Ridley, begun implanting polymethylmethacrylate (PMMA) IOLs in 1949 (Johns et al. 2003). The results were poor with numerous complications. The IOLs continued to improve with evolving designs, and today, foldable IOLs perform well with few complications. PMMA IOLs still are widely used since the foldable IOLs are unaffordable in many countries.
1.2 POSTERIOR CAPSULE OPACIFICATION

PCO is the most common complication after cataract surgery and represents a continuous challenge to surgeons despite advanced surgical techniques and different IOL designs (Stordahl & Drolsum 2003; Kugelberg et al. 2006; Katayama et al. 2007). PCO develops as the result of migration and proliferation of residual equatorial lens epithelial cells (LECs) towards the center of the posterior capsule (Neumayer et al. 2006). The visual axis then is blurred again. The posterior capsule can be ruptured by neodymium-yttrium aluminium garnet (Nd:YAG) laser capsulotomy (Figure 1).

Figure 1. Eye with posterior chamber IOL that underwent Nd:YAG capsulotomy. A clear visual axis is seen.

The visual axis then is clear. However, in some cases the posterior capsule must be totally clear and remain intact, for example, if the intention is to implant an accommodative IOL. This might be managed using a sealed-capsule irrigation with a substance toxic to the LECs.

In pediatric cataract surgery, LEC proliferation causes serious visual problems, the cells form quickly and progressively in the visual axis (Figure 2) (Arkin et al. 1992; Brar et al. 2001).
Amblyopia develops rapidly in children, especially in the youngest age group (Lundvall & Kugelberg 2002). The children are also at risk of more visual axis opacification (VAO). Secondary glaucoma is another vision-threatening complication of cataract surgery in children (Lundvall & Zetterstrom 2006; Trivedi et al. 2006). In children, Nd:YAG laser treatment after cataract surgery has been investigated with no encouraging results compared to adults (Stager et al. 2006). VAO in children generally requires a second surgical intervention, as closure of the capsulotomy made by Nd:YAG laser is very common. In addition, smaller children are not cooperative enough to sit still for Nd:YAG capsulotomy. In pediatric cases, many surgeons therefore perform a posterior capsulorhexis, and in smaller children perhaps also an anterior vitrectomy (Figure 3).
Figure 3. Eye in which a posterior capsulorhexis and a primary anterior vitrectomy were performed. A clear visual axis can be seen.

An additional anterior vitrectomy is needed in children younger than 5 to 7 years to avoid VAO (Vasavada & Desai 1997; Kugelberg et al. 2005). Removal of residual LECs during the primary surgery is a key factor in avoiding PCO. Several chemicals have been suggested in experimental settings to remove or kill residual LECs (Inan et al. 2006); however, the substances may be toxic to other ocular structures. Many research groups are searching for a device that can help selectively kill these LECs (Findl et al. 2007). There are complications associated with touching the vitreous and breaking the posterior lens capsule. If this can be avoided, it would be highly advantageous. This however, generates the need for a lens capsule without LECs. Perhaps the sealed-capsule irrigation also could be an option in pediatric cases.

1.3 PERFECT CAPSULE

The sealed-capsule irrigation device, Perfect Capsule, invented by Anthony Maloof (Agarwal et al. 2003; Maloof et al. 2003), consists of a silicone plate, 0.7 mm thick, 7 mm in outer diameter, and 5 mm in inner diameter (Figure 4).
It has an inflow tube, an outflow tube (latest version), and a tube for creation of a vacuum. The device can be folded and introduced through a normal small incision. It then is placed over the anterior capsulorhexis, and the vacuum is created with a syringe (Figure 5).
This creates a sealed system between the capsular bag and the device. The capsule can be irrigated with a substance through the inflow tube, and the substance does not come in contact with the other intraocular structures. The substance then is washed out with balanced salt solution (BSS), the vacuum is released, and the device is withdrawn. An IOL then can be placed in the bag.

Recently, human studies reported the safety of the device in adults undergoing cataract surgery (Rabsilber et al. 2007). In that study, the sealed system was irrigated with distilled deionized water (DDW), which did not prevent PCO development. Earlier in vitro studies had shown that the LECs died from osmotic lysis when they were exposed to DDW (Crowston et al. 2004). However, this did not work in vivo.

1.4 PEDIATRIC CATARACT

In Sweden, a study showed the incidence of all congenital cataract cases to be 36/100,000 (Abrahamsson et al. 1999). In addition, a few hundred children develop juvenile cataract each year in Sweden. Congenital cataract (Figure 6) is considered to be the most common cause of treatable blindness in children. It is present at birth and may be unnoticed until the visual function is affected or a whitish pupil is seen (Lambert & Drack 1996). The etiology encompasses a wide range of factors including hereditary, metabolic, intrauterine, and idiopathic causes. One third of cases are inherited and mostly autosomal dominant (Francis et al. 2000; Moore 2004). Metabolic causes such as galactosemia in which the galactose cannot be metabolized inside the body lead to oil-droplet cataract associated with vomiting and diarrhea. Maternal rubella infection during the first trimester is considered an important cause of cataract with poor visual ability due to associated systemic symptoms (Vijayalakshmi et al. 2003; Sharan et al. 2006).

Figure 6. Infant with congenital cataract.
Nuclear cataract is the most common congenital form and consists of a unilateral or bilateral central white opacity surrounded by a clear cortex. Microphthalmia and microcornea are commonly associated (Amaya et al. 2003). Nuclear cataract is visually relevant as it easily leads to deprivation amblyopia if not treated early, especially in unilateral cases (Taylor et al. 1979).

Lamellar cataract (Figure 7) is usually acquired rather than congenital and almost always bilateral with autosomal dominant transmission (Forster et al. 2006). Lamellar cataract has a better prognosis than the nuclear cataract due to the late development, which does not affect visual maturation as greatly (Lambert & Drack 1996).

Figure 7. Lamellar cataract in a child.

Posterior polar cataracts, which can be unilateral or bilateral, are surgically challenging because hydrodissection should be performed carefully to avoid posterior capsule tears (Vasavada et al. 2004).

The visual results after pediatric cataract surgery have improved in the last few decades due to modern surgical techniques and IOL materials (Lambert & Drack 1996).

1.4.1 Amblyopia and Congenital Cataract

Amblyopia, considered a serious challenge to the pediatric surgeons, differs according to the type and density of the cataract. Amblyopia results from abnormal visual stimulation to the lateral geniculate body and the striate cortex. Children younger than 2 months of age tend to
develop irreversible amblyopia. Visual deprivation and permanent visual loss can develop quickly if proper treatment is not provided in a timely fashion (Agervi et al. 2006). The cataract density and location are directly related to visual development and development of amblyopia. The sensitivity to amblyopia decreases by 6 to 7 years of age because visual maturation is almost complete (Wright 1997; Zetterstrom et al. 2005).

Early diagnosis and treatment of visually relevant cataract in the youngest age group are the most important factors to avoid deprivation amblyopia (Merula & Fernandes 2005). In neonates younger than 8 to 10 weeks with dense central cataract, surgery may minimize the deprivation amblyopia and enhance chances of reasonable visual maturity. The visually relevant cataract that obstructs the examiner’s view and is greater than 3 mm in diameter is considered to be sufficient reason for immediate surgery especially in unilateral cases.

1.5 SURGICAL TECHNIQUE IN PEDIATRIC CATARACT

Children’s small eyes represent a challenge during surgery and for IOL power calculation and implantation technique. The management of cataract surgery requires experienced surgeons and should preferably be done by a cataract surgeon who has sufficient experience to manage the specific difficulties in pediatric eyes.

1.5.1 Surgical Steps

The incision
The incision should be water tight, preferably tunnel-shaped, and sutured at the end of the procedure. A small incision can be created due to the foldable IOLs, which decreases iris prolapse and the inflammatory process. The location of the incision can be temporal or superior, the latter of which is preferred by many surgeons. Wilson reported in his survey results that 63.6% of the American Society of Cataract and Refractive Surgery and 84.3% of the American Association for Pediatric Ophthalmology and Strabismus members prefer the superior approach (Wilson et al. 2003).

Ophthalmic viscosurgical devices
Ophthalmic viscosurgical devices (OVD) are especially important in pediatric cataract surgery as they protect the corneal endothelium and other ocular structures during surgical manipulations (Arshinoff 1998). OVDs increase the space available for surgical work especially for the anterior capsulorhexis because children usually have a shallow anterior chamber due to high vitreous pressure. At the end of surgery, the OVD should be removed because of the risk of elevated intraocular pressure (IOP) in the early postoperative period (Arshinoff 2000).

Anterior capsulorhexis
Anterior capsulorhexis is difficult in infants and young children because of the high elasticity of the anterior capsule and vitreous pressure. Creating an intact manual continuous curvilinear capsulorhexis (CCC) is challenging for even the most experienced surgeons as reported by Vasavada and Chauhan (1994) when they reported failure of 80% of 21 infant eyes (Vasavada & Chauhan 1994; Wilson 2004). The manual CCC is preferable in pediatric cataracts rather than the diathermic capsulorhexis, since the latter is much weaker and less liable to tolerate surgical manipulations (Krag et al. 1997).
Lens aspiration

Lens aspiration is considered an easy step in pediatric cataract because the nucleus is soft and requires no phacoemulsification but instead irrigation/aspiration (I/A). It is important to clean the lens matter thoroughly to decrease the possibility of residual active LECs (Apple et al. 2000; Peng et al. 2000).

IOL implantation

IOL implantation in children and younger age groups is widely accepted due to improvements in the surgical techniques and IOL materials (Apple & Trivedi 2002). The age at IOL implantation has become lower and lower and is now well below 1 year of age with lower rates of glaucoma in comparison to aphakia (Mamalis et al. 2004; Lundvall & Zetterstrom 2006). The foldable acrylic hydrophobic IOLs have a lower complications rate than PMMA IOLs, which are still used in many developing countries (Kugelberg & Zetterstrom 2002). The single-piece AcrySof is considered a good choice for capsular bag implantation in small eyes (Vasavada & Nihalani 2006) but unsuitable for sulcus fixation as decentration and iris capture can occur postoperatively (Li et al. 2007). Kugelberg et al. (2005, 2006) reported that AcrySof SA30AL has proper central position, a minimal postoperative inflammatory response, and is tolerated by the pediatric eye. The single-piece AcrySof is considered an ideal choice for children, which encourages many surgeons to use as the first choice in pediatric cataract surgery (Trivedi & Wilson 2003).

Postoperative treatment

Topical steroid eye drops are used early and frequently in the newborn 8 to 10 times daily with slow tapering over 2 to 3 months. In younger children, cycloplegic drops also are used after surgery. In older children, the eye drop dose is much lower, administered 3 to 5 times daily, and tapered over 1 month. Systemic steroids in combination with frequent topical dexamethasone are used especially in children with uveitis (Zetterstrom & Kugelberg 2007).

1.5.2 Postoperative Complications

Visual Axis Opacification

The old technique involved keeping the posterior capsule intact after lens aspiration (Scheie 1960). In this technique, the remaining LECs proliferate and migrate onto the center of the posterior capsule, which could be worse for vision than the cataract itself (Lambert & Drack 1996; Nekolova et al. 2008). It affects the macular sensitivity in young children and induces...
amblyopia (Varga et al. 2008). VAO is the most frequent complication of pediatric cataract surgery particularly in the youngest children (Sharma et al. 1999). Age is an important factor, i.e., the younger the age, the faster and more VAO there is due to mitotically active LECs (Lambert et al. 1999). As discussed previously, there have been improvements in the technique and posterior capsulotomy combined with anterior vitrectomy results in better vision quality. However, VAO is a persistent problem in small children (Parks 1983; Alexandrakis et al. 2002). Despite removal of the posterior capsule, the anterior vitreous serves as a scaffold for LECs to migrate and form a secondary membrane and opacity to develop (Morgan & Karcioglu 1987; Cassidy et al. 2001). The IOL type and material also play an important role in VAO development (Cheng et al. 2001; Kugelberg & Zetterstrom 2002).

**Secondary glaucoma**

Secondary glaucoma is another vision-threatening complication after pediatric cataract surgery (Figure 8).

![Figure 8. Secondary glaucoma in the left eye.](image)

It is a serious complication that can occur early or late after surgery and more than 15 years postoperatively (Medow et al. 2004; Kang et al. 2006). The incidence of early glaucoma is relatively common now despite modern surgical techniques. Some surgeons perform peripheral iridectomies in every case but usually not in routine cases (Wilson et al. 2005). Late-onset open-angle glaucoma can develop without symptoms or gross changes in the ocular appearance and make matters worse as children often do not cooperate for IOP measurements, optic disc evaluation, and visual field assessments (Johnson & Keech 1996). The new instrument ICare, however (Pakrou et al. 2008), makes IOP measurements easier to perform on small children without anesthesia. ICare is based on rebound tonometry (Abraham et al. 2008). A small thin probe bounces on the cornea and the impedance that is
detected is used to calculate the IOP. It does not require topical anesthesia, and the child does not have to sit in front of a microscope. The tonometer is applied when the child is sitting on the parent’s knee or on a chair. The measurement takes only a few seconds and can be used in small babies.

Secondary glaucoma could be treated either medically or surgically. Goniotomy, trabeculotomy, or glaucoma shunts followed by medical treatment in refractory cases are the successful treatment choices of many surgeons (Walton 1995; Magnusson et al. 2000; Wormald 2004).

**Fibrinous anterior uveitis**

In children, there is high degree of inflammation and fibrin in the pupillary area even after a highly compatible IOL is implanted (Leung et al. 2000). Posterior synechia formation in the postoperative period is common especially in young children (Lambert 1999).

**Pupillary capture and decentration**

The incidence of pupillary capture after pediatric cataract surgery varies from 8.5% to 41%. (Vasavada & Trivedi 2000). Iris incarceration in the wound occurs due to high positive pressure and iris trauma. The wound should be designed as a long tunnel, and suturing with 10-0 sutures is recommended to avoid this complication. If the wound gaps widely and there is iris prolapse, surgical reposition is recommended.

**IOL decentration/dislocation**

IOL decentration occurs due to inadequate capsular support or traumatic zonular loss during surgery. Asymmetric IOL placement also can occur with one haptic in the bag and the other in the ciliary sulcus. In trauma cases, complete IOL decentration can occur and repositioning is recommended for visual stability (Brady et al. 1995).
2 AIMS

1. To evaluate the Perfect Capsule in lens extraction in small eyes in an animal model (I, II, III, IV).
2. To find a substance that with the Perfect Capsule can prevent VAO in young eyes after lens extraction (I, III).
3. To evaluate the safety of using these substances in relation to the other intraocular structures (II, IV).
3 MATERIALS AND METHODS

The experiments were approved by the Northern Stockholm Animal Experiments Ethics Committee and adhered to the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research.

3.1 ANIMALS (I, II, III, IV)

We chose young New Zealand White rabbits for our experiments because the eyes resemble pediatric eyes in ocular dimension and the exaggerated response to surgery. Rabbit eyes have more regenerative power of the corneal endothelium than pediatric eyes, which should be considered in the postoperative evaluation. The rabbits were either 4 weeks old (I, III, IV) or 8 weeks old (II).

3.2 SURGERY (I, II, III, IV)

All procedures were performed by one surgeon. Before surgery, full pupillary dilation was obtained by topical application of a combination of cyclopentolate 0.75% and phenylephrine 2.5%. General anesthesia was induced with an intramuscular injection of ketamine hydrochloride and xylocaaine chloride; the dose depended on the body weight of the animal. Topical anesthesia (tetracaine hydrochloride) was administered before positioning an eye speculum. A 2.8-mm corneal incision was created using a disposable bevel-up 45-degree slit knife, followed by intracameral injection of sodium hyaluronate (Healon GV), which is highly viscous and recommended in pediatric eyes to maintain the anterior chamber (Arshinoff 2000).

A CCC was created with a bent needle and capsulorhexis forceps. The capsulorhexis must be only about 3 to 4 mm since the inner diameter of the Perfect Capsule is 5 mm, and the capsulorhexis has to be covered to obtain vacuum before irrigation.

I/A of the clear lens matter was done and no phacoemulsification was needed. The solution used in I/A was BSS with heparin 10,000 IU/l to avoid fibrin formation in the anterior chamber during surgery. Healon GV was injected into the anterior chamber before the Perfect Capsule was folded and introduced into the eye without widening the incision. It then was pushed down and fitted above the anterior capsulorhexis opening; and the assistant then pulled the syringe fully back and locked it. The sealing effect was created and the capsular bag was isolated from the surrounding structures. The system was sealed in all eyes.

The sealed system was irrigated, and in most cases, washed out with BSS to remove all residual active substance from the eye. The substance exits the eye through an outflow tube, since the substance also might be toxic to the ocular exterior, the corneal epithelium, and the conjunctiva. The vacuum then is released, and the device pulled out through the incision. In the four current studies, we did not implant an IOL but this is of course possible after this step. The incision was closed with a continuous 10-0 nylon suture and an intracameral injection of 1 mg cefuroxime was administered.

Postoperative dexamethasone eye drops were given in a tapered dose of four times daily for 1 week, three times daily for 2 weeks, and twice daily for the following 3 weeks in studies I, III, and IV. In study II, no postoperative eye drops were administered since the endpoints were 24 and 48 hours postoperatively.
3.3 STUDY DESIGN (I)

In the first study of 30 4-week-old rabbits, both eyes had clear lens extraction. We used the sealed capsule device in one randomly selected eye and the other eye served as a control and underwent the same surgery, but the Perfect Capsule was not used, and there was no irrigation with a substance. All randomizations were done using the Excel computer program.

The animals were randomized into three groups that received three different substances: BSS, DDW, or 5-fluorouracil (5-FU) 50 mg/ml. The sealed system was irrigated for 5 minutes and we used approximately 40 ml of a substance followed by flushing of the system for 10 seconds with BSS. During irrigation, no leakage occurred, and a vacuum was obtained in all eyes. This can be seen since inside the capsule; debris is moving around, while in the anterior chamber, it is still.

3.3.1 Evaluation (I)

Slit-lamp examination under full pupillary dilatation was done 23 days postoperatively. The examination was done in a masked fashion, the examiner did not know which eye had the Perfect Capsule or what substance had been used in the eye. After-cataract was graded from 0 to 2.

After 36 days, the pupils were fully dilated and retroillumination photographs were taken for after-cataract evaluation, which was graded 0 to 3.

The animals were killed 6 weeks after surgery with an intraperitoneal injection of phenobarbital preceded by induction of anesthesia. The eyes were enucleated and fixated in formalin for histologic evaluation. For after-cataract evaluation, the slides were stained with hematoxylin-eosin. After-cataract was graded from 0 to 4. To evaluate the capsule thickness, the slides were stained with Giemsa. The capsule thickness was measured using the Axiovision v. 4.2 software using a microscope connected to a camera. The mean of three readings of the central part was calculated and used for statistical analysis.

3.3.2 Statistical analyses (I)

Kruskal-Wallis analysis of variance (ANOVA) was used to compare the after-cataract among the three groups. Statistics were based on the difference between the Perfect Capsule eye and the other eye within each animal. To evaluate the difference between the Perfect Capsule eye and the other eye within each group, a sign test was used. One-way ANOVA was used to compare the central posterior capsule thickness.

3.4 STUDY DESIGN (II)

In the second study, we used 30 8-week-old New Zealand White rabbits. Clear lens extraction was performed, and the animals were randomly divided into four groups. In group 1, both eyes had clear lens extraction, and one eye did not have irrigation with the Perfect Capsule. In groups 1, 2, and 3, the Perfect Capsule was applied and the system irrigated for 5 minutes with a substance.

1. Perfect Capsule applied, irrigation with BSS
2. Perfect Capsule applied, irrigation with 5-FU 50 mg/ml
3. Perfect Capsule applied, irrigation with 5-FU 50 mg/ml followed by BSS for 10 seconds
4. Perfect Capsule was not applied, instead, after lens extraction, 0.2 ml of 5-FU 50 mg/ml was instilled in the capsular bag, left for 30 seconds, and then washed out with BSS. The second group was created to mimic leakage from a vacuum drop with some 5-FU left in the eye after surgery. The fourth group was created to evaluate 5-FU toxicity.

3.4.1 Evaluation (II)

In this experiment, we evaluated the safety of irrigation through the sealed irrigation device to the corneal endothelium, the trabecular meshwork, and the central and peripheral retina.

We counted the corneal endothelial cells preoperatively and 24 hours postoperatively to compare the effect of different irrigating substances. Endothelial cell images were obtained with the anterior segment camera SP 1000 (Topcon) and the count analyzed using IMAGEnet-640 software (Topcon). The corneal thickness was measured preoperatively and 24 hours postoperatively using the SP2000 mini-pachymeter (Tomey).

All animals were killed 24 or 48 hours postoperatively, half of each group at each time point. The eyes were fixed in formalin for histology. Histopathology evaluation was performed by an ophthalmic pathologist after staining with hematoxylin-eosin (1 section) and periodic acid-Schiff (1 section). The sections were examined for tissue damage in a masked fashion. Apoptotic cells were examined using TUNEL staining to detect minimal damage. Sections from a human eye with retinoblastoma were used as a positive control.

3.4.2 Statistical analyses (II)

Statistical analyses were based on the difference between the preoperative measurement and the 24-hour postoperative measurement within each eye. Kruskal-Wallis ANOVA was used to compare the differences in pachymetry and the endothelial cell count between the groups. The Wilcoxon matched-pairs test was used in group 1 to compare pachymetry and the endothelial cell count between the Perfect Capsule eye and the control eye.

3.5 STUDY DESIGN (III)

In the third study, clear lens extraction was performed in one eye of 42 4-week-old New Zealand White rabbits. All eyes had the Perfect Capsule applied. They were divided into four groups that received different irrigation substances, i.e., BSS, thapsigargin, 5-FU 50 mg/ml, and 5-FU 25 mg/ml. The sealed system was irrigated for 2 minutes with approximately 20 ml of the substance, and the residual substance was washed out for 10 seconds with BSS. We wanted to evaluate the possibility of lowering the 5-FU concentration and irrigation with thapsigargin.

3.5.1 Evaluation (III)

A clinical evaluation was done by slit-lamp examination after dilating the pupils 3.5 and 5.5 weeks after surgery. The examination was done in a masked fashion, and the examiner did not know what substance had been used in the eye. We evaluated after-cataract and posterior synechiae, which were graded on a scale of 0 to 4.

After 5 weeks, the pupils were fully dilated and retroillumination photographs were taken to evaluate after-cataract and synechiae evaluation, which again were graded from 0 to 4.

At the endpoint 6 weeks after surgery, the animals were killed with an intraperitoneal injection of phenobarbital, preceded by induction of anesthesia. All eyes except two in each
group were enucleated and fixated in formalin for histologic evaluation. The slides were stained with hematoxylin-eosin. It is impossible to evaluate the synechiae histologically. The after-cataract was evaluated and graded from 0 to 4.

3.5.2 Statistical analysis (III)

Kruskal-Wallis ANOVA with multiple comparisons was used to calculate the difference between the groups regarding after-cataract and synechiae formation.

3.6 STUDY DESIGN (IV)

Two eyes from each group from study III were used for study IV. Six weeks after surgery, the eyes were enucleated and the capsular bag was extracted from the eye and fixed for transmission electron microscopy (TEM). We also enucleated the contralateral eyes from two rabbits in group 1 irrigated with BSS. These eyes had not had surgery and served as a control to observe an unaffected posterior capsule. In these eyes, the capsular bag including the crystalline lens was extracted and fixed for TEM.

3.6.1 Transmission Electron Microscopy (IV)

Small pieces of the capsular bags were fixed in 2% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M sodium cacodylate buffer containing 0.1 M sucrose and 3 mM CaCl₂ (pH 7.4) at room temperature for 30 minutes followed by 24 hours at 4°C. The small pieces were rinsed in 0.15 M sodium cacodylate buffer containing 3 mM CaCl₂ (pH 7.4) and postfixed in 2% osmium tetraoxide in 0.07 M sodium cacodylate buffer containing 1.5 mM CaCl₂ (pH 7.4) at 4°C for 2 hours. The specimens were dehydrated in ethanol, followed by acetone and finally embedded in LX-112. Semi-thin sections were cut and stained with toluidine blue and used for light microscopic analysis. After this analysis, ultra-thin sections of approximately 40 to 50 nm were cut and contrasted with uranyl acetate followed by lead citrate. These ultra-thin sections were examined in a Tecnai 10 TEM at 80 kV. Digital images were taken using a Mega View III digital camera (Soft Imaging System, GmbH, Münster, Germany).

Only two eyes irrigated with each substance were used and no statistical analysis could be performed.
4 RESULTS AND DISCUSSION

4.1 SURGICAL TECHNIQUE (I, II, III, IV)

The sealed capsule irrigation device was applied successfully in all operated eyes and the sealing effect was maintained throughout the procedure. This also had been reported in older rabbits (Maloof et al. 2005).

4.2 IRRIGATION SUBSTANCE (I)

It was believed initially that distilled deionized water prevents PCO formation. This was shown in vitro in human capsule samples (Crowston et al. 2004). However, a later human in vivo study showed that there was no difference when performing regular cataract surgery, compared to using the Perfect Capsule and irrigating with DDW (Rabsilber et al. 2007).

In the first study, we compared irrigation with DDW, BSS, and 5-FU 50 mg/ml. The first clinical control 23 days after surgery showed that 5-FU was associated with significantly less after-cataract than BSS but not compared with DDW (Figure 9).

\[ \text{Group 1: BSS} \quad \text{Group 2: DDW} \quad \text{Group 3: 5-FU} \]

\[ p = 0.49 \quad p = 0.11 \quad p = 0.0043 \]

Figure 9. Results of the first clinical control.

Evaluation of the images taken at 5 weeks showed that 5-FU was associated with less after-cataract than BSS and DDW (Figure 10). There was no significant difference between DDW and BSS.
Histologic evaluation after the endpoint showed that 5-FU was associated with less after-cataract than BSS but not compared with DDW (Figure 11).

A comparison within each group in study I using a sign test showed that in group 1 irrigated with BSS there was no difference between the eyes irrigated using the Perfect Capsule and BSS and the fellow eyes, in which the Perfect Capsule was not used (Figure 12).
Figure 12. On the left, an eye with the Perfect Capsule irrigated with BSS. On the right, the fellow eye without the Perfect Capsule and irrigation at surgery.

In group 2 irrigated with DDW, there was no difference between the eyes irrigated with the Perfect Capsule and DDW and the fellow eyes, in which the Perfect Capsule was not used (Figure 13).

Figure 13. On the left is an eye with the Perfect Capsule at surgery, irrigated with DDW. On the right is the fellow eye without the Perfect Capsule and irrigation at surgery.

In group 3, irrigated with 5-FU 50 mg/ml, there was significantly less after-cataract in eyes receiving irrigation using the Perfect Capsule and 5-FU and the fellow eyes, in which the Perfect Capsule was not used (Figure 14).
5-FU 50 mg/ml was the most effective agent compared with DDW and BSS. DDW was effective in \textit{in vitro} studies of human anterior capsule samples but not in \textit{in vivo} human studies performed later. This might be due to the fact that in \textit{in vivo} studies, there is probably a small amount of cortex that cover the LECs so that they are not fully exposed to the substance, while in \textit{in vitro} studies, all LECs are directly exposed to the substance.

The posterior capsule thickness did not differ significantly between the groups or within each group between the eyes with the Perfect Capsule and the fellow eyes.

4.3 IRRIGATION SUBSTANCE (III)

In study III, we evaluated the possibility of lowering the 5-FU concentration and still obtain a good antiproliferative effect on the LECs. We compared 5-FU 50 mg/ml and 25 mg/ml concentrations of 5-FU. We also evaluated thapsigargin, which is derived from a plant, thapsia gargencia. Duncan et al. performed some experiments with this substance and reported its efficacy against LEC proliferation in a human \textit{in vitro} model (Duncan et al. 1997; Duncan et al. 2007).

We analyzed the results of the evaluations at four time points; clinical examination 1 (Table 1a), clinical examination 2 (Table 1b), images (Table 1c) and histologically (Table 1d).
<table>
<thead>
<tr>
<th></th>
<th>BSS</th>
<th>TG 300 µM</th>
<th>5-FU 50 mg/ml</th>
<th>5-FU 25 mg/ml</th>
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<td></td>
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<tr>
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<td><strong>c. Photograph evaluation</strong></td>
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<td><strong>d. Histologic evaluation</strong></td>
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Table 1. The *P* values for comparisons of after-cataract and synechiae between groups, using Kruskal-Wallis ANOVA with multiple comparisons. The italic data (top right corner in the shaded boxes) show the *P* values for comparisons of synechiae. Data in the bottom left corner in the white boxes show *P* values for comparisons of after-cataract. *P* values below 0.05 are bold. Table a shows the results from the first clinical examination, table b from the second clinical examination, c the results from the photographic evaluation, and d from the histologic evaluation. In d, there are no figures for synechiae, since there was no histologic evaluation.
We concluded that thapsigargin was ineffective in this animal model since it did not significantly prevent either after-cataract or synechiae compared with BSS in any evaluation. Figure 15 shows an eye irrigated with thapsigargin.

Figure 15. After-cataract is seen in this eye irrigated with thapsigargin in the Perfect Capsule. The pupil is fully dilated with a great deal of synechiae.

5-FU 25 mg/ml was not as effective as 5-FU 50 mg/ml. The higher concentration of 5-FU was significantly better than BSS in all evaluations; however, 5-FU 25 mg/ml was not better in all evaluations. In this model, the higher concentration of 5-FU is needed to effectively prevent after-cataract, which agrees with the results of a previous study. 5-FU in a concentration of 12.5 mg/ml did not inhibit after-cataract formation in the rabbit eye (Chew et al. 2006).

4.4 SAFETY (II, IV)

It is important to look at the safety of the Perfect Capsule and the safety of the substance used together with the device. What happens if leakage occurs during irrigation? In the safety study, study II, we looked at four groups. The first group was a control group irrigated with BSS. The second group was irrigated with 5-FU 50 mg/ml and the 5-FU was left in the eye after irrigation with no BSS washout. The third group represented the normal situation, in which there was irrigation with 5-FU and then the substance was washed out. The fourth group was used to evaluate the toxicity of 5-FU in another way; the Perfect Capsule was not used, but 5-FU was instilled in the capsule after lens extraction and then removed by I/A.

We assessed the safety based on the endothelial cell count, pachymetry, and histology. Pachymetry values correlated well with the endothelial cell count after cataract surgery.
(Lundberg et al. 2005). The endothelial cells regrow rapidly in young rabbits, but by 24 hours after surgery, they should not have had time to regrow (Nuyts et al. 1992). Histology was performed by an experienced ophthalmic pathologist, who looked at minimal damage with TUNEL staining and damage to the intraocular tissues; the trabecular meshwork, and the peripheral and central retina.

There were no differences in endothelial cell counts (Figure 16) among the four groups or in pachymetry measurements.

Figure 16. Endothelial cell count image of an eye from group 3 irrigated with 5-FU 50 mg/ml followed by washout of the substance for 10 seconds with BSS: On the left is the preoperative image and on the right the 24-hour postoperative image.

Histology (Figure 17) showed no tissue damage resulting from the substances used. TUNEL staining for apoptotic cells was negative in all examined samples.
We also compared the eye with the Perfect Capsule irrigated with BSS and the fellow eye that did not receive irrigation with the Perfect Capsule to determine if just the use of the Perfect Capsule damaged the endothelial cells or other tissues. There was no difference between the eyes either in endothelial cell count, pachymetry, or histology.

5-FU has been reported to be safe (Nuyts et al. 1992; Creten et al. 2006). The activity of 5-FU is probably also dependent on the pH. When the pH drops below 8.52, 5-FU 50 mg/ml precipitates (Stiles et al. 1989). In our experiment, 5-FU mixed with the anterior chamber fluid or the irrigation fluid. The pH then decreased to about 7.4, and 5-FU might not be as toxic. This is the situation in vivo in cataract surgery, which might be an advantage.

4.4.1 Transmission Electron Microscopy (IV)

All TEM analyses were done by one person in a masked fashion. The evaluation showed an ultrastructure of thin collagen fibers arranged on the anterior and posterior part of the posterior lens capsule (Figure 18). These comprise about one third of the posterior capsule, the rest is an amorphic matrix.
There was no difference in the structure of the posterior capsule between the eyes irrigated with BSS, 5-FU 50 mg/ml, 5-FU 25 mg/ml, or thapsigargin. In addition, the ultrastructure did not differ from the eyes that had not had surgery. 5-FU has been used in ophthalmic surgery such as glaucoma filtering procedures and intravitreal injections. It is safe for the ocular tissues (Blankenship 1989; Lattanzio et al. 2005). 5-FU was reported to be safe for the cornea, capsular bag, and retina when used through an intracapsular ring after phacoemulsification in rabbit eyes (Pandey et al. 2002). TEM has been used to evaluate the posterior lens capsule and is considered a valid method to evaluate the posterior capsule morphology (Al-ghoul et al. 1998; Pandey et al. 2002).
5 CONCLUSIONS

VAO is the most common complication in the young children after cataract surgery. This might be managed by performing a posterior capsulorhexis and anterior vitrectomy at the primary surgery. However, the youngest children can develop VAO anyway. Also, it would be best if the vitreous could remain untouched for the future. We evaluated the Perfect Capsule, a sealed-capsule irrigation device that can be used in cataract surgery. In our young rabbit model, the device was used successfully in all operated eyes. Our experiments showed that 5-FU 50 mg/ml was the most effective substance evaluated to prevent after-cataract. The safety of 5-FU 50 mg/ml as an irrigation substance with the Perfect Capsule device also was investigated; the corneal endothelium, trabecular meshwork, and central and peripheral retina were not damaged. We did not detect damage when looking at the posterior capsule on TEM.

We hope that this device can be used in the future with children with cataract who need surgery, and hopefully complications such as VAO can be avoided.
6 ACKNOWLEDGEMENTS

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7 REFERENCES


